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Abstracts are listed in alphabetical order by the last name of the senior author.

Analysis of *Plasmodium* gene expression during its development in the mosquito, *Anophleles stephensi*

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Plasmodium, the causative agent of malaria, has to complete a complex developmental program in its mosquito host for transmission to occur. However, little is known at the molecular level about mosquito-parasite interactions. By use of subtraction libraries, we have begun an analysis of mosquito and parasite genes expressed during *Plasmodium berghei* development in *Anopheles stephensi*. The libraries were obtained by subtracting mRNA of non-infected blood-fed mosquito guts and/or *Plasmodium* asexual stages from mRNA of *P. berghei* ookinetes or infected mosquito guts. Libraries enriched for genes preferentially expressed at 4 developmental stages were prepared: 1) *in vitro* cultured ookinetes, 2) early oocysts, 3) middle oocysts, and 4) late oocysts. A total of 1,657 unique sequences were identified among the 2182 clones that were sequenced. Of these, 1032 were of presumptive mosquito origin (<55% AT) and 625 of *Plasmodium* origin (>55% AT). Northern blot analysis of several randomly selected genes indicates upregulation at a time of development corresponding to the library of origin. A polyclonal antibody to a putative cell surface ookinete protein containing a von Willebrand type A adhesive domain inhibited oocyst formation by 60-79%, making this a candidate for transmission-blocking antigen. Clones encoding three different mosquito caspases were isolated. Interestingly, the three genes are induced in the midgut epithelium at the time of parasite invasion.

Reduced vector competence of transgenic mosquitoes expressing bee venom PLA2 from a gut-specific peritrophic matrix protein-1 (*Ag-Aper1*) promoter

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Ag-Aper1 encodes a major *Anopheles gambiae* peritrophic matrix (PM) protein. The PM is an acellular matrix secreted by the midgut epithelium in response to a blood meal. The *Ag-Aper1* protein is stored in vesicles prior to the blood meal and is secreted immediately upon blood ingestion. This makes the *Ag-Aper1* promoter an attractive candidate to drive the expression of molecules, such as the bee venom PLA2, which can interfere with *Plasmodium* differentiation (Zieler et al. 2001). To test this hypothesis, we transformed into the *Anopheles stephensi* germ line a *piggyBac*-based construct containing a 2.5 kb *Ag-Aper1* promoter and signal sequence fused to the PLA2 coding sequence. Abundant gut-specific expression of PLA2 mRNA and protein was detected in four independent transgenic lines. As is the case for *Ag-Aper1*, the PLA2 protein was stored in the midgut epithelial cells of transgenic mosquitoes and secreted into the lumen upon blood ingestion. Significantly, formation of *P. berghei* oocysts was strongly (73-91%) inhibited in transgenic mosquitoes. These findings demonstrate the feasibility of genetic manipulation of mosquito vector competence.

Instability of the piggyBac element in transformed *Aedes aegypti*

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The piggyBac element has been used to transform a number of different insect species, including the mosquitoes *Anopheles stephensi* and *Aedes aegypti*. A piggyBac plasmid (p3xP3EGFPUbD2FB) was microinjected into *Ae. aegypti* embryos (Higgs white eye strain) along with a helper plasmid containing the piggyBac transposase gene. Five independent transpositions were identified as G, individual animals positive for the marker gene (EGFP+). Two of these individuals died before they could produce progeny, while two produced numerous progeny following mating, but none were EGFP+. The last family, # 104, was the only family to give EGFP+ progeny in the Gz. Surprisingly, only 10% of progeny from both an outcross and intercross were EGFP+. Gene amplification of pooled genomic DNA confirmed that the inserted piggyBac element was not present in EGFPindividuals, only in EGFP+ mosquitoes, ruling out a simple loss of EGFP protein expression. Southern analyses of line #104 showed multiple copies of the inserted piggyBac element in both Gz and G3 individuals. Genomic DNA fragments containing transposonchromosome junctions also were observed to contain plasmid DNA, while others did not, indicating multiple integrations, both precise and imprecise. Plasmid rescue experiments failed to detect episomal transposons, but full-length plasmid was easily recovered when genomic DNA was digested by a specific restriction endonuclease, religated and transformed into *Escherichia coli.* Line # 104 has been carried to GS by intercrossing and continues to produce 1030% EGFP+ individuals. Multiple outcrosses of this line have repeatedly led to complete loss of the inserted element. This work is supported in part by grant A148740 from NIAID.

RNAi based inhibition of salivary gland invasion by *Plasmodium gallinaceum* sporozoites using recombinant double subgenomic Sindbis viruses.

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RNA interference (RNAi) has been used in organisms such as plants, flies, and worms to generate loss of function mutant phenotypes. RNAi has also been shown to repress specific gene expression in the mosquito, *Aedes aegypti,* and in the protozoans, *Trypanosoma* and *Paramecium*. We have tested whether RNAi induced in *Ae. aegypti* could affect another protozoan, *Plasmodium gallinaceum*, the causative agent of avian malaria. Recombinant Sindbis viruses were generated that contained a portion of the P. gallinaceum TRAP or CSP gene in either sense or antisense orientation (TRS, TRAs, CSS, CSAs). Both TRAP and CSP are surface proteins involved in sporozoite mobility and salivary gland invasion. Recombinant viruses were injected into newly emerged *A. aegypti* female mosquitoes. At 710 days post injection, mosquitoes fed on *P. gallinaceum* infected chicks. After 13 days the number of sporozoites per pair of salivary glands (spz/gl) was determined. Mosquitoes injected with L15 medium or with a control virus contained an average of 10,000 spz/gl, as did uninjected mosquitoes. In contrast, 8/27 and 12/27 (30 and 44%) of TRS and TRAs injected mosquitoes contained between 1500 spz/gl. Similarly, 12/61 and 7/50 (20 and 14 %) of CSS or CSAs injected mosquitoes had 1500 spz/gl. These data show that RNAi induced in the mosquito host can affect development of *P. gallinaceum*. This work is supported in part by grant AI29746 from NIAID.

Characterization of the pyrokinin/pban receptor

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The pyrokinin (PK)/pheromone biosynthesis activating neuropeptide (PBAN) family of peptides is a multifunctional family that plays a major role in the physiology of insects. The presence of PK/PBAN peptides has been demonstrated in a variety of moths and in other non-lepidopteran species, and their mode of action has been studied extensively. Despite these studies, very little is known about the endogenous mechanism, and much remains to be determined concerning the structural, chemical and cellular basis of their activity. Most of these studies were performed with synthetic peptides and involved exogenous application (mainly by injection) of the tested compounds, which does not necessarily reflect the endogenous natural mechanisms. Currently, it is still not known which endogenous peptide(s) mediate(s) each of the *in vivo* functions, whether each function is mediated by a different peptide, and whether each peptide mediates one or several functions. It is also not clear whether these functions are mediated by the same receptor or by different receptors, or whether the receptors of the various PK/PBAN peptides share functional homologies. One way to obtain a better insight on the mode of activity of this family of peptides is by the characterization of their receptors. Currently very little is known on the receptors of the PK/PBAN family. Recently, we have developed a binding assay and characterized the properties of the PK/PBAN receptor using female moths (*Heliothis peltigera)* pheromone glands. A summary of our recent findings will be presented. A complementary approach for studying receptors is by means of receptor-selective agonists and antagonists. We have developed a novel approach, termed the backbone cyclic neuropeptide-based antagonist (BBC-NBA) for the discovery of neuropeptide antagonists. This approach has been applied to the insect PK/PBAN family and resulted in the discovery of highly potent, metabolically stable antagonists, devoid of agonistic activity. The application of the antagonists for the study of the functional diversity and characterization of the PK/PBAN receptors as well as their possible application as insect control agents will be discussed.

RNAi mediated knockout of a GATA type transcriptional factor in *Aedes aegypti*

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In *Aedes aegypti* the reproductive cycle is tightly linked to the acquisition of a blood meal. Upon initiation of the reproductive cycle, tissue specific transcription, translation and secretion of yolk protein precursor genes (YPPs) from the fat body occurs on a massive scale. This process is called vitellogenesis. In the period prior to blood meal activation (previtellogenesis) YPP genes are repressed to prevent premature expression, this repressed state is called the state of arrest. Analysis of the promoter from the major yolk protein vitellogenin (Vg), revealed multiple binding sites for GATA type transcription factors. A fat body specific GATA factor (AaGATAr) was cloned and characterized. This factor is expressed during the previtellogenic period, and in cell transfection studies has been shown to have transcriptional repression activity. We hypothesize that this GATA factor maintains the previtellogenic state of arrest prior to blood feeding. To test this hypothesis we are using RNAi to knockout AaGATAr function. RNAi stimulates the degradation of a specific mRNA in response to the presence of a double stranded RNA homologus to the mRNA. Double stranded RNA was delivered by a recombinant Sindbis virus that expressed a single stranded transcript containing inverted repeats from AaGATAr. The inverted repeats cause the RNA to fold over into a double-stranded conformation thereby inducing the RNAi response. To test the effects of this knockout we used RT-PCR to measure levels of YPP transcripts in response to the knockout of this gene. Preliminary results indicate that knockout of AaGATAr caused a modest increase in expression of Vg indicating premature derepression of this gene in RNAi treated mosquitoes.

Initial characterization of a cDNA library from diapausing pupae in the fleshfly, *Sarcophaga crassipalpis*.

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Diapause is an optional, inducible developmental state in the flesh fly, *Sarcophaga crassipalpis*. We are interested in the molecular controls underlying the developmental control of the initation and maintenance of this state. A cDNA library was constructed from the brains of diapausing pupae of *S. crassipalpis*. Initial cDNA was constructed using RT-PCR and the cDNA inserts were cloned into bacteriophage vectors. Plasmids were rescued from the phage vector and initial characterization of the inserts has begun. We are attempting to assemble a set of representative genes for use in a microarray analysis of diapause.

Linkage mapping in a multiresistant strain of diamondback moth *Plutella xylostella* reveals separate genetic mechanisms of Cry1A and Cry1C resistance.

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Linkage mapping with AFLPs was conducted in a backcross family originating from a cross between susceptible diamondback moths and a field-derived strain showing resistance to both Cry1Ac and Cryl1C. A single linkage group was responsible for the majority of the Cryl1Ac resistance. This is the same linkage group as previously identified in the NO-QA strain from Hawaii. Finer-scale mapping is underway to investigate whether the same chromosomal region is involved. Two separate linkage groups were associated with Cry1C resistance. Four aminopeptidases have been tested as candidate resistance genes and none of them map to the three resistance-associated linkage groups. We discuss the use of denaturing HPLC in rapidly mapping additional candidate loci.

Understanding the mechanism of juvenile hormone action in coleopterans

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The mode of action of juvenile hormone (JH) is one of entomology's longest standing mysteries. In *Dendroctonus jeffreyi* and *Ips pini*, JH III induces an increase in pheromone biosynthetic gene expression, especially *3-hydroxy-3-methylglutaryl-CoA reductase* (*HMG-R*), in male midgut cells. These genes offer a new system to study JH regulation independent of complex processes involved in development and female reproductive maturation. To exploit the system, an EST library (currently 384 unique cDNAs) of male midgut cDNAs is being constructed using high-throughput technology. JH-responsive clones are being identified using microarrays. Preliminary northern blots prepared from beetles at various time points (within the first 8 hours) after JH treatment revealed some that are rapid, primary JH-responders, with transcripts visible after only 45 minutes. Curiously, *HMG-R* may be a secondary responder. The 5' flanking region of *D. jeffreyi HMG-R* has been isolated and sequenced. Important regions for induction will be mapped by transcriptional assays of reporter constructs transfected into isolated midgut cells. Tissue incubation studies are underway to determine the requirements for JH action on cells. Also, a genomic library is being prepared from *I. pini* in order to clone the 5' flanking regions from JH-inducible genes, including *HMG-R*. The isolation of JH-inducible genes and the promoter sequences that regulate them will provide important information about the mechanism of JH action in these two Coleoptera.

LaCrosse virus and *Aedes triseriatus* – molecular determinants of the most remarkable of arbovirus-vector interactions.

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Transovarial transmission (TOT) of LaCrosse virus by *Aedes triseriatus* is very efficient. TOT maintains, amplifies, and promotes evolution of LAC virus in nature. The molecular determinants of this remarkable host-parasite relationship are being revealed. Co-regulation of host and virus transcription modulates viral virulence during ovarian quiescence and embryo diapause. Virus transcription, which requires scavenging of caps from host mRNAs, may target an inhibitor of apoptosis mRNA, thereby perturbing the apoptotic response and promoting viral persistence. LAC virus does not induce apoptosis in vector cells and eludes the host RNAi response, resulting in long term persistent infection of vectors. These long term infections and TOT greatly enhance the evolution of LAC and related viruses both by antigenic shift and drift, and may account for the amazing evolutionary potential of viruses in the family Bunyaviridae.

Immunocytochemical localization of ecdysis-triggering hormone in the epitracheal system and silk glands of the parasitic wasp *Cotesia congregata*

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The release of ecdysis-triggering hormone (ETH) induces shedding of the cuticle during the final stages of a molt. In the present study we used antibodies directed against *Drosophila melanogaster* ETH to localize sites of synthesis of this peptide hormone in the epitracheal endocrine system of the parasitic wasp *Cotesia congregata*. This wasp undergoes its last larval ecdysis during emergence from the host larva. Pre-emergent wasps were found to have substantial amounts of ETH in both the epitracheal cells and silk glands, and the hormone appeared to be released during emergence of the wasps from the host. This report constitutes the first evidence that the hormone is present outside of the insect epitracheal system. Pre-absorption of antibody with *D. melanogaster* ETH abolished its binding to epitracheal cells and the silk gland lumen, indicating binding to ETH-containing cells was specific. The release of ETH appears to temporally correlated with wasp emergence, providing evidence for the hormonal regulation of the parasitoid's ecdysis and emergence behaviors. The release of ETH and emergence of the wasps is suppressed if the host is treated with the JH analogue methoprene, suggesting high levels of juvenile hormone have an inhibitory influence on the cascade of events triggering the wasp's emergence behavior.

A novel malaria gene, Pg4, is expressed preferentially in sporozoites isolated from mosquito salivary glands

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Malaria sporozoites exhibit infectivity for mosquito salivary glands and vertebrate host tissue, and are excellent targets for efforts designed to prevent malaria transmission. In this study, *Plasmodium gallinaceum* sporozoites were isolated from mosquito salivary glands and a cDNA library was constructed. Following a heterologous screening technique, a gene, designated, Pg4, was isolated from this sporozoite cDNA library. Pg4 is a novel gene as determined by BLAST analysis of its DNA sequence and translated protein sequence. It contains a signal peptide sequence that encodes a transmembrane domain as determined by PSORT, a protein sequence analysis program, and located near the carboxy terminus of the protein are ten six-amino acid tandem repeats. Transmembrane domains and repetitive sequences are characteristic of other *Plasmodium* surface antigens, suggesting that Pg4 may be a novel sporozoite surface antigen. Recombinant Pg4 protein (rPg4) was made and used to generate polyclonal antibodies that then were used in immuno-localization studies. Immuno-fluorescence assays suggest that Pg4 is a surface molecule and immuno-electron microscopy is being performed to confirm this cellular location. Interestingly, initial data suggest that Pg4 is preferentially expressed, at the mRNA and proteins levels, in the sporozoites isolated from mosquito salivary glands as compared to those isolated from oocysts located on the mosquito midgut. Future studies include the use of the anti-Pg4 polyclonal antibodies as well as rPg4 in *in vivo* blocking studies to determine if Pg4 may play a role in the invasion of mosquito salivary glands or vertebrate host tissue.

Molecular and evolutionary analysis of Juanα and Juanβ: Two widely dispersed non-LTR retrotransposon families in mosquitoes

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Juan,a retrotransposon originally discovered in *Culex pipiens quinquefasciatus* (Mouches et al. 1991), was found in 18 species of five genera. A new family related to Juan was found in eight species of three genera. This new family is named Juanβ while the original Juan is called Juanα. Juanβ comprises divergent subfamilies, three of which were found by searching the sequenced *Anopheles gambiae* genome. However, Juanα was not found in *An. gambiae* and appears to have been lost from this species. Phylogenetic analysis supports the grouping of Juanβ with Juanα in the Jockey clade along with BS, Doc, and Jockey elements of *Drosophila melanogaster*. Tree topology of Juanα has an overall congruence with mosquito phylogeny, supporting vertical transmission of these elements. No support was found for horizontal transfer of Juanα among *Aedes* species as was previously proposed (Mouches et al. 1991). This demonstrates the first example of a non-LTR element without target site specificity that has been sustained in a major Dipteran lineage by vertical transmission. A conclusion regarding transmission cannot be made for Juanβ due to having sequences from few taxa and the

presence of subfamilies. GC content analysis shows Juanβ sequences having an overall high GC content and G/C mutational bias in the 3rd codon position, similar to that of host coding sequences. In contrast, Juanα sequences are AT rich and have an A/T mutational bias in the 3rd codon position. Also, a Juanβ copy was found inserted in close proximity to a histone gene in *Culex quinqefasciatus*. The apparent difference in mutational pressure between Juanα and Juanβ is discussed. In a few species, a two to 100 fold higher copy number was determined for Juanα by library screening. The differences described make for an interesting evolutionary study of two related retrotransposons in the mosquito. Finally, data reported here suggest recent activity of both Juanα and Juanβ in some species. These include high sequence identity, intact open reading frames, a high ds/dn ratio among sequences, and ESTs that correspond to Juanα in *Aedes aegypti* and Juanβ in *An. gambiae*. In addition, a RT-PCR product for Juanβ was found using *An. gambiae* cultured cells. With evidence of activity, these retrotransposons could contribute to the development of mutagenesis tools or transformation vectors for the study of medically important mosquitoes.

Using transposon display to identify polymorphic markers in the African malaria mosquito, *Anopheles gambiae*

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Transposon display (TD), a modified form of amplified fragment length polymorphism (AFLP), has been successfully used to identify transposable element insertion polymorphisms (TEIPs) in *Anopheles gambiae* individual mosquitoes. TD differs from AFLP in that one of the primers is designed according to a transposable element. This technique was shown to be both specific and reproducible using several transposons in *Anopheles gambiae*. Re-amplification and sequencing of bands from a TD gel verified the presence of both transposon and genomic sequence, which allowed mapping of the transposon insertions onto the *Anopheles gambiae* genome. We also showed a relatively high level of insertion polymorphism of some of these transposons. With the completion of the *An. gambiae* genome sequence in the near future, the establishment of a robust TD assay for mosquitoes is invaluable. It will be a powerful new tool for the study of transposon activity and for population analysis of this medically important mosquito species.

cDNAs encoding putative odorant binding proteins and other ligand carrier proteins from the antennae of *Anopheles gambiae.*

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To obtain a better understanding of the olfactory processes that allow mosquitoes to identify human hosts, a molecular study has been performed to identify and characterize molecules in the olfactory signaling pathway of the African malaria vector *Anopheles gambiae*. cDNA libraries from antennae of females and males were established and characterized. We report the isolation and preliminary characterization of eight cDNAs from these antennal libraries that encode putative odorant binding proteins (ODP). Their conceptual translation products show extensive sequence similarity to known insect OBP, especially to those of *Drosophila melanogaster*. The *A. gambiae* OBPs are expressed at different levels in the antennae of both genders. In addition, other cDNAs were found encoding small putative ligand carrier proteins. Two of these novel proteins show sequence similarity to the *D. melanogaster* protein TAKEOUT that is under control of a circadian clock and is linked to feeding behavior. A large-scale survey of 20,000 cDNA antennal clones on filter arrays revealed quantitative but not qualitative differences in antennal gene expression in males vs. females. OBPs are the most abundantly expressed genes in A. *gambiae* antennae, accounting for about15% of cDNAs.

Sphingomyelinase D from venoms of brown spiders: evolutionary insights from gene structure.

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Loxosceles spider venoms are famous for causing dermonecrotic lesions in human tissues. The enzyme sphingomyelinase D (SMD) is a central causative agent for lesion formation. This enzyme is not known elsewhere in the animal kingdom, but is found in some pathogenic bacteria. We cloned a cDNA sequence from *L. arizonica* with homology only to previously reported N-terminal amino acid sequences for SMD from *Loxosceles*. We used this sequence to screen a *L. arizonica* genomic library. The gene spans at least 6,500 bp, contains 5 introns, and has at least one paralog. The presence of a signal sequence indicates SMD is expressed as a zymogen with a trypsin cleavage activation site. Weak similarity suggests SMD is a divergent member of the glycerophosphodiester phosphodiesterase family. These data suggest an evolutionary origin by gene duplication rather than horizontal transfer from bacteria.

The two *Drosophila melanogaster* receptors for tachykinin related peptides are differentially distributed in the brain

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In *Drosophila melanogaste* two different G protein coupled receptors, NKD and DTKR, have been identified with tachykinin related peptides (TRPs) as likely ligands (Li et al., *EMBO J. 10:* 3221 322, 1991; Monnier et al., J. *Biol. Chem. 267:* 1298 1302, 1992). Transcripts of both receptor genes were shown to be expressed in the central nervous system. However, until now the distribution of the receptor proteins has not been studied. To identify possible

sites of action of TRPs in the *D. melanogaste* brain we have raised antisera to portions of the NKD and DTKR proteins. As antigens for NKD we used two different peptides coupled to Keyhole limpet hemocyanin: (1) a portion of the 3rd intracellular loop and (2) part of the C terminus. For DTKR we used a portion of the C terminus coupled to bovine serum albumin as antigen. The receptor antisera were used for westem blots on brain extracts. Both NKD antisera recognized protein bands of the same molecular weight (around 60 kDa) and the DTKR antiserum identified a band at about 65 kDa. The antisera were also employed for immunocytochemistry on sections of brains. The NKD protein was seen in neuronal varicosities in brain neuropils of the protocerebrum (central body, dorsal and lateral protocerebrum) and subesophageal ganglion, as well as in a small number of neuronal cell bodies. DTKR immunoreactivity was more abundant: labeled varicosities were detected in the central body, dorsal and lateral protocerebrum, the antennal lobes and part of optic lobe and cell bodies in the median neurosecretory cell group and a few other sites of the brain. Although the two receptor proteins in some cases are localized to the same general brain areas, it is clear that their neuronal distributions are mutually exclusive. Only DTKR was seen in antennal lobes and optic lobes and different layers of the central body contain NKD and DTKR immunoreactive varicosities. Taken together the distribution of the two receptors correlate well with the distribution of TRP containing neuronal processes. Thus the TRP signalling system appears to employ two distinct receptor types with a differential distribution. The functional consequences of the dual receptor system may in the future be approached in *D. melanogaste* by genetical disruption of each of the two receptors.

Patterning insect gnathal and thoracic appendages

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The development of the proximal distal axis of *Drosophila melanogaste* legs depends on the relative spatial amount of the morphogens wingless (wg) and decapentapiegic (dpp) in the early leg imaginal disc. High WG and DPP signals activate the expression of Distal less (D11), which is necessary for the development of distal leg structures. While D11 and *wg* expression patterns are similar between the grasshopper *Schistocerca americana* leg and the *D. melanogaste* imaginal disc, expression patterns of *dpp* vary greatly between the two species. Although the *dpp* expression pattern differs in grasshoppers, high WG and DPP levels could nonetheless be required to set up the morphogenetic gradient. However, pSMAD, the downstream transducer of the DPP signal is localized in the same banding pattern as *dpp* in the grmhopper leg. Hence, it is more likely that D11 expression is not regulated exclusively by high levels of WG and DPP. In addition, the role of *dpp, wg,* and, D11 in the formation of branches was studied by looking at their expression in the ventral branches of the grasshopper mouthparts. The patterns of wg and dpp expression seen in the leg are similar in the grasshopper mouthpart during its patterning. wg is similarly expressed in the grasshopper mouthparts as it is in the leg, and there is no apparent modulation of wg expression during branch formation. pSMAD is expressed only in the distal tip of the mouthpart and not in the branches. These results suggest other factors likely establish branches in the grasshopper mouthpart. Thus although *dpp, wg,* and *D11* are all involved in establishing the proximal distal axis during insect leg development, the specific roles of these gene products may vary.

Biochemistry and molecular biology of JH-regulated pheromone biosynthesis in pine bark beetles

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Important pheromone components of the pine bark beetles *Ips pini* and *Dendroctonus jeffreyi* are ipsdienol and frontalin, respectively. Bark beetle monoterpenoid pheromones have historically been thought to arise via simple modifications of host tree precursor molecules. A growing body of evidence now supports the *de novo* production of certain components. Since ipsdienol and frontalin synthesis in males is regulated by juvenile hormone (JH), we investigated the effects of JH on pheromone-related gene expression and metabolism in *D. jeffreyi* and *I. pini*. JH treatment induced a large increase in 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-R) gene expression, and a modest increase in HMG-CoA synthase mRNAs in both beetles, all of which correlate with increased pheromone production. HMG-R enzyme activity is also elevated by JH treatments, and radiolabeled mevalonate is incorporated into ipsdienol and frontalin in *I. pini* and *D. jeffreyi*, respectively. These data are strong evidence that the monoterpenoid pheromone components are derived *de novo* via the mevalonate pathway. They provide the basis for a paradigm shift of bark beetle pheromone production from the model in which host-tree precursors are modified to pheromone components to one where JH regulates *de novo* biosynthesis of key isoprenoid pheromone components.

Modified *Jc*DNV somatic transformation vectors provide an efficient means of assessing promoter activity in insects

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Plasmids containing a modified genome of the *Junonia coenia* lepidopteran densovirus (*Jc*DNV) integrate into the genome of a host insect cell rather than becoming infectious. These plasmids have been utilized to achieve somatic transformation in *Drosophila melanogaster* that is stable from embryo to adult (Royer et al, 2001, Insect Molcular Biology 10, 275). We have assessed the effect of additional modifications including different expression cassettes on the efficiency of *Jc*DNV plasmid somatic transformation activities in three orders of insects. The plasmid pJDsRed[3xP3EGFP]H,

which contains the Densovirus P9 promoter controlling expression of DsRed fluorescent protein and the 3xP3EGFP expression cassette (Berghammer et al., 1999. Nature *402*, p370), was injected into syncytial embryos of the fruitfly *D. melanogaster* w^m, the moths *Plodia interpunctella* and *Ephestia kuehniella*, and the beetle, *Tribolium castaneum*. Somatic transformation was observed on the basis of either DsRed and/or GFP fluorescence in G0 embryos and larvae of all four species at rates from 40-95%. Where comparisons could be made, the expression patterns were consistent with germ line transformed insects. Removal of the *Jc*DNV coding sequences for nonstructural proteins or the right inverted terminal repeat had no effect on the rate of somatic transformation. Cloning of 3xP3EGFP in the unique *Pvu*I site outside the *Jc*DNV sequence did not affect the transformation rate either. When compared with observed production from the *piggyBac* gene vector, similar frequencies and patterns of DsRed and GFP expression were observed for pJDsRed[3xP3EGFP]H in *D. melanogaster* G0s. However, no detectable expression was observed from the *piggyBac* gene vector in *P. interpunctella* or *E. kuehniella* G0s while up to 90% of the insects expressed DsRed after somatic transformation with pJDsRed[3xP3EGFP]H. These experiments demonstrate the utility of *Jc*DNV vectors in assessing promoter activity in insects.

piggyBac transposon-derived gene vector system activity in production of transgenic insects

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The *piggyBac* transposon gene vector can be used to achieve germ line transformation in three insect orders. We have established protocols that result in consistently high transformation rates in *Drosophila melanogaster* by adjusting the timing of microinjection to a period of embryogenesis when there are few nuclei to transform. In addition, we have demonstrated that transformation with *piggyBac* is supported over a forty fold range of helper concentration in *D. melanogaster* $w^{[m]}$ and is not subject to feedback inhibition at the highest concentrations of helper tested. We utilized the pB[3xP3EGFP]af (Berghammer et al., 1999, Nature 402, 370-371) transformation vector in combination with the phsp-pBac helper (Handler and Harrel, 1999, Insect Molcular Biology 8, 449-457) and varied the helper concentration from 0 to 600 ng per µl. A 20% transformation rate was maintained from 10 to 400 ng/µl. These observations suggest that the *piggyBac* gene vector can be used to achieve genetic transformation with a high probability of success because the transposition of the gene vector is not restricted by the mechanics of the transposase activity. In order to provide a more stable helper, the phsp-pBacwc helper was constructed by removal of both inverted terminal repeats. The phsp-pBacwc helper supports transformation rates identical to those of phsp-pBac. To establish the activity of an expression cassette containing a lepidopteran viral promoter IE1 (Jarvis et al., 1996, Protein Expr. Purif. 8:191-203), the vector pB[3xP3EGFP-IE1DsRed] was constructed and tested for activity in *D. melanogaster*. The IE1 promoter was active in the pupal and adult stages but not the embryonic or larval stages. The activity of this expression cassette is being assessed in lepidopteran and coleopteran species.

Forward genetic isolation of synaptic mutants in *Drosophila melanogaster*: *slug-a-bed* and *rolling blackout* in presynaptic mechanisms

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Genetic screens in *Drosophila melanogaster* for paralyzed, or conditionally paralyzed, mutants have elucidated a number of fundamental neuronal mechanisms. Proteins identified in this way include founding members of ion channel families, e.g. *paralytic* (Na^+ channel), and fundamental components of the synapse, e.g. *shibire* (Dynamin GTPase). In this seminar, I will present two new genes identified as paralytic mutants in flies: 1) *slug-a-bed* (*slab*) which encodes the sole ceramidase in the fly genome, and 2) *rolling blackout* (*rbo*) which encodes a novel 3-pass transmembrane protein. The *slab* gene was identified as an unconditional paralytic mutant with severely impaired presynaptic neurotransmitter release at the glutamatergic neuromuscular junction. Ultrastructural analyses reveal that synaptic vesicles in *slab* mutants are arrested in a tethered cytoplasmic state ("reserve pool") and lost from the docked state at presynaptic active zones ("readily releasable pool"). The function of *slab* ceramidase is to catalyze the conversion of ceramide to sphingosine. Thus, *slab* is required in the sphingosine lipid pathway for determining the ratio of reserve: docked vesicles at the synapse, and hence the efficacy of synaptic transmission. The *rbo* gene was identified as a temperature-sensitive paralytic mutant with a complete, reversible loss of both phototransduction in the eye and synaptic transmission at the nmj. Null mutants are embryonic lethal and nearly paralyzed. The expression of *rbo* is nervous system specific and the protein is present in the plasma membrane in most/all neurons (including motor neurons, interneurons and sensory neurons). In CNS neurons, the protein is restricted to axons and the synapse-rich neuropil; the protein also localizes to presynaptic neuromuscular junction boutons. In photoreceptors, *rbo* is acutely required for Ca^{2+} influx mediated by the *transient receptor potential* (*trp*) and *trp-like* (*trpl*) Ca^{2+} channels. We have hypothesized that *rbo* mediates Ca^{2+} influx through this class of Ca^{2+} channels (13 genes in flies) throughout the nervous system. Thus, *rbo* acts as a novel TM protein to acutely regulate Ca^{2+} channels essential for both sight and movement. On-going studies on *slab* and *rbo*, along with an extensive collection of other genes identified in the lab, will provide the foundation for dissecting mechanisms of synaptogenesis and neurotransmission.

Linkage analysis of resistance to *Baciflus thuringknsis* in cotton bollworm *Heficoverpa armigera.*

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The BX strain of *H. arinigera is* resistant to CrylA type Bt toxins and can complete development on transgenic cotton. To investigate the genetic basis of this resistance, a linkage map based on AFLPs was constructed from a backcross derived from a cross between BX and a susceptible strain. Only one linkage group showed an association with Bt resistance; an AFLP from this linkage group was cloned and sequenced for development as an STS marker. The *H. armigera* homologue of the 11 domain cadherin responsible for resistance in the YHD2 strain of the related species *Heliothis virescens* (Science 293: 856 (2001)) was mapped in this family, and found to reside on a different linkage group. This shows that BX does not have the YHD2 type of resistance mechanism. Several aminopeptidases were also mapped to separate linkage groups; and the search for a candidate resistance gene is still continuing.

Tribolium Hox genes repress antennal development in the gnathos and trunk.

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Homeotic genes determine developmental fate along the anterior-posterior axis in animals. Homeotic mutations alter developmental fate, and result in the misspecification of segmental identity in insects. Work in *Drosophila melanogaster* suggests that in their normal expression domains, some homeotic genes repress antennal development while directing segment-specific identity during adult development. Our embryonic analysis of *Tribolium* homeotic mutants indicates that repression of antennal development by homeotic genes is conserved and that it occurs throughout the gnathos and trunk. Antennae develop on posterior segments in the absence of homeotic gene function. In *D melanogaster* embryos, anterior transformation is most complete when other genes such as *teashirt* are also removed. However, our analysis of a putative *Tribolium teashirt* homolog suggests that while its role in leg development is conserved, it does not play a role in determining trunk identity. Therefore, *Tribolium* homeotic proteins may not require modifiers such as *teashirt* to determine developmental fate.

Odor and taste: Receptors, genes, and cells

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We developed a computer algorithm to identify odor and taste receptors from the *Drosophila melanogaster* genome database. The algorithm identifies proteins with a particular structure, as opposed to a particular sequence. It maps the predicted products of open reading frames into an n-dimensional protein space and then determines which predicted proteins map into a portion of the space occupied by known G-protein-coupled receptors. We have isolated two large families of genes, the *Or* genes and the *Gr* genes, which are likely to encode odor and taste receptors. The molecular basis of odor coding has been studied by analyzing the expression and function of *Or* genes. The cellular basis of odor coding has been explored through physiological analysis of individual receptor neurons. We are now integrating the molecular and cellular maps of the olfactory system by recording from transgenic animals in which the promoters of individual *Or* genes are used to drive GFP expression. We record from neurons that are marked by GFP, thereby correlating particular *Or* genes with neurons exhibiting particular odor sensitivities. The goal is to understand the functional organization of the olfactory system at the molecular and cellular levels, and thereby to yield new insight into mechanisms of odor coding.

Factors controlling the retention or elimination of protein meals by the midgut of *Aedes aegypti* females.

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There is a relationship between the progress of digestion and the retention or elimination of the meal by the midgut of female *Aedes aegypti*. We are trying to identify factors involved in the regulation of this physiological process. The addition of Soy trypsin inhibitor (STI) to a protein meal prevented digestion and resulted in a rapid elimination of the undigested protein from the midgut. SDS-PAGE analysis of feces collected at different times after feeding showed the presence of undigested protein. The largest amounts of proteins in the feces were detected as soon as 2-5 hours after feeding; with most of the protein eliminated before 10 hours. The addition of free amino acids to a protein meal together with STI results in a significant increase in the retention of the meal by the midgut during the first 10 hours after feeding. The undigested meals were ultimately eliminated, but the “peak of elimination” was shifted from 2-5 to 15-24 hours after feeding. If digestion is not preceding normally the mosquito needs to vacate the midgut contents to make room for a new blood meal. The presence of free amino acids in the midgut lumen seems to be an important signal used by the mosquito to regulate the retention or elimination of the meal. Decapitation of the females immediately after feeding changed the patterns of the retention or elimination of the meal.

Modulation of vertebrate immune responses by saliva of the mosquitoes *Aedes aegypti* and *Anopheles stephensi*

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Successful blood feeding requires that mosquitoes inject saliva containing antihemostatic enzymes and other proteins. Exposure of these antigenic proteins to the host may be expected to elicit an

immune response that could be deleterious to the mosquito. We have found that saliva of two important vector species, *Aedes aegypti* and *Anopheles stephensi*, inhibits several aspects of the host immune response, including T-cell and B-cell proliferation and production of TH1 and TH2-type cytokines from T-cells. Marked decreases in the proportions of both CD4 and CD8 positive T-cells were observed. In contrast there appears to be little effect on MHCII positive cells including professional antigen presenting cells. The activity appears to be due to a single component of the saliva, a large (280-300 kDa) glycoprotein. This component is present in saliva of females but not males, and it is depleted from the salivary glands following a blood meal, indicating a role in blood feeding. It is possible that by modulating the host response to salivary antigens, this protein protects the feeding mosquito from potentially harmful host immune responses.

Detection of carbohydrate-binding molecules implicated in the establishment of mosquito infection on *Plasmodium berghei* ookinete

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Interactions between the invasive *Plasmodium berghei* ookinete and the midgut epithelium are believed to be critical for the establishment of infection of the parasite to the mosquito vector. Specific sugar inhibitors, such as N-acetyl-galactosamine (GalNac), incorporated in the mosquito blood meal containing *in vitro* cultured ookinetes significantly reduced the infectivity of the parasite to the midgut of mosquitoes, suggesting that carbohydrate-lectin interactions play important roles during infection. An *in vitro* binding assay and direct cell autoradiography with radiolabelled neoglycoproteins of known sugar moieties and the mosquito midgut microvillar protein extracts were developed to examine the nature of such carbohydrate-lectin interactions between the parasites and mosquitoes. Results showed that the radiolabelled neoglycoprotein, N-acetyl-galactosamine-BSA (GalNac-BSA), and the mosquito midgut microvilli (Mv) membrane proteins could specifically bind to all immature, retort ookinetes, but not to mature ookinetes and other parasite forms, such as merozoites and shizontes. Furthermore, the binding of the radiolabelled GalNac-BSA or midgut Mv membrane proteins to the ookinetes was inhibited with the addition of competitive sugar inhibitors, GalNac or mannose, in a dose-dependent manner, in both the *in vitro* binding assay and ookinete whole cell autoradiography. In contrast, radiolabelled N-acetyl-glucosamine-BSA (GlcNac-BSA) could bind to merozoites, shizontes and, to a less extent, to the immature ookinetes, but not to mature ookinetes. The binding of GlcNac-BSA to parasites was only inhibited by GlcNac itself, but not by either GalNac or mannose at 200 mM concentrations. In addition, deglycosylated midgut Mv proteins failed to bind to any parasite forms, as observed in ookinete cell autoradiography. We postulate that an N-acetyl-galactosamine/mannose-specific lectin is present on the immature transforming ookinetes, and is responsible for interactions with possible carbohydrate-receptor ligands from the mosquito midgut microvilli. Further experiments with plant lectins of known sugar-specificity support the presence of several unique glycoproteins between 25 and 32 kDa, as examined by autoradiography of isolated, radiolabelled Mv proteins in the SDS-PAGE under the reducing conditions, which contain GalNac and mannose moieties as potential ligands for the ookinete lectin.

Molecular characterization of bilin binding protein (BBP) from *Hyphantria cunea*

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The bilin-binding protein is a blue pigment protein binding heme-related compound in insects. The amino acid sequence from the bilin binding protein (BBP) of the fall webworm *Hyphantria cunea* has been determined. The cDNA has a length of 1333 bp coding for a 288 residue protein with a predicted molecular mass of 33kDa. This cDNA has a homology with human apolipoprotein D and other insecticyanin. Northern blot analysis clearly showed a ca 1.5kb transcript in the epidermis, brain, and fat body. Developmental expression profiles clearly showed that BBP transcripts are present in the whole body at only 4- and 6-day-old pupae. Computer searches of data banks yielded in a new member of this superfamily, lipocalin protein superfamily whose other members transport small hydrophobic ligands in a wide variety of biological context.

Very similar yolk protein genes in *Hyphantria cunea*

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Yolk protein 1 and 2 in the fall webworm, *Hyphantria cunea*, were detected in large amounts from the ovaries of 10-day old pupae and accumulated in the eggs. We isolated and sequenced cDNA clones corresponding to the two yolk proteins. The cDNAs for YP1 (1.2 kb) and YP2 (1.1 kb) code for 290 residue proteins. The sequence identity between YP1 and YP2 was very high of 79.9%. Two *H. cunea* YPs were most closely related to the follicle specific yolk protein 4 from the moths, *P. interpunctella* and *G. mellonella*, but not related to Vg sharing similarity with vertebrate lipase. Northern blot analysis showed YP1 and YP2 transcripts were present in only female fat body and at trace level in the ovary. YP1 and YP2 cDNAs began to express from 10-day-old pupae and increased to adult stage. This result suggests that *H. cunea* YP1 and YP2 genes are expressed in sex-, tissue- and stage- specific way. The possibility of gene duplication and overlapping function for two very similar YPs in *H. cunea* will be discussed..

Pheromone Biosynthetic Pathways in the moths *Helicoverpa zea* and *Helicoverpa assulta*

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Sex pheromones of many lepidopteran species have relatively simple structures consisting of a hydrocarbon chain with a functional group and usually one to several double bonds. The sex pheromones are usually derived from fatty acids through a specific biosynthetic pathways. We investigated the incorporation of deuterium-labeled palmitic and stearic acid precursors into pheromone components of *Helicoverpa zea* and *Helicoverpa assulta*. The major pheromone component for *H. zea* is (Z)11-hexadecenal (Z11-16:Ald) while *H. assulta* utilizes (Z)9-hexadecenal (Z9-16:Ald). We found that *H. zea* uses palmitic acid to form Z11-16:Ald via Δ11 desaturation and reduction, but also requires stearic acid to biosynthesize the minor pheromone components Z9-16:Ald and Z7-16:Ald. The Z9-16:Ald is produced by Δ11 desaturation of stearic acid followed by one round of chain-shortening and reduction to the aldehyde. The Z7-16:Ald is produced by ˆ9 desaturation of stearic acid followed by one round of chain-shortening and reduction to the aldehyde. *H. assulta* uses palmitic acid as a substrate to form Z9-16:Ald, Z11-16:Ald and 16:Ald. The amount of labeling indicated that the Δ9 desaturase is the major desaturase present in the pheromone gland cells of *H. assulta*; whereas, the ˆ11 desaturase is the major desaturase in pheromone glands of *H. zea*. It also appears that *H. assulta* lacks chain-shortening enzymes since stearic acid did not label any of the 16-carbon aldehydes. It will be of interest to determine the evolutionary relationship between the desaturases present in the *Helicoverpa* complex.

Comparative genomics of protein-coding genes: *Anopheles gambiae* vs. *Drosophila melanogaster*.

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The genome of the malaria vector, *Anopheles gambiae*, was submitted to a Whole Genome Shotgun procedure to establish an assembly of the complete euchromatic portion of the genome. Libraries were made from the inbred PEST strain provided by Frank Collins (Notre Dame). DNA was size fractionated to produce libraries of 2 kb, 10 kb and 50 kb inserts. 2,059,200 of these clones were sequenced at Celera, and an additional 195,345 were sequenced at Genoscope. In addition, a total of 27,324 BAC clones were end-sequenced at TIGR and Genoscope. Final sequence coverage was 10.15 X. At Celera Genomics these fragments were submitted to their whole genome assembly programs, which produced 8,997 scaffolds representing 278 Mbp of eukaryotic portion of the genome. The median scaffold size was 6.1 Mbp. These scaffolds were submitted to the Genome Crawler, a series of programs that perform automatic gene finding, megaBLAST pre-computes, and a variety of automated annotating functions. The starting point for the analysis presented in this talk is the set of inferred transcripts derived from *Drosophila melanogaster* and from *Anopheles gambiae*. These were submitted to an all-vs-all BLAST search, and the reciprocal best blast hits were then aligned. The resulting xxx alignments were examined for both qualitative assessment of presence/absence of gene sharing in pathways of interest, and for quantitative levels of divergence. Overall the nonsynonymous divergence rate was xxx, while the synonymous divergence was xxx, or essentially saturated. Some patterns of divergence between *Drosophila* and *Anopheles* in known developmental pathways will be illustrated. Single nucleotide polymorphisms (SNPs) were detected in the inbred strain in a highly clustered manner, suggesting heterozygosity for ancient polymorphic inversions and/or the possibility that there are segmental introgressions from other members of the species complex. SNPs were identified from alignments with a large collection of ESTs, and their distribution is also highly nonhomogeneous.

Sequence analysis of candidate genes associated with longevity in lines of *Drosophila melanogaster*.

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In recent years, sequencing analysis has become an important tool in molecular biology. Additionally, the completion of the *Drosophila melanogaster* genome has also become a valuable resource for researchers. The information that is available regarding the nucleotide sequences of genes that are associated with longevity has provided us with an excellent tool for comparing genetic differences. A long-standing selection experiment (Rose, 1984) has produced populations of flies with divergent life spans. From these populations, we produced a series of long and short-lived inbred lines. The present study entails comparing the genetic sequences among those inbred lines and the comparison of our lines with the published sequence for several candidate genes. If a candidate gene affects longevity differences in our experimental populations, then we would expect to see nucleotide and amino acid differences when we compare long and short-lived inbred lines. Any differences observed between the published sequence and those lines with increased longevity are subject to further investigation. A significant difference may be defined as a change in amino acid sequence that results in a possible change of function at the protein level. All changes that occur at the level of the amino acid do not result in altered function. The purpose of this study is to aid in the identification of new candidate genes for longevity and to provide additional evidence for the existence of current candidate genes associated with longevity.

The insect cytokine plasmatocyte spreading peptide (PSP) interacts with its receptor through highly specific domains

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Plasmatocyte Spreading Peptide (PSP) is a 23 amino acid cytokine that induces a class of insect immune cells called plasmatocytes to spread on foreign surfaces. PSP consists of an unstructured N-terminus (residues 1-6) and a well-defined core (residues 7-23) stabilized by a disulfide bond and a short β-hairpin. Mutagenesis studies have identified several domains critical for activity. These include the cysteines used to form the disulfide bond, charged residues within the β-hairpin and, most importantly, residues Glu^1 and Phe^3. The structure of Phe^3 is essential, as adding a hydroxyl (Tyr), or altering the chirality (D-Phe), or replacing the aromatic ring with a branched aliphatic chain (Val) each destroyed the activity. Activity was partially restored by the addition of a methylene group to Val (to make Leu), whereas removal of a methylene group from Phe^3 (phenyl-Gly) destroyed activity, indicating a branched carbon chain with a methylene spacer as the minimum functional structural motif. We also tested alterations of Glu^1 and Asn^2. Ala substitutions at Glu^1 or Asn^2 both increased activity, but a Glu^1 deletion eliminated activity, indicating that only the charged N-terminal amine is essential. Mutant peptides lacking activity were assayed for antagonism of wild-type PSP activity. Phe^3 substitutions were unable to antagonize PSP, whereas mutants lacking the charged N-terminal amine were effective. These data suggest that Phe^3 is essential for PSP/receptor binding. This Phe^3-mediated interaction likely serves to stabilize the normally unstructured N-terminus and therefore facilitates binding and activation of the receptor by the N-terminal amine.

Race to death: The encapsulation response by insect hemocytes is mediated by the surface coat proteins and cuticular proteins of *Heterorhabditis bacteriophora*.

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Entomopathogenic nematodes, like *Heterorhabditis bacteriophora,* have evolved a lifecycle in which survival depends upon overcoming insect immunity for survival and reproduction. After the nematode invades the insect hemocoel and before an extensive cellular immune response by the insect, the symbiotic bacterium *Photorhabdus luminescens* must be released from the nematode gut and established for nematode reproduction. The underlying mechanisms used by the nematode to evade the insect immune response and the countermeasures used by the insect present a unique system for study of host/pathogen coevolution and for discovery of key regulators of cellular immunity. Evidence is presented that the initial, immediate insect immune response is critical and that the nematode itself is contributing to overcoming this defense. The interactions of the nematodes and the hemocytes from a series of resistant and susceptible hosts (*Manduca sexta, Galleria mellonella, Popilla japonica,* and *Acheta domesticus)* were visualized by light microscopy in sterile, *in vitro* cultures, and captured with time-lapse computer-generated movies (available via web-site access). In addition, the cellular interactions were examined using scanning electron microscopy. Initial recognition of the nematode by the hemocytes determines success of nematode. In the resistant host *M. sexta*, hemocytes rapidly recognize the nematode ends and encapsulate the entire nematode, while producing reactive oxygen species. In a semi-permissive host (*P. japonica*), recognition is also rapid but directed first at the middle of the nematode and then the ends, permitting release of the bacteria. In the susceptible host *G. mellonella*, hemocyte recognition is weak, allowing release of the bacteria and survival of the nematode. Preliminary data suggest that less than 15 major proteins are present in the surface coat proteins, and that these can disrupt melanization and coagulation by *M. sexta* hemocytes. The nematode/bacterium produce factors eliminating reactive oxygen species that underlie the killing of invaders by insect hemocytes. In a semi-permissive host like *P. japonica* these factors may permit the nematode to survive until the bacterium can act. Thus, a triad of interactions governs the fate of the nematode/bacterium versus insect.

Immune mechanisms in mosquitoes: Cells, gene expression and melanin

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The innate immune response of insects is receiving considerable attention from the general immunology community, primarily because of the striking similarity with acute phase responses of vertebrates. The primary emphasis in recent years has centered on the insect humoral response and the regulation of immune peptide production. Recently we have begun studies of the cellular response of mosquitoes and the role hemocytes play in phagocytosis and melanotic encapsulation reactions against malaria parasites and filarial worms. Circulating hemocytes of *Aedes aegypti* and *Armigeres subalbatus* consist of granulocytes and oenocytoids, with granulocytes being the predominant cell type and also the hemocyte responsible for phagocytosis. Oenocytoids are less numerous, but these hemocytes are responsible for the production of key enzymes involved in the production of melanin. Transmission electron microscopy and colloidal gold labeling studies determined that two of the rate-limiting enzymes, phenoloxidase and phenylalanine hydroxylase, are produced in oenocytoids. We have been able to use the Sindbis virus transducing system in gene silencing experiments to verify the role specific genes play in melanotic encapsulation. It is anticipated that our successful construction of cDNA libraries from immune-activated hemocytes from these two mosquito species will greatly enhance our ability to better understand gene expression involved in the cellular immune response of mosquitoes against the parasites they transmit. Supported by NIH grant AI 19769 and AI 46032.

A type III secretion system facilitates the conversion to

mutualism in a lineage of insect endosymbionts.

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The view that parasites can develop cooperative symbiotic relationships with their hosts is both appealing and widely held, however, there is no molecular genetic evidence of such a transition. Here we demonstrate that a mutualistic bacterial endosymbiont of grain weevils maintains and expresses *inv*/*spa* genes encoding a type III secretion system (TTSS) homologous to that used for invasion by bacterial pathogens. Phylogenetic analyses indicate that the *inv*/*spa* genes were present in a pre-symbiotic ancestor of the weevil endosymbionts, occuring at least 50 million years ago. The function of the *inv/spa* genes in maintaining symbiosis is demonstrated by the up-regulation of their expression under both *in vivo* and *in vitro* conditions that coincide with host cell invasion.

The epiproctodeal glands and their apparent release of an ecdysterostatic peptide in *Manduca sexta.*

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In immature insects the release prothoracicotropic hormone (PTTH) from the brain activates the prothoracic glands to cause a rapid increase in ecdysone titer that initiates the molting process. For normal development to continue, the ecdysteroid titer must then decline rapidly. Recently, it has been shown that a myoinhibitory peptide, first identified in *Manduca sexta*, acts as an ecdysterostatic factor to block PTTH stimulated secretion of ecdysone in *Bombyx mori*. Using an antiserum to *M. sexta* myoinhibitory peptide (MIP), we have found a pair of epiproctodeal glands, that are MIP -immunoreactive. In 4th instar larvae these glands apparently release MIP into the hemolymph at a time that corresponds to the decline in ecdysteroid titer in *M sexta.* The structure of these gland cells was described in a previous study, and their cells were shown to be multinucleate, and the gland is located on the proctodeal nerve at the junction of the hindgut and rectum. We have demonstrated that the glands have a very extensive array of varicose, neurohemal processes extending on the surface of branches of the proctodeal nerve. The glands exhibit a distinct secretory cycle that is correlated with the molting cycle. In the fourth larval instar, the glands become depleted at about the time of the ecdysone peak, and this depletion is followed by a phase of synthesis of MIP for release in the next instar. These glands are also found in pupae and adults, and we have found similar glands in *B. mori.*

DNA polymorphisms detected in a population of glassy-winged sharpshooters (*Homalodisca coagulata*) from Weslaco, Texas by PCR-based DNA fingerprinting methods

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The glassy-winged sharpshooter is a large and slender xylem feeding leafhopper measuring about half an inch in length that was native to the southeastern United States, including Texas. Recently, this sharpshooter has become a concern in California because it vectors a strain of *Xylella fastidiosa*, a bacterium that causes Pierce's Disease in grapevines. This disease poses a potentially serious threat to the wine and table grape industry in California. The current work was undertaken to develop molecular genetic makers for the glassy-winged sharpshooter by various PCR-based DNA fingerprinting methods for the purpose of estimating the level of genetic variation within and among populations with the aim of achieving genetic information useful for improving biological control of this leafhopper. These fingerprinting methods included RAPD (Random Amplification of Polymorphic DNA), RAMP (Randomly Amplified Microsatellite Loci), and SAMPL (Selective Amplification of Microsatellite Polymorphic Loci). A combined total of about 204 polymorphic bands were detected with the three methods and three insects per primer combination (77 total), specifically 73, 79, and 52 polymorphic bands were generated by RAPD, RAMP, and SAMPL, respectively. We than applied and compared these PCR-based DNA fingerprinting methods to a local population of thirty glassy-winged sharpshooters and demonstrated extensive genetic variation within the population.

Peritrophic matrix proteins of the mosquitoes *Anopheles gambiae* and *Aedes aegypti*.

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The PM is a thick extra cellular structure secreted by the mosquito midgut epithelial cells, which completely surrounds the blood meal and is a potential barrier to malaria parasite development. Antisera were raised against newly identified PM proteins from *Aedes aegypti* and *Anopheles gambiae* and used to study their secretion and localization by immunofluorescence and immunoelectron microscopy. In *Ae. aegypti* midgut epithelial cells, RER cisternae are often assembled into characteristic whorls that unfold after a blood meal, correlating with activation of protein synthesis. Thus mRNAs encoding PM proteins (rather than the proteins themselves) are thought to be stored in midgut epithelial cells and their translation induced by blood feeding. Consistent with this view, mRNAs encoding the *Ae. aegypti* PM proteins AEIMUC and Aa-Aper50 are present before and after blood feeding, while the corresponding proteins can only be detected after blood feeding. In contrast, midgut epithelial cells of anophelines contain a large number of apical secretion granules before the blood meal. Upon blood feeding, the

apical granules disappear and their contents are presumably released into the lumen. Thus, at least some of the PM proteins of anophelines are thought to be stored prior to blood feeding. In agreement with this model, two cloned *An. gambiae* genes, *Ag-Aper1* and *Ag-Aper14*, are both expressed and translated prior to blood feeding and co-localize to secretory vesicles lying beneath the epithelial cell apical region. After blood feeding both *Ag-Aper1* and *Ag-Aper14* localize to the PM and are depleted from the epithelial cells.

Molecular identification of bursicon, the insect cuticle sclerotizing hormone

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Bursicon is the key hormone responsible for triggering the darkening and hardening (sclerotization) of new cuticle after the insect has shed its old one during ecdysis. Its actions and timed release are of critical importance to the survival of all insects and, most likely, all arthropods. We obtained four partial amino acid sequences of bursicon from the cockroach *Periplaneta americana* by microsequencing protein from one of four bursicon-bioactive spots in two dimensional gels. All of the P. *americana* peptide sequences align with the *D. melanogaster CG13419* gene product. Evidence supporting the hypothesis that CG13419 codes for bursicon will be presented. The CG13419 gene is found on the third chromosome at position *93F*. Its gene product is predicted to be a 19 kDa protein and has a C terminal cystine knot like domain (CTCK), which is predicted for formation of homodimers. Bursicon functions as a dimer. We have identified partial sequences from *Anopheles gambide* (mosquito) and *Apis mellifera* (honey bee), which are *92%* and *83%* identical, respectively, to the *CG 13419* gene product. We identified the cellular localization of CG.13419 in the ventral nervous system of third larval instar *D. melanogaster* using in situ hybridization analysis. The CG]3419 labeled neurons were also crustacean cardioactive peptide (CCAP) immunoreactive. We are currently producing recombinant *CG13419*, which we expect will show bioactivity in the ligated fly bioassay, a well known assay for bursicon activity. A positive result will provide additional evidence that the sequence *CG13419* is the bursicon sequence of *D. melanogaster*. Elucidation of the sequence and structure of this hormone will finally resolve a major unsolved problem in insect endocrinology.

Spatial and temporal binding sites of sex-peptide pheromones in *Drosophila melanogaster* females.

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Mating in *Drosophila melanogaster*, as in many other insects, significantly affects female reproductive behavior and physiology. Two conspicuous changes are 1) elevation of egg laying and 2) decreased receptivity. Sex-peptide and DUP99B, the peptides of the Sex-peptide pheromone family, elicit these two post mating responses. To elucidate the mechanism of action, we identified the target sites and compared the binding properties of the peptides. Cryostat sections of adult animals were incubated with alkaline phosphatase labelled peptides. In virgin females, the peptides have widespread but specific target sites located in the nervous system and in the genital tract. Incubation of sections of mated females with AP-peptides showed that some of these target sites are blocked by the peptides transferred during copulation. These results suggest that the binding sites characterized in the virgin females are indeed the *in vivo* targets of the two peptides. Neuronal binding is dependent on the C-terminal parts of the peptides, binding in the genital tract is less specific in terms of peptide sequence. On affinity blots AP-peptides bind to proteins extracted from abdomen and head+thorax, respectively. These proteins are membrane bound and are separable on PAGE. Taken together, we conclude that the binding proteins of the neuronal system and the genital tract differ in their molecular properties. Calculation of Kd's and the minimum concentrations necessary for binding, suggest that SP is the key player. DUP99B may act synergistically with SP to produce a maximal post mating response.

Cloning and developmental expression of cDNAs encoding putative laccases from *Manduca sexta* and *Anopheles gambiae.*

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We have cloned 2 cDNAs for the phenoloxidase laccase from *Manduca sexta* and 1 from *Anopheles gambiae* by a combination of RT-PCR, RACE-PCR, and library screening methods. The *M. sexta* laccase 1 and 2 cDNAs encode 801 amino acid (aa) and 760 aa proteins respectively, while the *A gambiae* laccase cDNA encodes a 1009 aa protein. All 3 cDNAs contain putative secretion signal sequences and the 10 His and 1 Cys residues shown to be necessary for copper binding in fungal laccases. Novel to the insect laccases is a larger amino terminal sequence, with the *A. gambiae* laccase nearly 350 aa longer and the *M sexta* laccases 200 aa longer than the fungal proteins in this region. These extensions appear to be unique as they share no sequence similarity to each other. Northern blot analysis has identified a single transcript of ~4.4 kb for the *A. gambiae* laccase, and ~3.6 kb for both *M. sexta* laccase 1 and 2. Northern blot analysis has also shown the *A. gambiae* laccase to be expressed in all life stages. RT-PCR was used to examine the tissue distribution and developmental profile of laccase gene expression during the last larval stage of *M. sexta*. Laccase 1 was most abundant in the midgut, Malphigian tubules and epidermis, with reduced expression in the fat body and very low expression in hemocytes. Laccase 1 was constitutively expressed but showed enhanced expression in the pharate pupal and pupal epidermis, consistent with

its presumed role in cuticle sclerotization. Laccase 2 was most abundant in the epidermis, followed by low expression in the midgut and Malphigian tubules, and very low expression in the fat body and hemocytes. Similar to laccase 1, laccase 2 is also constitutively expressed with enhanced expression in the epidermis during periods of molting. The expression of laccase in the fat body led us to examine if either gene was stimulated upon microbial challenge. Preliminary experiments indicate that laccase 1 is up regulated by the gram-negative bacteria *Escherichia coli*, but not by the gram-positive bacteria *M. luteus* or by yeast. In contrast, laccase 2 was induced by all 3 microbial challenges. Future experiments are planed to further investigate the potential role of laccase in the immune response.

Comparison of two cloned monoamine transporters from the CNS of the cabbage looper moth *Trichoplusia ni*

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The Na^+/Cl^- dependent family of neurotransmitter transporters includes those responsible for the uptake of monoamines. We recently characterized a novel phenolamine transporter from the nervous system of the cabbage looper (*Trichoplusia ni)* that takes up octopamine with high affinity. To demonstrate that this lepidopteran octopamine transporter (OAT) is distinct from known dopamine transporters characterized in other organisms, we have also cloned the *T. ni* dopamine transporter (DAT) for comparison. Here we show that the *T. ni* DAT has greater similarity to other known DAT's and has a different pattern of expression in caterpillar nervous system compared to that of *T. ni* OAT. We also compare the function and pharmacology of the two transporters expressed in cultured insect cells and show that they have different affinities for various biogenic amines as well as distinctive pharmacological properties.

A novel gene, clone 50, expressed at specific stage of *Maduca sexta* is down-regulated by juvenile hormone and 20E

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A novel gene, clone 50, was cloned from the epidermis of tobacco hornworm, *Manduca sexta*, using PCR-based Suppression Subtractive Hybridization. It belongs to a gene family that includes *takeout* of *Drosohila melanogaster* and several JH-binding proteins. Clone 50 mRNA first appears in the epidermis on day 0 of the 5th instar and rises to its peak expression by mid-day 2, then declines rapidly on day 3 and is gone by the onset of wandering. No expression was seen in the fat body, muscle, eye and wing discs. After allatectomy early in the 4th instar that induced precocious metamorphosis, clone 50 mRNA increased to high levels by 33h, then disappeared by the onset of wandering. Allatectomy after the critical period for juvenile hormone (JH) in the larval molt caused a more rapid upregulation on day 1 of the 5th instar. The JH mimic pyriproxifen suppressed most expression both in normal and allatectomized larvae, indicating that the appearance of clone 50 mRNA is initiated by the decline in JH in the final larval instar. Infusion of 20-hydroxyecdysone (20E) into ligated abdomens of day 2, 5th instar larvae and culture of day 2, 5th abdominal epidermis with 20E *in vitro* caused a rapid decline of clone 50 mRNA. A slower and variable decline also occurred in controls treated with hormone-free media, indicating that another factor may be present in the larva that sustains high expression. Thus, clone 50 mRNA is expressed only in epidermis of the final instar larva and only when JH declines. Its expression is terminated by the commitment peak of ecdysone as metamorphosis begins. The role of the protein is currently under study. Supported by NIH.

Characterization of heme oxygenase and biliverdin reductase homologues from *Drosophila melanogaster* and *Anopheles gambiae*

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In vivo heme is degraded by heme oxygenases (HOs) yielding biliverdin, carbon monoxide and iron. The released biliverdin can be further converted to bilirubin by biliverdin reductases (BVRs). Oxidative degradation of heme is essential for iron reutilization and the cleavage products of the porphyrin ring play important physiological roles as antioxidants and/or signaling molecules. While heme catabolism in vertebrates is well understood, little is known about how insects deal with endogenous and exogenous heme. In an attempt to investigate insect heme degradation, we have identified genes and cDNAs encoding homologues of the vertebrate heme oxygenase and biliverdin IXβ reductase in the genome sequences and EST collections of *Drosophila melanogaster* and *Anopheles gambiae*. The genomes of both dipterans encode single HO homologues, in contrast to mammalian genomes, which have three HO genes. Conservation of sequence motifs and catalytically important residues in the deduced proteins suggest possible conservation of function between the insect and vertebrate enzymes. Northern blotting experiments were employed to study the developmental and organ specific expression patterns of HO and BVR messages. In *D. melanogaster* both messages are better represented in the larval midgut than in the fat body and are abundant in the testes and ovaries of adult flies. Iron and/or heme enrichment of the diet leads to increase in the message abundance. In female *A. gambiae* mosquitoes BVR messages are detected only in the midgut, Malpighian tubules and ovaries. The abundance of BVR messages increases in the midgut 24h after blood feeding. We also report the production of recombinant HO and BVR from *D. melanogaster* and BVR from *A. gambiae.* The recombinant BVR enzymes effectively

converted β, γ, and δ, but not αbiliverdin isomers into the corresponding bilirubins and also displayed flavin and ferric reductase activities, much like the vertebrate biliverdin β reductases. In addition, we found that insecticyanin, a biliverdin IXγ chromoprotein from the hemolymph of *Manduca sexta*, is a good substrate for these dipteran BVRs. The results of these studies are discussed in the light of the possible functions of these enzymes in insect iron and heme homeostasis as compared to their vertebrate counterparts.

Expression studies on *Drosophila melanogaster* G-protein coupled receptors

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A wide range of seven transmembrane spanning (7-TM) G-protein coupled receptors have been cloned and expressed from the fruitfly, *Drosophila melanogaster*. Many of them can be specifically activated by either biogenic amines or neuropeptides and some show a high degree of structural conservation with their vertebrate counterparts. Multiple receptor subtypes have been demonstrated for particular receptor classes. The power of *D. melanogaster* genetics should allow the identification of the functional roles of such receptors. However, it is clear that functional expression studies on such receptors in heterologous expression systems, such as insect or vertebrate clonal cell lines or *Xenopus* oocytes, can provide much information about their potential endogenous ligands, pharmacology and potential coupling to second messenger systems. A number of examples of such studies will be discussed to illustrate some of the general phenomena that have been discovered using this approach. The concept of the agonist-specific coupling of specific receptors to different second messenger pathways will be discussed in relation to studies on a cloned octopamine/tyramine receptor (Arakawa et al., 1990, Neuron 2:343-354; Robb et al., 1994, EMBO, J., 13:1325-1330) and on a novel cloned Neuropeptide F-like receptor (Feng et al., 1999, Soc Neurosci. Abstr., 25:183). Recent expression studies on a novel cloned *D. melanogaste* β–adrenergic-like receptor (Yu et al., 2000, Society Neuroscience Abstracts, 26:916) will also be discussed. The completion of the sequencing of the *D. melanogaster* genome makes the identification of potential 7-TM receptor sequences much easier, but functional genomic studies will also be required to assess the physiological roles of these receptors.

***Plodia interpunctella* 0 1,3 glucan recognition protein Properties of the N terminal carbohydrate binding domain**

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Bacteria and fungi possess specific molecular pattern molecules that form essential structural scaffolding. Typical molecular patterns associated with Gram negative bacteria, Gram positive bacteria, and fungi include lipopolysaccharide, peptidoglycan, and 0 1,3 glucan, respectively. Invertebrate pattern recognition molecules function to stimulate the host innate immune response after encountering such nonself moieties. The pyralid moth, *Plodia interpunctella*, possesses a 0 1,3 glucan recognition molecule (PiPGRP) that may function to continuously survey the hemolymph for the presence of foreign fungal cells. The PiPGRP possesses two putative domains consisting of a novel N terminal carbohydrate recognitioni'domain and a C terminal glucanase like domain, which can be separated by an *in vitro* proteinase treatment. The PiPGRP C terminal domain sequence shares similarity with other recognition proteins and 0 1,3 glucanases from bacteria and a sea urchin, whereas the N terminal sequence is unique to members of the arthropod GRP family and lacks sequence similarity with the 0 1,3-glucanases. The full length PiPGRP and constructs corresponding to 118 and 18 I residues from the N terminus and 290 residues from the C terminus of PiPGRP were expressed as recombinant proteins using an *Escherichia coli* heterologous expression system. Circular dichroisin (CD) analysis of the 118 and 18 1 residue N terminal constructs indicate that the recombinant proteins are folded and possess primarily a helical secondary structure. The full length protein binds to insoluble P 1,3 glucan and causes *in vitro* aggregation of Gram positive and Gram negative bacteria, as well as yeast. The 181 residue N terminal construct causes significant aggregation of yeast cells but no aggregation of bacteria. These data suggest that the PiPGRP functions as a pattern recognition molecule in the innate immune system of P. *interpunctella* and that the N terminal domain possesses a carbohydrate binding site necessary for recognition of non self.

Analysis of dietary proteins derived from prey eggs and an embryonic cell line and their effects on the fecundity of *Orius insidiosus*

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The fecundity of the insidious flower bug, *Orius insidiosus* (Hemiptera: Anthocoridae), was poor when reared on a minimal artificial diet (control diet) composed of brewers yeast, soy protein hydrolysate and chicken yolk. Consequently, we supplemented test diets with homogenates of eggs from the Indian meal moth (*Plodia interpunctella*), proteins or lipids extracted from *Plodia* eggs, or an embryonic cell line (PIE) derived from *Plodia* eggs. Test diets were also supplemented with each of three fatty acids identified to be predominant in prey eggs (palmitic, linoleic and oleic acid), bovine serum albumin (BSA), chicken liver, beef liver, or chicken egg white albumin. Diets were compared against an optimal standard, *Plodia* eggs, and the control diet on the basis of the average total number of eggs a female oviposited during her lifetime. Only proteins derived from *Plodia* eggs and the cell line produced significant improvements in fecundity over the control diet at relatively low concentration of protein, indicating the quality of protein is important in selecting

supplements. Proteins extracted from prey eggs and the cell line were further separated by preparative isoelectric focusing and are being evaluated in the artificial diet.

Genomic approaches to the evolution and function of a large multigene family

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The cytochrome P450 gene family is represented by 90 sequences in the *Drosophila melanogaster* genome. Clues to the evolution of this large multigene family are provided by an analysis of the sequences, of their organization on the chromosomes and by comparisons to other species. Strategies used to unravel the functions of these multiple genes in the fruit fly and in other insect species includethe production of functional proteins in heterologous systems and DNA microarray analysis of expression patterns.

Molecular characterization of a family of candidate odorant receptors from the malaria vector mosquito *Anopheles gambiae*

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Olfaction plays an important role in many behaviors, including host preference, feeding, mating and socialization of many insects. We are interested in the molecular biology of olfaction as it impacts upon host selection in the major human malaria vector mosquito, *Anopheles gambiae*, where host preference selection makes a significant contribution to the vectorial capacity of this insect. It is hoped that in addition to providing insight into insect chemosensory pathways, a detailed examination of the olfactory signaling cascade in this critically important disease vector insect may lead to novel strategies to reduce the incidence of malaria. We have recently identified and are in the process of characterizing a large family of candidate odorant receptors (ORs) that are presumed to initiate olfactory signaling in *A. gambiae*(AgORs). In some instances, amino acid alignments between AgORs and putative ORs from *Drosophila melanogaster* display significant homology although it is seldom possible to assign orthology between specific receptors from these two highly divergent flies. The possible evolutionary and biological implications of these relationships will be discussed. Furthermore, data will be presented concerning the developmental, spatial and temporal expression patterns of AgORs along with the effect of the initiation of blood-feeding in adult female mosquitoes. Future studies that address questions as to whether host preference is directly prescribed by the type of odorant receptors expressed in a mosquito's sense organs will be discussed.

Induction of mosquitocidal immunity in mice immunized with *Anopheles gambiae* midgut cDNA

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Vaccines that can kill mosquitoes may have a profound effect toward limiting the transmission of certain mosquito-borne diseases, especially vaccines against African malaria vectors. In an attempt to generate and characterize anti-mosquito immunity, we immunized groups of mice with two individual *Anopheles gambiae* midgut cDNAs that are induced in the midgut upon bloodfeeding; Ag-Aper1 (a secreted peritrophic matrix protein) and AgMuc1 (a midgut-bound mucin). We also immunized two separate groups of mice with an *An. gambiae* midgut cDNA library from bloodfed mosquitoes; one of these groups received a final boost of midgut protein. Humoral and cellular immune profiles were recorded from the immunized mice and cages of *An. gambiae* mosquitoes were fed on these same mice and monitored for rates of mortality and fecundity. We observed consistent and significant increased mortality from mosquitoes that fed on either the AgMuc1 (Muc) or the cDNA library (Lib) immunized mice as compared with controls, but no differences from those that fed on either Ag-Aper1 immunized mice (PM1) or the midgut protein-boosted mice (Lib+Prot). Western blots revealed that all experimental groups produced antibodies that recognized protein corresponding to the cDNA(s) with which they were immunized. However, ELISA measurements of the midgut-specific antibody titers showed that mice immunized with DNA alone produced very low to undetectable antibody titers. Antigen re-stimulation assays using immunized mouse splenocytes showed that the quantity of Type 1 cytokines (TNF-α, IFN-γ) secreted from Muc, Lib, and Lib+Prot groups was significantly higher than either the control groups or the PM1 group. However, the quantity of Type II cytokines (IL-5, IL-10) secreted from the Lib+Prot and PM1 groups was significantly greater when compared to all other groups. Acellular immune sera from each immunization group was pooled and fed *in vitro* to mosquitoes but all failed to increase mosquito mortality. Our results show that mosquitocidal immunity can be consistently generated from midgut DNA immunization and they suggest that this DNA-induced mosquitocidal immunity is cell-mediated.

Foreign gene expression and endogenous gene repression in mosquitoes using recombinant Sindbis virus vectors

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Sindbis viruses (*Alphavirus: Togaviridae*) are enveloped viruses that contain single-stranded, positive sense RNA genomes and are normally transmitted between birds and arthropod vectors. We have pioneered Sindbis virus expression systems to transiently express proteins in mosquitoes and for silencing the expression of endogenous genes in mosquito gene function studies. We are constructing recombinant, infectious DNA clones based on a Malaysian strain of Sindbis MRE16 that have a viral subgenomic promotor inserted either upstream or downstream of the viral structural genes. This second subgenomic promotor drives the expression GFP, which can be visualized in infected insect or mammalian cell culture, as well as in infected mosquitoes. We are currently characterizing the growth and stability of our infectious clones. Our results based on the creation of MRE16 and AR339-like *Sindbis* strain chimeras, reveal that the structural E2 protein is the primary determinant of *per os* mosquito infectivity, and that foreign genes are more likely to be retained if cloned upstream of the virus structural proteins. These new generations of Sindbis viruses can be powerful tools for the characterization of gene function within mosquitoes and other arthropods, and for examining the molecular determinants of pathogen infection within vector insects.

Enhanced TPRT activity by 3' end modification of retrotransposon, R2Bm

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R2Bm is a non-long-terminal-repeat (non-LTR) retrotransposon that was identified at a specific target site in the 28S rRNA genes of the silkworm, *Bombyx mori*. Although in vitro analysis has revealed that the 3' end of R2Bm is integrated into the target site by means of target primed reverse transcription (TPRT), the mechanism of the 5' end integration is not well understood. We established a novel *in vivo* system to assay the insertion mechanism of R2Bm using a cultured cell line, C65, and a baculovirus, AcNPV, as host and vector, respectively. The 3' end of R2Bm integrated at the target site in the rRNA genes of C65 cells when an AcNPV containing both the full-length 3' UTR and the whole ORF of R2Bm was introduced while the 5' end integration was incomplete. R2Bm alone may have only the ability to integrate the 3' end by TPRT. When a sequence homologous to 3'-side at the endonuclease cutting point of the target site of 28S RNA region was added at just after the 3' UTR of R2Bm, more enhanced TPRT activity was observed. This result suggests that a sequence homologous to 3'-side of the target site added to R2Bm functions as a holding apparatus stably keeping the transcript of R2Bm to the target site and results in assistance for TPRT.

***Helicoverpa zea* exhibits differential tolerance to Cry1A(c) during larval development**

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Cry1A(c) is a *Bacillus thuringiensis* protein that has specific activity against Lepidoptera. *Helicoverpa zea* larvae were tested for their response to high doses of Cry1A(c) protein at each instar. The first four instars had high mortality and the survivors had delayed development. Fifth instar individuals showed significantly less mortality, although the pupa were significantly smaller than the control. The results illustrate that physiological changes that occur during development can influence the action of anthropomorphic compounds.

Development of DNA diagnostic methods to screen for resistance to *Bacillus thuringiensis* toxins in field populations of tobacco budworm *Heliothis virescens*

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Resistance to Cry1A-type Bt toxins in the YHD2 strain of *Heliothis virescens* is conferred by disruption of a gene encoding an 11-cadherin-domain protein expressed in the larval midgut (Science 293: 856 (2001)). The gene was disrupted by insertion of an LTR-type retrotransposon, but it is not clear whether this event occurred in the field, or in the laboratory after the YHD2 strain was established. To resolve this issue, we have developed PCR-based diagnostic methods to specifically detect the disrupted version of the gene, as well as other mutant alleles that might also confer Bt-resistance. We will discuss the benefits and drawbacks of this approach and present the results of screening field samples to date.

Identification and mapping of a second major gene conferring resistance to *Bacillus thuringiensis* toxins in tobacco budworm *Heliothis virescens*

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Resistance to Cry1A-type Bt toxins in the YHD2 strain of *Heliothis virescnes* is conferred by disruption of a gene mapping to Linkage Group 9 (LG 9) (Science 293: 856, 2001). Linkage mapping with a different resistant strain, CP73, shows no effect of that linkage group. Instead, a factor or factors on LG 10 is responsible for 50-80% of

the resistance in CP73. Resistance conferred by the LG 10 factor is semidominant, and has an additive or greater effect on resistance when combined with the LG 9 genes. Tests of linkage to candidate genes have ruled out the 11-domain cadherin and four separate aminopeptidases, but other data suggest a likely candidate for the LG 10 resistance mechanism.

Identification of LRLRFa peptides and their receptor from *Drosophila melanogaster*

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Peptides bearing the signature C-terminal sequence, LRLRFamide, have been isolated from *Aedes aegypti*, *Leptinotarsa decemlineata*, and *Periplaneta americana*. A gene encoding two candidate LRLRFamides was identified in the *Drosophila melanogaster* genome database. Oligonucleotide primers were designed to amplify the open reading frame from mRNA isolated from *D. melanogaster* adults. A cDNA product of approximately 800 bp was amplified by RT-PCR, the resulting cDNA was cloned and sequenced, and the encoded LRLRFamide sequences (AQRSPSLRLRFamide and WFGDVNQKPIRSPSLRLRFamide) were confirmed. For use in RIA and receptor binding assays, novel *D. melanogaster* LRLRFamide analogs containing D-tyrosine were synthesized and radiolabeled with ^{125}Iodine. The RIA was used to screen HPLC separated extracts of hemolymph and of whole bodies for the presence of LRLRFamide peptides in adult *D. melanogaster*. We identified two potential NPY-like G protein-coupled receptors (DmNPFR1 and DmNPFR2) in the *D. melanogaster* genome database, and later showed DmNPFR1 to be a functional receptor for *D. melanogaster* NPF, a full-length (36 amino acid) member of the NPY family. For the present study, CHO-K1 cells were stably transfected with DmNPFR2 cDNA, and ^{125}I-labeled LRLRFamide analogs were used to determine specific binding. Membranes prepared from stable transfectants exhibited high affinity (IC_{50} = 0.2 nM) binding for LRLRFamide peptides; membranes from mock-transfected cells lacked specific binding altogether. Assay findings indicate that *D. melanogaster* LRLRFamide occurs in hemolymph and tissues of adult flies, and that DmNPFR2 is an LRLRFamide receptor. Supported by NIH AI33108 and USDA-CSREES GEO00786

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Sequence and expression of mosquito collagen IV during development and *Plasmodium* infection of the midgut

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A crucial stage in the life cycle of the malaria parasite occurs at the basal lamina (BL) of the mosquito midgut epithelium, when the ookinete develops in to the oocyst. Very little is known about the molecular structure of the mosquito BL and its role in triggering oocyst development. We have undertaken a study to characterise one of the main structural components of the BL, collagen type IV. An *Anopheles gambiae* pupal cDNA library was constructed and screened with a probe generated by degenerate PCR against the C-terminus of the collagen IV cDNA. Specific primers were then used in a semi-quantitative RT-PCR approach to study collagen IV expression during mosquito development and in response to an uninfected and a *Plasmodium* infected blood meal. A cDNA of 5433 bp was sequenced, which coded for a protein of 1811 aa. Amino acid similarity to *Drosophila melanogaster* collagen α1(IV) was 66% for the whole cDNA and 83% for the conserved NC1 carboxyl domain. Collagen expression was low during larval stages, peaked in the pupae then declined within 12 hours of emergence of the teneral adult. Expression was induced from 48 hours after a blood meal and remained constant before increasing 3-fold 21 days after feeding. An additional 3-fold increase in expression occurred 5 days after an infectious feed, coinciding with the growth of the young oocyst between the basal lamina and midgut cells.

Molecular characterization of the *ecdysoneless* gene in *Drosophila melanogaster*

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The *ecdysoneless* (*ecd*) locus of *Drosophila melanogaster* was mutagenized both chemically (EMS) and with gamma-ray irradiation. The several resulting alleles exhibit phenotypes ranging from a thermosensitive lethal or female sterile (*ecd*^{1}) to non-conditional larval lethal (*ecd*^{2} and all other alleles). *ecd*^{1} mutants are characterized by low ecdysone titer, which seems to be the cause of at least some of the defects. Cell autonomous defects in sensory bristle formation suggest that *ecd* is not simply participating in ecdysone synthesis but may play a more general role in development. Although *ecd* mutants have been used for studies of ecdysone response for decades, the molecular identity and thus the mechanism of *ecd* action remained unknown. As the initial step to understand Ecd action, we set out to clone *ecdysoneless*. Using complementation tests between *ecd*^{2} and deficiencies affecting the 62D region, we localized *ecd* into a 60-kb sequence containing 7 to 8 predicted genes. Using P-element mediated germline transformation, we generated *D. melanogaster* lines carrying four partially overlapping genomic fragments containing these genes. One of the genomic rescue constructs allowed the otherwise larval lethal *ecd*^{2} mutants to mature as homozygous adults. Genes within the rescuing DNA fragment were sequenced from *ecd* mutant lines, revealing either short deletions or single base substitutions in one of the candidate genes. The molecular identity of *ecd* will be reported. Northern blot analyses showed a single *ecd* transcript of 2.35 kb, which was present throughout development with highest levels during pupal stage and in adults. Closer examination of the *ecd* expression pattern is underway using in situ hybridizations and an *ecd-lacZ* transgenic reporter fusion.

Response of *Aedes aegypti* larval cells to iron

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We are evaluating the iron metabolism pathway in *Aedes aegypti* larval cells. As a first step in this work, we cloned and sequenced the A. *aegypti* iron regulatory protein I (IRP I) and showed that the recombinant IRP1 binds to the A. *aegypti* putative ferritin beavy chain (FHC) iron responsive element (IRE). Further, IRP1/IRE binding activity was upregulated in response to infection of mosquitos. We now have evaluated iron response of mosquito larval cells, in order to establish that this mosquito cell line can be used as a model to study relationships between cellular iron metabolism and infection. We have found that iron increases the level of ferritin in the cytoplasm and secreted ferritin in the culture medium. We also have found that the IRPI binding activity for the FHC IRE is modestly downregulated by iron at high doses administered for 18 hours. We have shown that in vitro translation of ferritin is downregulated by the presence of recombinant A. *aegypti* IRP1. When IRP1/IRE interaction is evaluated in mammals, reducing agent is added to the electrophoretic mobility shift assays to measure total binding activity. We evaluated the effect of reducing agent on binding activity of mosquito cell cytoplasmic extracts and found that contrary to the mammalian system, addition of reducing agent caused loss of binding activity. We are testing recombinant A. *aegypti* IRP1 for the effects of reducing agent on binding activity at this time. We also are currently evaluating a full dose response and time course for iron effects on IRP1 and ferritin mRNA levels, as well as protein and binding activity.

***Anopheles gambiae* secreted ferritin: Characterization of cDNAs encoding two subunits, mRNA expression pattern, and gene organization**

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Ferritins are large multimeric shell-like proteins that serve as repositories for iron, keeping it in a bioavailable form and preventing its toxic effects. In female mosquitoes that take blood meals rich in heme iron, secreted ferritin apparently plays a dual function: to sequester iron and prevent oxidative damage on one hand, and on the other to provide iron store in the eggs. To better understand the roles of ferritin in the malaria mosquito *Anopheles gambiae* we have identified among the ESTs obtained from immune competent cell lines several cDNAs encoding secreted ferritin subunits. The deduced amino acid sequences revealed that these are homologues of the vertebrate heavy and light ferritin chains (HCH and LCH) with high similarity to other insect secreted ferritin subunits. An iron responsive element was located in the 5'UTR of the HCH mRNA but not in the LCH mRNA. Probing Northern blots with these cDNAs revealed a single mRNA species for each subunit type, in contrast to *Drosophila melanogaster* and *Aedes aegypti*, which produce ferritin messages of different lengths. Developmental Northern blots revealed that HCH and LCH mRNAs are equally represented at all stages and are most abundant in fourth instar larvae and in adult mosquitoes. Both ferritin messages are well represented in the ovaries and become very abundant in the midgut of blood fed female mosquitoes 24h and 48h after a blood meal. Microarray analysis did not show regulation of the ferritins in malaria and bacteria infected mosquitoes but HCH transcription is repressed in microbially challenged immune competent cell lines. Analysis of *A. gambiae* genome sequences from the public databases revealed that the genes encoding these secreted ferritin subunits are located in a head to head fashion with their open reading frames separated by 1005 bp. The arrangement of the *A. gambiae* HCH and LCH genes in a cluster with possibly shared regulatory regions is strikingly similar to the organization of their *D. melanogaster* orthologs. In addition, a third gene encoding a ferritin subunit apparently belonging to a cytosolic ferritin was also found in the genome sequence. These results are discussed in the light of the possible functions of *A. gambiae* ferritins and are compared to their counterparts from *D. melanogaster* and *A. aegypti*.

Transgenic *Spodoptera exigua*: Possibilities for their use.

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Transgenic *Spodoptera exigua* are being developed by microinjection of a *piggyBac* vector. The vector expresses green fluorescent protein (GFP) under the control of the actin promoter. Forty percent of the first-instar larvae that hatched from the injected eggs were green fluorescent. However, after backcrossing none of the G1 first-instar larvae was fluorescent and a transgenic line could not be established. Several possibilities for the use of transgenic insects are discussed.

Evolution of desiccation resistance in laboratory poopulations of *Drosophila melanogaster*.

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We are performing a large stress (desiccation) selection experiment in *Drosophila melanogaster*, using multiple starting populations and two types of selection control. The founding populations differ in lipid and carbohydrate storage, two parameters that have evolved rapidly in previous selection studies. Metabolic and biochemical measurements reveal that flies use different energy sources under different stresses. These physiological changes probably involve differential expression of genes involved in energy storage (e.g.

glycogen and TAG synthases) and metabolism (e.g. glycolytic enzymes, IGFs). We will test this hypothesis by using microarrays to identify which genes are turned up or down under desiccation stress.

Olfactory receptor cells use nitric oxide signaling to influence antennal lobe structure in *Manduca sexta*.

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Nitric oxide synthase (NOS) is present at olfactory receptor cell (ORC) axon terminals throughout development of the adult antennal (olfactory) lobe of the brain of the moth *Manduca sexta* (Gibson & Nighorn, 2000, *J. Comp. Neurol.* 422: 191-205). To test the possible importance of nitric oxide (NO) in development, we have investigated the effect of blocking NO signaling. Animals treated with L-NAME (a NOS inhibitor) or carboxy-PTIO (CPTIO, a NO scavenger) develop abnormal antennal lobes in which neuropil-associated glia fail to migrate to surround ORC protoglomeruli properly and in which the dendrites of the multiglomerular 5-HT interneuron arborize beyond their normal boundaries. Glial migration is only halted when blockade of signaling precedes glial migration by several days, thus allowing for the possibility that NO affects gene transcription. NO is known to stimulate soluble guanylyl cyclases in some systems, but neither antennal-lobe glia nor the 5-HT neuron exhibit cGMP immunoreactivity following NO stimulation. In addition, continuous infusion of the soluble guanylyl cyclase inhibitor, ODQ, has no effect on antennal-lobe morphology. Treatment of developing animals with the antibiotic Novobiocin, which blocks ADP-ribosylation of proteins, results in antennal lobes similar to those seen with L-NAME and CPTIO, raising the possibility that NO mediates posttranslational changes in proteins. In summary, ORC axons appear to stimulate glial cell migration and limit 5-HT neuron dendrite outgrowth via NO signaling, and NO-stimulated ADP-ribosylation of target-cell proteins may play a role. Preliminary attempts to duplicate these results *in vitro* (Eric Tucker, this laboratory) have so far proven inconclusive. Supported by NIH grant P01-NS 28495.

The role of hemolymph proline as a nitrogen sink during blood meal digestion by the mosquito *Aedes aegypti*.

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The blood meal that mosquitoes ingest is a unique source of nutrition: it is rich in protein but relatively deficient in carbohydrate and lipid. Amino acids from blood meal protein are used to produce egg proteins. In addition, they can be utilized to produce lipid necessary for egg maturation or be oxidized for energy production, but either process results in the release of ammonia, which is highly toxic. Therefore, amino acids must be processed in such a way that the ammonia can be released and incorporated into non-toxic waste products without accumulation of ammonia. Proline is the predominant amino acid in the hemolymph of the adult female mosquito *Ae. aegypti*. After feeding on porcine albumin, to mimic blood feeding, hemolymph proline levels increase 5 fold over unfed levels. Hemolymph proline levels increase as the concentration of protein in the meal increases. In addition, hemolymph proline levels reach a maximum in the first few hours after feeding, and remain high through ovipostion. When starved of sugar in the 24 hours prior to feeding on an albumin meal, hemolymph proline levels increase to almost 4 fold over the proline levels of non-sugar starved mosquitoes. Proline levels after feeding on a protein deficient in essential amino acids, pike parvalbumin, increase to twice the levels of albumin fed mosquitoes. Based on these observations, we discuss the role of proline as a transient nitrogen sink and transport molecule in the mosquito during blood digestion.

Partial purification of two activators of immune related serine proteases from *Manduca sexta*

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Serine proteases mediate insect immune responses, including cellular encapsulation and melanization of pathogen surfaces. Hemocyte spreading during cellular encapsulation is controlled by cytokine-like peptides of the ENF family. In *Manduca sexta*, plasmatocyte spreading is induced by paralytic peptide (PP), which is released into the plasma after proPP is cleaved by an unknown serine protease. We have developed an immunoblot-based assay for detecting proPP processing by hemolymph fractions. Because PP could not be detected on immunoblots using antibodies against proPP or PP, we expressed proPP tagged with a myc epitope and a poly-His tag. Cleavage of this recombinant protein can be assayed by immunoblot analysis using anti-myc or anti-his antibodies. We have used this assay to partially purify an activator of proPP from larval hemolymph. During cellular encapsulation, melanization of pathogen surfaces often occurs. This process involves the activation of prophenoloxidase activating proteinases (PAPs), which cleave prophenoloxidase, leading to melanin formation. The three *M. sexta* PAPs are clip domain serine proteases that are expressed as zymogens and activated by proteolytic cleavage. We are currently in the process of identifying the activator of a constitutively expressed hemolymph PAP, PAP3. We expressed and purified proPAP3 to use as a substrate in assays for its activation. Progress towards the purification and characterization of the activator of proPAP3 from prepupal hemolymph will be reported.

Interrupting malaria transmission by genetic manipulation of anopheline mosquitoes

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The mosquito is an obligatory vector for malaria transmission. *Plasmodium* undergoes a complex series of developmental events in the mosquito that includes the crossing of two different epithelia: midgut and salivary gland. Circumstantial evidence suggests that this requires specific interactions between *Plasmodium* and surface molecules. By use of a phage display peptide library we have identified a peptide, SM1, that specifically binds to both salivary glands and the midgut lumen and strongly inhibits (90~95%) *Plasmodium* invasion of both organs (Ghosh *et al.*, *PNAS* 98:13278–13281, 2001). Recently, we demonstrated that a carboxypeptidase promoter could be used to drive the expression of foreign genes in the mosquito (Moreira *et. al*., *PNAS* 97:10895-10898, 2000). Constructs containing either a gut-specific carboxypeptidase promoter, or a fat body-specific vitellogenin promoter driving the expression of a SM1 tetramer, were transformed into *Anopheles stephensi* using a *piggyBac* transposon. The transgenes are strongly expressed with the correct temporal and tissue specificity. Importantly, *P. berghei* development was inhibited by 85~95% in the transgenic mosquitoes and transmission of the parasite to naïve mice was drastically reduced. We will report on progress toward the identification of parasite molecules involved in interactions with the mosquito. Zieler *et al*. (*Journal of Experimental Biology* 204:4157-4167, 2001) discovered that snake venom phospholipase A2 (PLA2) interferes with *Plasmodium* invasion of the midgut epithelium. We report that transgenic *An. stephensi* mosquitoes that express PLA2 from the carboxypeptidase promoter are strongly impaired in sustaining *P. berghei* development and transmission. In summary, we have shown that *Plasmodium* development and transmission can be blocked by two independent effector genes: the SM1 peptide and PLA2. These findings have important implications for the development of new strategies for malaria control.

Short day and long day expression patterns of genes involved in the flesh fly clock mechanism: period, timeless, cycle and cryptochrome

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Though our knowledge of the molecular details of the circadian clock has advanced
functional elements of the photoperiodic clock remain unknown. As a first rapidly, the f step to approach this issue, we have sequenced and examined the expression patterns of period (per), timeless (tim), cycle (cyc) and cryptochrome (cry) mRNAs in the flesh fly *Sarcophaga crassipalpis*. per and tim mRNAs were especially responsive to daylength and temperature. The peak of per mRNA expression shifted in concert with onset of the scotophase, while tim mRNA was little affected. The amplitude of tim mRNA was severely dampened under long daylength, but that of per mRNA was not affected. At lower temperatures, the abundance of per increased, but that of tim decreased. These distinct patterns of expression suggest that this information could be used to determine photoperiodic responses such as diapause.

Toward increased and more reproducible transgene expression levels in the yellow fever mosquito, *Aedes aegypti*

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Aedes aegypti is a key vector of both the yellow fever and dengue fever viruses throughout many parts of the world. Low and variable transgene expression levels due to position effect and position effect variegation are problematic to efforts to create transgenic laboratory strains refractory to these viruses. Transformation efficiencies are less than optimal, likely due to failure to detect expression from all integrated transgenes. The IE1 promoter (*Autographa californica* MNPV) and Hr3 enhancer (*Bombyx mori* NPV), in combination with the IE1 gene product, gave significantly increased reporter gene expression levels in cell culture experiments. Additionally, studies are underway to identify an endogenous boundary element that will insulate transgenes from neighboring regulatory elements and the silencing effects of encroaching heterochromatin. A combination of these strategies should result in higher transformation efficiencies and more consistent transgene expression.

Construction and analysis of a cDNA library from larval midguts of cotton bollworm *Helicoverpa armigera*.

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A cDNA library from midguts of fourth-instar larvae of *H. armigera* was constructed in lambda-ZAP. A subset of the library was subjected to mass excision and propagated as a plasmid library, which was gridded onto high-density filters and subjected to 5' end sequencing. Analysis of the resulting EST dataset, and comparison to EST collections from midgut of domesticated silkworm *Bombyx mori*, will be described.

The role of resource availability in allocation patterns between growth and storage in the grasshopper, *Schistocerca americana*.

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Many organisms have been shown to change resource allocation

strategies in response to resource availability. This flexibility allows organisms to maximize fitness in different environments by changing allocation patterns. Body size and nutrient reserves are essential life history correlates of fitness. In insects, body size is fixed at the larval-adult moult, at which point resources accumulated by the larvae are invested into either growth or storage for future use. It is unknown if allocation strategies between growth and nutrient storage at the larval-adult moult in insects are fixed or flexible. This work determines if the grasshopper, *Schistocerca americana*, has resource-mediated flexibility in its allocation strategy to growth and nutrient storage at the larval-adult moult. Resources were manipulated by rearing nymphs on artificial diets differing in total nutrient content. The most nutrient poor diet contained 14% carbohydrates + proteins in a 1:1 ratio, and the most nutrient rich diet contained 84% carbohydrates + proteins. Adult body size showed a positive quadratic relationship with nutrient content, and growth rate showed a negative quadratic relationship with nutrient content. Animals feeding on diets with intermediate nutrient levels grew the largest, and did so most rapidly. Patterns of allocation to growth and storage, and their impact on life history strategies are discussed.

Anterior midgut tissue synthesizes monoterpenoid pheromone components in male pine engraver, *Ips pini*, and male Jeffrey Pine Beetle, *Dendroctonus jeffreyi*

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For over three decades the site of bark beetle aggregation pheromone production had remained elusive. Recent studies of pheromone production in the pine engraver, *Ips pini* (Say), and the Jeffrey Pine Beetle, *Dendroctonus jeffreyi* Hopkins, demonstrated *de novo* biosynthesis of pheromone components via the mevalonate pathway. In the present work, the gene encoding a key regulated enzyme in this pathway, 3-hydroxy-3-methylglutaryl-CoA reductase (*HMG-R*), was utilized as a probe to localize the site of pheromone biosynthesis by *in situ* hybridization. The male anterior midgut of both *D. jeffreyi* and *I. pini* showed high HMG-R transcript levels that were sex and juvenile hormone dependent. Additionally, isolated midgut tissue from juvenile hormone III-treated males readily incorporated [1-^{14}C]acetate into ipsdienol in *I. pini* and frontalin in *D. jeffreyi*, providing strong evidence that midgut tissue is the site of *de novo* pheromone biosynthesis. Pheromone-producing midgut cells are distinguished by abundant and highly ordered arrays of smooth endoplasmic reticulum, consistent with highly elevated HMG-R protein levels. These examples are, to our knowledge, the first definitive demonstrations of pheromone production by insectan alimentary tract tissue, inviting the possibility that frass-associated isoprenoid pheromones of other scolytid species are produced in a similar manner.

Perspectives on the distribution and use of the piggyBac transformation vector

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Nondrosophilid germ line transformation has been achieved with piggyBac transposon vectors marked with white, GFP, DsRed, and NPT IL With an hsp70 regulated helper transposase several dipteran species have been transformed including Ceratitis capitata, Anastrepha *suspensa, Bactrocera dorsalis, Drosophild melanogaster* and *Anopheles albimanus*. Other laboratories have transformed several other dipterans, lepidopterans and coleopterans indicating a broad range of vector function. Southern analysis of *B. dorsalis* transformants and host strain DNA revealed the presence of 8 to 10 piggyBac elements in the host genome. Sequence analysis of PCR products and genomic clones indicated greater than 95% nucleotide identity among the elements and the *Tricoplusia ni* piggyBac, as well as conservation of the 13 bp terminal inverted repeat sequences and TTAA insertion site. More degenerate piggyBac related elements were also detected in *Spodoptera frugiperda* by hybridization, indicating that the transposon has undergone very recent horizontal transmission, and its appearance in divergent species is consistent with its wide range of function. The existence of piggyBac, or related transposons in various insect species has important implications for piggyBac transgene stability and horizontal transmission, and the use of piggyBac transgenic strains for field applications may require strategies to ensure vector stability.

Functional dissection of the hexamerin receptor and its ligand arylphorin in the blowfly *Calliphora vicina*

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Endocytosis mediated by cell-surface receptors is an essential process in all eukaryotes and is required for the uptake of nutrients and the recycling of membranes and membrane proteins. In insects, the best known examples are the uptake of low-density lipoprotein (LDL) and the uptake of yolk by growing oocytes. In both cases the uptake process follows a common scheme in vertebrates and in invertebrates. The receptors involved belong to the LDL receptor supergene family characterized by a highly conserved arrangement of cystein-rich repeats which form the ligand-binding domain. By contrast, the process of receptor-mediated uptake of hexamerin storage proteins from insect haemolymph by fat body cells is a unique feature of the class *Insecta* involving a receptor which does not belong to the LDL receptor family. We identified the binding domains of the hexamerin receptor and the hexamerin ligand arylphorin in the blowfly, *Calliphora vicina*, by means of the yeast-two-hybrid-system. The receptor-binding domain of arylphorin was located within an epitope of 49 amino acids (aa) in the domain 3 of the arylphorin monomer. The ligand-binding domain of the hexamerin receptor was mapped to the first 24 aa of the N-terminus of the receptor. The binding domains identified exhibit no similarity to any functional domains known to date and therefore represent new species of interaction-domains. We identified two previously

unknown protein-interactors of the hexamerin-receptor, the anterior fat body protein (AFP) and a delta-adaptin subunit (δ-AP3). The results of this study provide further insight in the mechanism of the receptor-mediated endocytosis of storage proteins in insects.

EST-cDNA clones were mapped on 28 chromosomes based on RFLP in the silkworm, *Bombyx mori*

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We have developed the genetic analysis by using molecular markers mapped on the chromosome based on restriction fragment length polymorphism (RFLP) in the silkworm, *Bombyx mori*. In the silkworm there is no crossover on female chromosomes, so (RF02 x RF50) female was crossed to RF02 male and from this BF_1 segregant individual DNAs were prepared and used for Southern blot hybridization to know their genotypes. Every clone gave homozygous or heterozygous type for each individual and the patterns for each clone of 15 BF_1 segregants could be identified from each other. After the 28 patterns for every chromosome were made, every new clone was easily to know their linkage group from the genotyping of 15 individuals based on its RFLP pattern. This effective linkage analysis system was named Scanning Linkage Analysis. After linkage analysis, all the clones were mapped by using the 100 segregants of the cross between (RF02 x RF50) male and RF02 female. Every clone gave homozygous or heterozygous pattern on the same BF_1 segregants. From all of the genotypes for every clone of 100 individuals, non-recombinant individuals are those with either all A or B but recombinant individuals have both A and B. The linear order of clones is known after data are sorted for recombinants and the distance between clones is determined by counting the recombinants. From this sorted data, it is easy to show the crossover point in between the genes, double crossover individual and graphical genotypes of the clones for each individual. Finally 195 cDNA clones have been mapped on 28 chromosomes in the silkworm, *Bombyx mori*.

Expression pattern and gene structure of *Aedes aegypti* transferrin

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Homologues of transferrin, the major iron transport protein in vertebrate serum, have been identified and characterized from various insects. Based on their upregulation upon bacterial infection, the dipteran transferrins appear to play antibiotic rather iron transport roles. One such transferrin was cloned from the mosquito *Aedes aegypti*. To understand the possible functions of this transferrin homologue in the mosquito iron homeostasis and immune defense, we studied the expression pattern of the transferrin mRNA and protein. Developmental Northern blots revealed that transferrin messages are present in the pharate first instar larvae and are moderately abundant in the late larval stages, in pupae and in adults. Transferrin protein is present at all developmental stages. In adult females, 24h after a blood meal, transferrin messages are highly abundant in the fat body and in the thorax and are also detected in the ovaries. Transferrin protein is present in the hemolymph and it is also found in the fat body and ovaries in female mosquitoes before and after blood feeding. Raising mosquito larvae in iron-rich water leads to a decrease in transferrin message abundance, an effect also observed in *Drosophila melanogaster*. In contrast, feeding adult female mosquitoes with natural or artificial blood meals results in transferrin message upregulation. These results are discussed in the light of the possible dual function of this mosquito transferrin as iron carrier and antibiotic agent and are compared to similar observations with *D. melanogaster*. The *A. aegypti* transferrin gene was cloned and sequenced and its structure was compared to the orthologous transferrin genes identified in the genomes of *D. melanogaster* and *Anopheles gambiae*. While the fly gene has four introns, the mosquito genes have only one. This is in contrast to vertebrate transferrin genes, which have up to 17 introns. Putative NF-κB-like binding sites were located in the promoter regions of the fly and mosquito genes, which is consistent with the induction of these genes upon bacterial infection.

Insecticidal activity and risk assessment of a recombinant baculovirus expressing a basement membrane-degrading protease

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Basement membranes surrounding the tissues of lepidopteran larvae are a potential barrier to baculovirus movement and establishment of systemic infection. Hence, one potential approach to improving the insecticidal activity of baculoviruses is to perforate or eliminate the basement membranes of their hosts. Towards this end, we constructed two recombinant clones of *Autographa californica* nucleopolyhedrovirus (AcMNPV) that expressed a basement membrane-degrading cathepsin L protease from the flesh fly, *Sarcophaga peregrina*. The recombinant viruses expressed *S. peregrina* cathepsin L from either the *ie-1* promoter (AcIE1TV3.ScathL) or the *p6.9* promoter (AcMLF9.ScathL). In survival time bioassays, AcMLF9.ScathL killed neonate *Heliothis virescens* 50% faster than wild-type virus and 30% faster than viruses expressing the scorpion toxins AaIT and LqhIT2 (AcMLF9.AaIT, AcMLF9.LqhIT2). Co-infections of larvae with AcMLF9.ScathL and a second insecticidal protein-expressing recombinant virus (AcMLF9.AaIT, AcMLF9.LqhIT2, AcJHE-SG, or AcJHE-KK) failed to produce additive or synergistic effects on host survival time. Baculovirus-expressed *S. peregrina* cathepsin L was optimally active at pH 5.0 and not active at pH 6.0, suggesting that *S. peregrina* cathepsin L may be operating at locally acidified microenvironments within the larvae. AcMLF9.ScathL caused premature cuticular melanization of 5th instar *Heliothis virescens*. Melanization of

internal tissues was also observed, suggesting that *S. peregrina* cathepsin L expression was activating host prophenoloxidase. Infections of 5th instar *H. virescens* with AcMLF9.ScathL yielded fewer polyhedra than wild-type AcMNPV and AcMLF9.AaIT, indicating that the insecticidal properties of *S. peregrina* cathepsin L may be independent of any effect on virus movement or systemic infection. Risk assessment studies were performed to determine if AcMLF9.ScathL had detrimental effects on two species of nontarget insect predators, the ladybeetle *Coleomegilla maculata* and the green lacewing *Chrysoperla carnea*. A diet of *H. virescens* was suboptimal for *C. maculata* larvae with a rate of survival to the adult stage of only 2%, but there were no differences in time to death of *C. maculata* fed *H. virescens* infected with AcMNPV or AcMLF9.ScathL. *C. carnea* larvae reared on *H. virescens* infected with AcMNPV and AcMLF9.ScathL exhibited rates of survival to the adult stage of approximately 70% and 86%, respectively. There were no significant differences in developmental rates between *C. carnea* fed *H. virescens* infected with AcMNPV or AcMLF9.ScathL. Results suggest that the use of AcMLF9.ScathL in pest management would pose no greater risk to nontarget insect predators in the environment than would the use of the parental virus AcMNPV.

The *Rachiplusia ou* multi-nucleocapsid nucleopolyhedrovirus genome sequence

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Rachiplusia ou multi-nucleocapsid nucleopolyhedrovirus (RoMNPV) was first isolated in 1960 from the mint looper, *Rachiplusia ou*. A different isolate of this virus was identified in 1985 from the celery looper, *Anagrapha falcifera*, and described as the *Anagrapha falcifera* multi-nucleocapsid nucleopolyhedrovirus (AfMNPV). RoMNPV is closely related to *Autographa californica* multi-nucleocapsid nucleopolyhedrovirus (AcMNPV). RoMNPV and AcMNPV have broad, overlapping host ranges, but RoMNPV is more virulent than AcMNPV against a number of agriculturally significant species, including the corn earworm (*Helicoverpa zea*), the European corn borer (*Ostrinia nubilalis*), and the navel orangeworm (*Amylois transitella*). As a preliminary step towards identifying the molecular basis for the greater virulence of RoMNPV against these species, we sequenced the RoMNPV genome. A set of overlapping restriction fragments of the R1 isolate of RoMNPV was cloned into plasmid vectors and sequenced by primer walking. The RoMNPV genome is 131,526 bp with a 39.1% G + C content. The RoMNPV nucleotide sequence is almost totally co-linear with the sequence of AcMNPV, and contains homologues of 149 of the 154 ORFs described for the C6 isolate of AcMNPV. The average sequence identity of RoMNPV ORFs with AcMNPV is 96.1%, with the lowest sequence identity observed with *ac70* (84.1%). Eleven RoMNPV ORFs are completely identical (100%) with their AcMNPV homologues. A 1275 bp region that in AcMNPV contains *ac2* (baculovirus repeated ORF, *bro*) and *ac3* (*ctl*) is missing from RoMNPV. No other *bro* homologues occur in the RoMNPV genome. Insertions, deletions, and substitutions have increased or decreased the size of 47 RoMNPV ORFs relative to their AcMNPV homologues. In three cases (*ac97, ac121,* and *ac140)*, the RoMNPV homologues have been reduced below 50 codons in length. Four pairs of ORFs (*ac20/ac21, ac58/ac59, ac106/ac107,* and *ac112/ac113*) have been fused into single ORFs in RoMNPV. All the homologous regions (*hr*s) present in AcMNPV-C6 are present in RoMNPV, but the RoMNPV hrs have one to four fewer palindromic repeats, and the spacing between the repeats is not well conserved.

***Drosophila melanogaster* insulin/IGF signaling mutations: Endocrinology, reproduction and life span.**

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Chico is a *Drosophila melanogaster* insulin receptor substrate protein that plays an integral role in insulin/IGR signaling. The Chico[1] null mutation results in a 40% extension of female longevity. We have investigated aspects of the reproductive system of Chico[1] and wildtype females to determine the number of nurse cells in egg chambers, number of ovarioles in ovaries, Northern blots to identify yolk protein gene mRNA and investigation of the mechanism of yolk protein uptake. Juvenile hormone biosynthesis and ecdysyteroid abundance was also investigated. The results provide insight into female reproduction and the longevity phenotype of insulin/IGR signaling mutations.

A DNA test for parasitization of whiteflies by native and exotic *eretmocerus* species

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The whitefly, *Bemisia agentifolii*, is a serious pest of fruit and vegetable, cotton and alfalfa crops in the south and southwest. One method to control this pest has been biological control, the use of natural enemies. The Aphelinidae wasps of the genus *Eretmocerus* parasitize whitefly nymphs and have been a favorite control method in fields and greenhouses. To fight the recent introduction of the prolific silverleaf whitefly the USDA has conducted a worldwide search for *Eretmocerus* species useful against this species of whitefly. With introduction of foreign exotic species into the environment it

becomes necessary to be able to follow the introduced species to determine its spread, its reproduction and its effect on native species. This is difficult with *Eretmocerus* because of its small size and the similar appearance of most species. No identification at all can be made on the embryonic wasps before they emerge as adults. We have developed a simple squash blot hybridization test using highly abundant but species-specific DNA probes that allows field tests of the rate of parasitization of whitefly nymphs and identifies the species of *Eretmocerus* parasites even at very early stages of development. We have used this in a large-scale test in the Imperial Valley of California. More than 26,000 whitefly nymphs and pupae were collected from 307 sites on four different crops over eighteen months. They were squashed on filters and hybridized with the labeled species-specific probes. Approximately 21% were parasitized with the native *E. eremicus* species and 6% were parasitized by a variety of imported European-African-Asian species. We were able to show that about 80% of the parasitization by exotic species was due to one species from Ethiopia. Much lesser amounts were due to species from the United Arab Emirates and Pakistan. About 15% was due to other unidentified exotic species. This method could provide a simple way to follow and evaluate large-scale field tests with these small insects.

Gene flow of *Pectinophora gossypiella* (pink bollworm) and *Spodoptera exigua* (beet armyworm) in Arizona.

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The use of genetically modified crops that express *Bacillus thuringiensis* (Bt) toxin genes can decrease use of insecticide sprays for pest management. During each of the past four years, Bt cotton has accounted for more than half of the >100,000 hectares of cotton grown in Arizona; therefore, proper management plans for Bt crops are needed to ensure that pests do not evolve resistance to Bt toxins too quickly. The EPA has mandated the high dose refuge strategy for delaying pest resistance to Bt crops. Refuges are composed of host plants that do not produce Bt toxins, thus enabling survival of susceptible pests. Refuges may substantially delay resistance if the following assumptions are true: 1) inheritance of resistance is recessive, 2) resistance genes are rare, and 3) extensive mating occurs between susceptible adults from refuges and resistant adults from Bt crops. My research will address assumption 3) by analyzing pest genetic population structure and dispersal. Understanding of gene flow and adult movement are essential for resolving key issues about these pests. Microsatellite markers will be used to assess gene flow among pink bollworm and beet armyworm populations. Genetic variation will be analyzed at 4 6 polymorphic loci in 50 60 individuals per population from 12 18 populations from Maricopa and Pinal Counties in Arizona because of their high adoption of Bt cotton. 6 10 additional populations will come from California, New Mexico, and elsewhere in Arizona. By combining an intensive survey in a small area with an extensive survey of three states, we will achieve a detailed and broad portrait of gene flow. Progress on microsatellite development will be presented.

Ecdysone receptor-dependent transcriptional activity is potentiated by juvenile hormone.

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The amino acid sequences within the ligand-binding domain of the *Drosophila melanogaster* ecdysone receptor and vertebrate farnesol X receptor (FXR) are about 40% identical. FXR-dependent transcriptional activity is increased by several compounds, including farnesol and juvenile hormone III, and we postulated that EcR and FXR may share similar activation properties. A heterologous mammalian cell line (Chinese hamster ovary; CHO) which displays no endogenous response to ecdysteroids or JHIII, was transfected with (1) a reporter plasmid controlled by several *D. melanogaster* hsp27 EcREs linked to the luciferase gene, (2) a plasmid expressing a chimeric *D. melanogaster* EcR, and (3) a plasmid expressing either the mammalian RXR or an insect USP. Transfected cells showed no response to JHIII alone but muristerone A-induced reporter activity was constituted in the cells. This response was further potentiated in a dose-dependent manner by either JHIII or methoprene. In several different chimeric EcRs, the EcR ligand-binding domain was necessary and sufficient for both muristerone A response and the potentiation caused by JHIII. The EcR-mediated response to muristerone A and the potentiation caused by JHIII also requires the presence of RXR or USP, though neither by itself confers cells with the ability to respond to ecdysteroids or JHIII. The elevated induction associated with JHIII was observed exclusively at submaximal levels of muristerone A induction. We conclude that JHIII interacts directly with the ecdysteroid-bound receptor complex to elevate its transcriptional activity further, or acts indirectly on the previously bound complex through a second protein or receptor modification pathway. The proposed mechanism is distinct from other additive and synergistic effects of various ligand combinations attributed to other nuclear receptor complexes.

Cell proliferation in *Anopheles albimanus* mosquito tissues *in vitro*, after yeast inoculation.

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In mammalian systems, the proliferation of responding cell types (T and B) is a common characteristic in both humoral and cellular aspects of adaptive immunity. Since cellular proliferation is central to the induction of vertebrate immunity, it is important to determine if insect hemocytes or any other tissue are able to proliferate. Important changes have been reported in *Aedes aegypti* hemocytes

subpopulations during the immune response against microfilariae. A possible hemopoietic tissue called "matrix tissue" was reported in *Simulium vittatum*.. The matrix tissue was present in higher quantities in the hemolymph after the treatment with microfilariae and LPS injected flies. The cell proliferation analysis was hampered because the lack of techniques and models *in vitro* that facilitate its evaluation. We have developed a strategy of primary cultures of *Anopheles albimanus* mosquito tissues for this propose. In the present study, we have evaluated the proliferative capacity of mosquito tissues by *in vitro* incorporation of 5-bromo-2-deoxyuridine (BrdU), using anti-BrdU fluorescein and anti-BrdU peroxidase antibodies, for epifluorescence microscopy and ELISA assays respectively. Mosquito tissues were dissected and cultivated in RPMI medium plus fetal bovine serum, antibiotics and BrdU. The tissues survived for two weeks at 22 ^{o}C. Cell proliferation was measured by epifluorescence in different mosquito tissues. Mosquitoes previously inoculated with yeast, dissected and incubated in RPMI medium for 5 days containing Con-A, showed highest BrdU incorporation in fat body, epithelial cells in pleural membranes, and the dorsal vessel. This proliferative response was inhibited with colchicin. On the other hand, the abdomens of control and inoculated mosquitoes with yeast, were cultivated in RPMI medium plus Con-A for 5 days. DNA was obtained by phenolic extraction and the BrdU incorporated was quantified by ELISA assays. The absorbance was recorded at 450 nm. The ELISA results correlated with the epifluorescence assays. The results showed the efficient proliferative capacity in different *An. Albimanus* tissues, mainly in epithelial cells of pleural membranes. We are currently evaluating the effect of different lectins and microorganism, included malaria parasites, on tissue proliferation response.

Anopheles pseudopunctipennis midgut produces nitric oxide in response to _Plasmodium berghei_ ookinetes.

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Recently it has been shown that the malaria vector mosquito *Anopheles gambie*, when infected with *Plasmodium*, is able to express different genes related to the immune response such as ISP13 (a putative serine protease), ISPL5 (serine protease like), GNBP, defensins and NOS (nitric oxide synthase). On the other hand, NOS expression and nitric oxide (NO) production in the mosquito *An. stephensi* limit malaria parasite development. In this work we characterized the NOS expression in the mosquito *An. pseudopunctipennis* and we evaluated the nitric oxide generation in response to *P. berghei* ookinetes, yeast, bacteria and L-Dopa. Using RACE- 5' and 3', we have obtained the ApNOS cDNA, which was cloned in pBS II KS. We have sequenced about 50% of *An. pseudopunctipennis* NOS (ApNOS) with high homology to *An. stephensi*, *An. gambie* and *Manduca sexta* NOS. ApNOS is only expressed in adults and we have observed transcriptional activation of NOS in mosquitoes after feeding on mouse blood. The treatment with antibiotics prevent the NO generation. NO generation was detected in midgut cultures, by Griess reaction. The results indicate that enzyme activity is induced by *P. berghei* ookinetes and yeast, but Gram negative bacteria did not induce it. We found that L-DOPA may regulate NOS transcription by hydrogen peroxide production. These data could improve our understanding of the NOS physiology in the mosquito *An. pseudopunctipennis* when it is infected with *P. berghei*.

Which isoforms of fasciclin 11 are involved in guidance of olfactory receptor axons in the developing olfactory system of _Manduca_?

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Using the olfactory (antennal) system of the moth, *Manduca sexta*, we are exploring mechanisms of guidance and target selection by olfactory receptor neuron (ORN) axons. We found previously that *M. sexta* fasciclin II (MFas 11) is present during development on the ORN axons that terminate in a subset of glomeruli, and that experimental disruption of the segregation of MFas 11 expressing axons is accompanied by mistargeting of the axons. Using antibodies that specifically recognize two isoforms of MFas 11, a GPI linked isoform (GPI MFas II) and a transmembrane isoform (TM MFas II), we have found that TM MFas 11 is expressed predominantly on antennal receptor axons, and expressed in.the same subset of glomeruli as we showed label with antibodies that recognize all forms of MFas 11. GPI MFas 11 is found predominantly on perineurial sheath cells and weakly on some of the glial cells that invest receptor axons in the antennal nerve. Western blots of antermal lobes reveal that TM MFas 11 expression is relatively low before glomerulus formation, attains peak expression during glornerulus formation, and then decreases; expression of GPI MFas 11 remains constant throughout development. Isoform complexity (numbers of protein bands that label with either GPI or TM MFas 11 antibodies) is highest during the period of glomerulus formation. We hypothesize that, as in the enteric nervous system, GPI MFas 11 functions to stabilize points of contact, while TM MFas 11 is instrumental in the fasciculation important for targeting to glomeruli. To this end we are synthesizing fasciclin 11 dsRNA fragments in order to perform RNA interference (RNAi) and thus knock out fasciclin 11 in the developing olfactory system.

Discovery of the first arthropod myokinin receptor in the cattle fever tick, _Boophilus microplus_, by functional analysis in a transfected mammalian cell line.

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We previously reported the cloning of a G protein-coupled receptor from the cattle fever tick *Boophilus microplus* that has high homology with the lymnokinin receptor from the pond snail *Lymnaea stagnalis*. Previous to our report, the lymnokinin receptor was the only identified myokinin receptor. The myokinins are multifunctional neuropeptides that have been found in several arthropod and invertebrate groups. They have myotropic and diuretic activity in insects, which is mediated through a cyclic nucleotide-independent signal transduction pathway involving increases in intracellular calcium. We transfected CHO-K1 cells with an expression construct encoding the tick receptor and isolated a clonal cell line functionally expressing the receptor protein. Challenge with several myotropic peptides induced increases in intracellular calcium measured in fluorescent assays performed with a laser cytometer. No calcium response was seen in negative control cells transfected with vector only. The calcium response was not affected by the absence of extracellular calcium, indicating action through intracellular stores. Our results provide functional evidence that the tick receptor is the first myokinin receptor cDNA clone available from an arthropod. Additionally, our *in vitro* assay provides a new system for testing hypotheses of structure-activity requirements in the myokinin neuropeptide family and a method of rapidly screening for compounds with potential applications as receptor agonists or antagonists.

Ecology of *Aedes aegypti* in Tucson

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Oviposition traps were used to follow changes in the population of *Aedes aegypti* in a seven-block area in the Sam Hughes region of Tucson, Arizona. About 20,000 eggs were collected over a period from 1 June to 14 October 2000. Peak numbers of eggs were correlated with the late summer rains. Mosquitoes seeking a blood meal were collected and dissected to determine if they had previously fed, i.e. if they were parous. Of the 241 females examined, 44% were parous, with a range from 0% to 80%. Mosquitoes containing a blood meal were collected and the source of the blood was determined using an ELISA. Preliminary data from 37 blood fed females showed that 80% had fed on humans. These data suggest that the reproductive history of Tucson populations of *Ae. aegypti* is favorable for transmission of dengue viruses.

Prothoracicostatic peptide: a novel neuropeptide inhibiting ecdysteroidogenesis in the silkworm, *Bombyx mori*

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Endocrine control of the activity of the prothoracic glands (PG) of insects, in which ecdysone is biosynthesized and released, is modulated by peptide hormones secreted from the brain. The biosynthesis of ecdysone by PG is considered to be stimulated by prothoracicotropic hormone (PTTH). Recently, we have identified a prothoracicostatic peptide (PTSP) from the brain of the silkworm, *Bombyx mori*, using a protocol that including four reversed-phase high performance liquid chromatography procedures. The primary structure of this peptide was determined to be H-Ala-Trp-Gln-Asp-Leu-Asn-Ser-Ala-Trp-NH_2. PTSP strongly inhibited ecdysteroidogenesis in the PGs of spinning larvae in a dose-dependent manner, and simultaneous application of PTSP and PTTH onto the PGs of feeding larvae inhibited the PTTH-stimulated ecdysteroidogenesis. Injection of PTSP into later 5^{th} instar larvae significantly reduced the ecdysone titer *in vivo*. These results indicate the importance of PTSP in the regulation of ecdysteroidogenesis. Using RT-PCR and 5'-RACE we have cloned PTSP cDNA from *B. mori* brain. The predicted open reading frame encoded 274 amino acids including five repeats of PTSP and seven other structure-related peptides based on the canonical endopeptidase cleavage and amidation sites. All the seven predicted peptides were prepared by chemical synthesis and some of them showed prothoracicostatic activity in the *in vitro* bioassay. One pair of neurosecretory cells in the brain was recognized by an antibody against Bom-PTSP. *In situ* hybridization revealed the same pair of cells that were positive in the immunohistochemistry. Northern blot analysis indicated that PTSP gene is expressed in the nervous system, namely the brain and nerve cord, but not in other tissues, suggesting that Bom-PTSP is a neuropeptide.

DUP99B, a new sex-peptide pheromone of *Drosophila melanogaster*.

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In *Drosophila melanogaster*, mating leads to two characteristic behavioral responses in females, increased egg laying and reduction of receptivity. Both responses persist for about one week. Three compounds of the male genital tract, sex-peptide (SP), ovulin, and DUP99B (Ductus ejaculatorius peptide, cytological localization 99B) are involved in the initiation of these responses. Ovulin, a protein synthesized in the accessory glands (Herndon and Wolfner, PNAS 92, 10114, 1995), elicits a partial increase in egg laying on the first day after copulation. When ectopically expressed or injected into virgin females, SP, a peptide also synthesized in the accessory glands, elicits an increase in egg laying and reduction of receptivity for one day. DUB99B also induces the two post-mating responses for one day after injection into virgin females. The gene *Dup99B*, as shown by promoter analysis, is expressed in the ejaculatory duct of the male genital tract and in the cardia (an organ situated in the thorax

and thought to be involved in sucking behavior) of both sexes. The expression is first seen in the late pupal stage. The question we address is the possible functional redundancy of the two peptides, SP and DUP99B. To study this problem, we used males lacking accessory glands and a mutant with deleted *Dup99B* genes. We show that *in vivo* ejaculatory duct and accessory gland components contribute to the increase of the egg laying rate after mating. The contribution of DUP99B, however, seems to be minor. From *in vitro* experiments we conclude that some functions of the two peptides are not redundant.

Generation of a hypothetical three-dimensional model of *Manduca sexta* eclosion hormone

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Eclosion hormone (EH) is a 62 amino acid insect neuropeptide that regulates the behavioral process of ecdysis by way of a positive feedback loop in conjunction with ecdysis triggering hormone (ETH). EH has been isolated from two Lepidopteran species, *Manduca sexta* and *Bombyx mori*, and cloned in *Drosophila melanogaste*r. Since no analogue has been identified in vertebrates, the EH loop may provide an elegant means of controlling insect populations. In order to begin a study of the structure-function relationship of *M. sexta* EH (Manse-EH), a three-dimensional model was generated using *ab initio* molecular modeling techniques. The resulting peptide structure contains three α-helical regions (residues 12-24, 38-41, 50-62) joined by disulfide bridges between Cys14-Cys38, Cys18-Cys34, and Cys21-Cys49 and is thermodynamically stable. Constraining the residues of Manse-EH that corresponded with helical regions previously published for *Bombyx* EH (Bommo-EH) resulted in a model that had greater helical content but was thermodynamically less stable. Despite the 81% sequence identity exhibited by the two peptides, Manse-EH was unable to assume the published Bommo-EH conformation. This observation was further substantiated by protein threading techniques. This suggests that EH activity may be the result of a conserved core region with a common conformation. Also, biologically active synthetic Manse-EH exhibited surprising stability when treated with pepsin and Glu-C, suggesting the possible efficacy of utilizing ingestable EH and EH analogs.

Chitin binding activity of *Oryctes rhinoceros* antimicrobial peptides

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Oryctes rhinoceros defensin and scarabaecin are antimicrobial peptides isolated from the coconut rhinoceros beetle, *O. rhinoceros*. Scarabaecin shows both antifungal and antibacterial activity whereas *O. rhinoceros* defensin shows only antibacterial activity. Scarabaecin binds to chitin but not to curdlan or peptidoglycan. Although the action mechanism of scarabaecin is not known, its binding activity to chitin is reasonable for its antifungal activity. Scarabaecin shows homology with a putative chitin binding domain of the horseshoe crab antibacterial peptide, tachycitin. The functional domain might be conserved through evolution. *O. rhinoceros* defensin also shows chitin binding activity although it does not have antifungal activity and shows no homology with other chitin binding proteins. The biological significance of chitin binding activity might be attributed to accumulation around wounds where exoskeletal chitin is exposed to hemolymph as a defense against microbial invasion. Defensin binds to peptidoglycan that is widely distributed as a bacterial cell wall component. As the bacterial membrane is assumed to be a target of defensin, its binding activity to peptidoglycan may be important for the antibacterial activity.

Antennae-specific chemosensory protein from the Argentine ant, *Linepithema humile*

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Chemosensory proteins (CSPs) have been previously identified from various insect species (moths, locustus, phasmid**)**. CSPs have been demonstrated to bind physiologically relevant ligands, but because they lack the hallmark of odorant-binding proteins (the six cysteine residues), they have been segregated in a group of olfactory proteins apart from odorant-binding proteins and pheromone-binding proteins. Although CSPs have been found in antennae and other sensory tissues (tarsi and labrum), they are neither tissue-specific nor expressed as the major olfactory protein. We have now identified a CSP in the Argentine ant, which is the major antennae-specific protein in workers. Although the N-terminal amino acid sequence showed 64% identity to a previously identified CSP from antennae of *Mamestra brassicae* (CSP-MbraA6), cDNA cloning showed that the two proteins have only 33% identity. As with other CSPs identified to date, the Argentine ant CSP has four cysteine residues.

Regulation of expression of digestive protease genes in *Aedes aegypti*: A complex pattern with basic rules?

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Blood proteins are digested in the midgut by the combined action of endo- and exo-peptidases. Twenty-one protease cDNAs have been identified in the midgut of female *Aedes aegypti*. They can be grouped into fourteen different gene-families belonging to the trypsin, chymotrypsin, aminopeptidase and carboxypeptidase groups of

peptidases. Changes in the steady state of mRNA levels were studied by northern hybridization and PCR. Four different patterns of expression can be recognized: a) highly expressed before feeding and reduced after a meal is taken, b) expression induced during the first 6 h after feeding, c) expression induced with a maximum at 18-24 h after feeding, and d) highly expressed before and after feeding. The midgut activities of trypsin, chymotrypsin, aminopeptidase and carboxypeptidases were studied using synthetic substrates. Ingestion of a blood meal induces two phases of digestion; the first phase, which encompasses the first 4-6 hours following a blood meal, is involved in assessing the quality of the meal. The second phase, which occurs between 8 and 36 hours after blood feeding, is responsible for the digestion of the meal proteins. Understanding the regulation of synthesis of proteases during these two phases is the aim of our research. Our working hypothesis is that during the first phase there is a protease that acts as a master regulator of gene expression (tentatively identified as early trypsin). This protease "senses" the quality and quantity of protein present in the meal, and controls the expression of a battery of protease genes that are expressed during the second phase of digestion. Several of the enzymatic activities induced during the second phase of digestion have in common: a) stimulation by feeding a blood or protein meal but not by feeding an amino acid meal, b) the amount of activity induced is proportional to the protein concentration of the meal, and c) the induction of enzymatic activities is blocked by the addition of STI to the meal.

Expression of anti sporozoite, single chain antibodies in transgenic *Aedes aegypti* blocks transmission of *Plasmodium gallinaceum* to the vertebrate host.

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Transgenic mosquitoes resistant to malaria parasites were developed to test the hypothesis that they may be used to control transmission of the disease. Transformation technology was used to insert into the yellow fever mosquito, *Aedes aegypti*, a gene encoding a recombinant single chain antibody, N2, that recognizes the circumsporozoite protein (CSP) on the surface of *Plasmodium gallinaceum* sporozoites. We report efficient germ line transformation using the piggyBac transposable element vector and EGFP (enhanced green fluorescent protein) as a transformation marker. Three transgenic lines, 21, 17 and P12, each containing the blood inducible vitellogenin gene promoter ft) driving the expression of the N2 transgene, were established and characterized. N2 single chain antibodies block sporozoite invasion of salivary gland by 99.7 % in line 21, but did not block in lines 17 and P12. Immunoblot analyses demonstrated stronger N2 expression in line 21 than in 17 and P12, possibly accounting for the different numbers of sporozoites observed in the salivary glands. Surprisingly, even mosquitoes that had very low levels of sporozoites in their salivary gland (21 59 sporozoites) compared with controls (5500 6500 sporozoites) were able to infect 7 day old chickens. In contrast, mosquitoes that had 20 sporozoites in their salivary glands did not infect 14 day old chickens. In summary, expression of the N2 single chain antibodies in mosquitoes can block sporozoite transmission and this approach has the potential to be a powerful antiparasite effector system. This work is supported by grant AI44238 from NLAJD.

Expression, purification, characterization, and in wtro activation of proPAP 1 and proPAP 2 from baculovirus infected insect cells

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Analogous to human thrombin, prophenoloxidase activating proteinase (PAP) is a terminal enzyme of a putative serine proteinase cascade in the tobacco hornworm, *Manduca sexta*. We previously isolated PAP 1 and PAP 2 from integuments and hemolymph, respectively, and cloned their cDNAs. In order to purify and study the unknown activating enzyme(s) for the PAPs from this insect, we produced proPAP 1 and 2 in the *Escherichia coli* and baculovirus insect cell expression systems. Polyclonal rabbit antisera were generated against the affinity purified proteins from the bacteria. Immunoblot analysis indicated that low, constitutive expression of proPAP 1 was induced six to eight fold after *M. sexta* larvae were challenged with killed bacterial cells. Induced synthesis of proPAP 2 was detected in plasmatocytes; by immunofluorescence labeling. In the eukaryotic expression system, the recombinant proPAP 1 and 2 were secreted into the media at a concentration of approximately 0.4 and 14.8 mg/liter, respectively. We developed simple, efficient schemes to enrich and purify the zymogens by affinity chromatography. Immunoblot analysis and MALDI TOF mass spectrometry indicated that the recombinant proPAPs are nearly identical in size to the zymogens from *M. sexta* hemolymph. Other properties, such as isoelebic point and glycosylation, were determined to further characterize the two proteins. When mixed with *a* larval hemolymph sample, the recombinant proPAP 1 was activated along with the endogenous proenzyme in the presence of *Micrococus lysodeikticus.* ProPAP 2 was also readily processed when incubated with hemolymph of *M. sexta* pharate pupae in the presence of a fungal cell wall component, 0 1,3 glucan.

Polyhedral envelope protein mutants of *Rachiplusia ou* multi-nucleocapsid nucleopolyhedrovirus

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Rachiplusia ou multi-nucleocapsid nucleopolyhedrovirus (RoMNPV) has great potential as an environmentally benign control agent for certain lepidopteran (moth) pests. To increase the virulence

of RoMNPV, we mutated the RoMNPV polyhedral envelope protein gene (*pep*) to prevent formation of the polyhedral envelope. The *pep* ORF was disrupted by insertion of *lacZ* to produce the recombinant virus RoPEPlacZ. Two additional *pep* mutant recombinant viruses were produced by site-directed mutagenesis that eliminated the *pep* initiation codon (RoPEPΔATG) or inserted a stop codon at position 13 of the *pep* ORF (RoPEPTAG). A fourth *pep* mutant virus, RoPEPΔATG-TAG, carried both the ablation of the *pep* initiation codon and the insertion of the stop codon. Western blot analysis revealed that PEP was still expressed during infection of Sf9 cells with RoPEPΔATG. A greatly reduced quantity of PEP was evident in Sf9 cells infected with RoPEPTAG or RoPEPΔATG-TAG, indicating that translation occurred past the inserted stop codon. No PEP was detected in Sf9 cells infected with RoPEPlacZ. Transmission, scanning, and immunogold electron microscopy indicated that the polyhedral envelope was absent from polyhedra of RoPEPTAG, RoPEPΔATG-TAG and RoPEPlacZ. Although immunogold electron microscopy suggested that a polyhedral envelope was still present on RoPEPΔATG polyhedra, scanning electron microscopy showed that polyhedra of this mutant has a pitted surface consistent with the absence of an envelope. In bioassays, *pep*-mutant viruses showed reduced potency against neonate larvae of a semi-permissive host, the European corn borer *Ostrinia nubilalis*. The activity of the mutant viruses was unaltered relative to wild-type RoMNPV against a permissive host, the cabbage looper *Trichoplusia ni*.

Antiapoptotic action of Deterin, the *Drosophila melanogaster* homolog of cancer-related survivin

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Deterin, a new apoptosis inhibitor from *Drosophila melanogaster*, possesses an unusual structure of only a single baculovirus inhibitor of apoptosis (IAP)-type repeat and no RING finger motif. The biochemical actions of deterin are demonstrated in SF9 and S2 cell transfection assays, in which the expressed protein acts in the cytoplasm to inhibit or deter cells from apoptosis otherwise induced by the caspase-dependent apoptosis activator reaper or by cytotoxicants. Cell death in cancer-derived HeLa cells induced by Survivin antisense oligonucleotide could be partially complemented by Deterin, the *D. melanogaster* homolog of Survivin. Reciprocally, a chimera of the Deterin BIR domain and Survivin C-terminus could rescue *D. melanogaster* Kc cells from death induced by transfection of a human caspase-7-expressing plasmid. These results indicate common components of Survivin and Deterin antiapoptotic action in the vertebrate and invertebrate phyla.

Transfer of cholesterol and diacylglycerol from lipophorin to *Bombyx mori* ovarioles: Role of the lipid transfer particle

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The objective of this study was to characterize the transfer of diacylglycerol (DAG) and cholesterol from larval *Bombyx mori* lipophorin to oocytes. Transfer studies were carried out by incubating pupal oocytes (day 5) with [^{3}H]-cholesterol and [^{3}H]-DAG-labeled lipophorin under different conditions. Transfer of both cholesterol and DAG exhibited hyperbolic dependency on lipophorin concentration with an apparent Km of about 1.2 mg/ml. Pretreatment of oocytes with anti-lipid transfer particle (LTP) IgG significantly inhibited (75%) transfer of labeled DAG to oocytes but had no effect on the transfer of cholesterol from lipophorin, indicating that LTP facilitates DAG transfer to oocytes. Injection of *B. mori* pupae (day 4) with anti-LTP IgG significantly affected the weight (65%), number of eggs (49%), amount of lipid (74%) and protein (65%) of the adult ovaries compared to control insects that were injected with non-immune rabbit serum IgG. In addition, the eggs had very faint yellow color and deformed shape compared to control. This inhibitory effect demonstrates the active role LTP plays in ovary growth, development and oogenesis. Similarly, normal growth of ovaries was halted when the ovaries were implanted in male pupae that lack vitellogenin, demonstrating that vitellogenin, the female specific protein, is needed as an amino acid source for normal egg development. Thus, restricting lipid or protein delivery to developing ovaries would stunt growth and dramatically affect choriogenesis.

Pheromone biosynthetic pathway of the gypsy moth, *Lymantria dispar*, involves oenocyte formation of the alkene precursor and transport to pheromone gland for epoxidation.

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The pheromone biosynthetic pathway for production of disparlure, 2-methyl-7R,8Sepoxy-octadecane, was investigated in the gypsy moth. Deuterium labeled precursors and intermediates were utilized to follow specific reactions in the pathway. It was determined that the alkene precursor, 2-methyl-Z7-octadecene, is most likely made in oenocytes cells. This pathway uses valine to contribute the methyl carbons for chain initiation, followed by chain elongation to 19-carbons. The double bond is introduced with an unusual “12 desaturase followed by decarboxylation to the hydrocarbon. The alkene is then transported to the pheromone gland through the hemolymph. At the pheromone gland the alkene is unloaded and transformed into the epoxide. This last step is regulated by PBAN. Each step in this pathway was followed using deuterium labeled

compounds that could be identified using GC/MS. This provides unequivocal determination of specific reactions in the pathway.

Construction of BAC contigs covering densonucleosis virus-nonsusceptibility genes, *Nid-1*, and *nsd-2* of *Bombyx mori*

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Bombyx mori densonucleosis virus (BmDNV) multiplies in the columnar cell nuclei of the mid gut epithelia in the silkworm, *Bombyx mori*. It is classified into 2 types, DNV-1 and DNV-2 based on their symptoms, serological characters, genome size and structural proteins. Some silkworm strains were identified as nonsusceptible against DNV-1 and/or DNV-2. So far four non-susceptibility genes have been reported; *nsd-1*, *Nid-1*, *nsd-2* and *nsd-Z*. However, none of them have yet been isolated. Previously, we carried out linkage and mapping analysis of *Nid-1* and *nsd-2* by using cDNA markers on the RFLP linkage map. Both were mapped on linkage group 17 and four closely linked markers were also identified. A *Bombyx* BAC library was screened with these markers. Positive BAC clones were subjected to DNA fingerprinting, resulting in four contigs. The longest contig spanning 800 kb contained three out of four cDNA markers. The remaining marker was located on a separate contig. The results concur with the genetic linkage distance among 4 markers. The sequencing of the longest BAC contig is in progress.

Possible roles for Eph/ephrin signaling in the development of primary olfactory pathway of *Manduca sexta*

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Eph-family receptor tyrosine kinases (RTK) and their ligand ephrins have been shown to play important roles in axon guidance. In the current study, we explore the possibility that Eph/ephrin signaling is involved in the guidance, sorting, and/or targeting of olfactory receptor cell (ORC) axons in the moth, *Manduca sexta*. Using RT-PCR with degenerate oligonucleotide and screening of a pupal antenna cDNA library, we cloned MsEph, an Eph-like RTK, and MsEphrin, a putative ligand for MsEph. Predicted amino acid sequences of MsEph and MsEphrin are most similar to Eph and ephrin in *Drosophila melanogaster*, resepctively. Messenger RNA levels of both MsEph and MsEphrin are upregulated in the pupal antenna during the period when ORC axons grow into the CNS. Ephrin-Fc, a recombinant protein in which the extracellular domain of MsEphrin is tagged with IgG Fc, bound to and induced tyrosine phosphorylation of MsEph expressed in HEK cells, sugesting that MsEphrin acts as a functional ligand. Eph-Fc and Ephrin-Fc were used to examine the localization of ligand(s) for Eph and of receptor(s) for ephrin, respectively, in whole-mount brain preparations. The receptor(s) and the ligand(s) were detected on ORC axons, and were particularly abundant on axon terminals in a subset of glomeruli. The staining was most intense during the peirod of glomerular formation, disappearing after their establishment. The distribution of the receptor- and ligand-positive glomeruli appeared to be complementary to each other among identifiable glomeruli. Effects of Eph/ephrin signaling on ORC axon behaviors were examined using explant cultures of olfactory epithelium in (1) substratum choice assay and (2) neurite outgrowth assay. In substratum choice assays, we examined how ORC axons growing on concanavalin A/laminin behaved upon encontering substrate-bound MsEphrin-Fc. Neurites of antennal explants, when confronted with Fc-containing substratum, freely crossed the border and grew onto the Fc control region. In contrast, MsEphrin-Fc-containing substratum was largely avoided by neurites, suggesting that MsEphrin acts as a repulsive cue. In neurite outgrowth assays, we examined the effect of disrupting endogenous Eph/ephrin interactions on neurite outgrowth by adding Eph-Fc to the culture medium. Treatment with Eph-Fc inhibited neurite outgrowth in a dose-dependent manner, suggesting that the MsEph/MsEphrin interactions are required for optimal axon outgrowth. These results indicate that MsEph and MsEphrin expressed in the developing ORC axons may have distinct functions depending on the stage of axonal growth. Supported by Flinn Foundation and Charles E. Culpeper Biomedical Initiative 99-294.

Immunolocalization of aquaporin 5-like water channel protein in salivary glands of *Amblyomma americanum*

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The purpose of this study was to determine the cellular and sub cellular localization of aquaporin 5 (AQP5) in the tick salivary glands by immunobloting and immunofluorescence using peptide derived rabbit antibodies to rat AQP5. Dopamine is a neurotransmitter at the neuroeffector, junction regulating fluid secretion via an increase in cAMP in the salivary glands, of ixodid ticks. Aquaporin 5 (AQP5) is a water channel protein and is considered to play an important role in water movement across the plasma membrane. An antibody to mammalian Aquaporin 5 cross reacts with a protein in tick salivary glands. Mercuric chloride, an inhibitor of aquaporins in other epithelia inhibits fluid secretion in salivary glands. Confocal microscopy was used to localize the AQP5 protein in the salivary glands. The molecular mechanisms for water movement in salivary glands are not yet elucidated in ticks. Results suggest that AQP5 is one of the candidate molecules responsible for the water movement in the salivary glands.

SNARE and cell trafficking regulatory proteins in the salivary glands of *Amblyomma americanum*

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The Ca^{2+}-triggered release of proteins in the saliva via salivary glands is mediated by fusion of secretory vesicles with the plasma membrane. The molecular machinery that translates the Ca^{2+} signal into exocytosis is only beginning to emerge. The soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) and the proteins syntaxin, SNAP-25 and synaptobrevin, are central components of the fusion apparatus. Prostaglandin E_2 (PGE_2) stimulates secretion of tick salivary gland proteins via a phosphoinositide signaling pathway and mobilization of intracellular Ca^{2+} (Qian Y et al., 1998. Insect Biochemistry and Molecular Biology 28: 221-228; Yuan et al., 2000. Insect Biochemistry and Molecular Biology 30:1099-11060). Proteins in the salivary glands of partially fed female lone star ticks cross-react individually with antibodies to synaptobrevin-2 (vesicle (v)-SNARE), syntaxin-1A, syntaxin-2 and SNAP-25 (target (t)-SNAREs), cytosolic α/β SNAP and NSF (N-ethylmaleimide-sensitive fusion protein), Ca^{2+} sensitive synaptotagmin, vesicle associated synaptophysin, and regulatory cell trafficking GTPases Rab3A and nSec1. v-SNARE and t-SNARE proteins form an SDS-resistant, boiling sensitive core complex in the salivary glands. To better understand the functional significance of SNARE complex protein expression in salivary acini, we used confocal microscopy to localize SNARE proteins in salivary gland cells. Antibodies to SNARE complex proteins inhibit PGE_2-stimulated secretion of anticoagulant protein in permeabilized tick salivary glands. We conclude that SNARE and cell trafficking regulatory proteins are present and functioning in the process of PGE_2-stimulated Ca^{2+} regulated protein secretion in tick salivary glands.

Chitin biosynthesis and the mosquito peritrophic matrix

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Glusosamine:fructose-6-phosphate aminotransferase (GFAT) catalyzes the formation of glucosamine 6-phospate and is the first and rate-limiting enzyme of the hexosamine biosynthetic pathway. The final product of the hexosamine pathway, UDP-N-acetyl glucosamine, is an active precursor of numerous macromolecules containing amino sugars, including peptidoglycan and lipopolysaccharides in bacteria, glycoproteins and glycolipids in eukaryotes, and chitin in fungi and insects. Chitin is the main component of the insect cuticle and peritrophic matrix. The peritrophic matrix is produced in the midgut of mosquitoes in response to bloodfeeding, and may affect vector competence by serving as a physical barrier to pathogens. GFAT controls the flux of glucose into the hexosamine pathway, and thus formation of hexosamine products, regulating the availability of precursors for amino sugar containing macromolecules. Therefore, it is hypothesized that GFAT also plays a regulatory role in biosynthesis of chitin and the peritrophic matrix formation. We cloned and sequenced GFAT gene (*AeGfat*) and its promoter region from *Aedes aegypti*. *AeGfat* message is constitutively expressed but is gradually up-regulated in the midgut after bloodfeeding. We are currently studying the putative promoter region of gene to explore the *AeGfat* regulation and the molecular mechanisms of induction of peritrophic matrix formation following bloodfeeding. Supported by NIH grant AI44461-04.

***Heterorhabditis bacteriophora* surface coat proteins disrupt coagulation, encapsulation and melanization immune response by insect hemocytes.**

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To reproduce, the entomopathogenic nematode *Heterorhabditis bacteriophora* (Oswego) needs to find, invade, and overcome the host immune response system. The initial host immune response is critical in determining the outcome of the interaction. We have shown that *H. bacteriophora* elicits a rapid cellular immune response in a resistant host, *Manduca sexta*. The nematode, along with its symbiotic bacteria *Photorabdus luminescens* both have roles in overcoming the host defense. In this study we have characterized surface coat proteins from the nematode that we hypothesize are essential to evasion of the host cellular immune response. To determine the role of surface coat proteins in host evasion, the effects of these cuticular proteins were tested upon the *in vitro* coagulation, encapsulation and melanization response of hemocytes from *M. sexta and Galleria mellonella*. Our data supports a role of these proteins in determining host suitability. We have successfully separated these proteins and further biochemical and sequence homology studies using MALDI are underway.

Development of SCAR markers for the detection of Asian long-horned beetle, *Anoplophora glabripennis*

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The introduction, establishment and spread of non-indigenous insect pest such as the Asian long-horned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera:Cerambycidae) is growing threat to North America's forests, which has major implications for forest health, biodiversity, the North American forest industry and international trade. Morphological similarities and nuances between native and non-native immature larval taxonomy make identification difficult. An important step in limiting the import and spread of this non-native beetle will be providing a sensitive and reliable detection system based on unique molecular markers. DNA markers were identified for ALB detection based on sequence characterized

amplified regions (SCARs) derived from a randomly amplified polymorphic DNA (RAPD) band. A 2740-bp DNA fragment that was present only in ALB, and not in other Cerambycids, was identified after screening 230 random primers. Three pairs of extended 22-bp oligonucleotide primers were designed based on the sequence of this fragment and used to perform diagnostic PCR. The first pair of primers (SCAR1) amplified a single 745 bp fragment of ALB DNA, but this did not differentiate ALB from other species. The other two pairs of SCAR primers (SCAR2 and SCAR3) amplified bands of 1237- and 2720-bp, respectively, that were capable of differentiating ALB from other closely related non-native and native Cerambycids, such as *A. chinensis* (Forster), *A. malasiaca* (Thomson), *A. nobilis* (Ganglbauer), *Monochamus scutellatus* (Say), *Plectrodera scalator* (Fab), *Saperda tridentata* (Olivier) and *Graphisurus fasciatus* (Degeer). These two SCAR markers can be amplified using DNA from body parts such as wing, leg, and antenna as well as the tissues in all developmental stages including egg, larva, pupa and adult. They were also capable of identifying ALB from the DNA extracted from frass. These results demonstrate that DNA markers can be used for unambiguously identifying ALB from other closely related invasive and native Cerambycids. This study was supported in part by the Canadian Biotechnology Strategy Fund.

Molecular characterization and developmental expresion of acalcium-activated k+channel gene in the tobacco hornworm, *Manduca sexta.*

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Studying ion channels in identified native cells will greatly enhance analysis of their function and modulation. Insect nervous systems are well suited for such analysis as most insect channels have mammalian counterparts but are expressed in a much smaller and more accessible set of neurons. We report the molecular characterization of a *Manduca sexta* homologue of *slowpoke* (*msslo*), a Ca^{2+}-activated K+ channel. RT-PCR of larval body wall muscle total RNA using degenerate PCR primers to conserved sites in the *slo* sequence identified a 1.2 kb fragment of *msslo* that spans the S9 transmembrane domain through the calcium binding motif. Portions of this fragment were used to design species-specific RT-PCR primers and for probes used in a Southern blot, Northern blot, and RNA *in situ* hybridization. A nick translated P^{32} labeled DNA probe was used in the Southern blot while a transcribed digoxigenin-UTP labeled RNA probe visualized with an alkaline phosphatase reaction was used in both the Northern blot and RNA *in situ* hybridization analysis. A single copy of *msslo* is present per haploid genome. Northern blot hybridization has revealed differential expression of the transcript in the CNS during the larval-pupal transition. RNA *in situ* hybridization detected *msslo* mRNA in discrete cells in thoracic and abdominal ganglia in larvae. Research supported by NSF IBN9905697 to JLW.

The nucleocapsid protein of sigma virus: Sequence similarity and phylogenetic relationship with insect-associated negative-strand RNA viruses

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Negative-strand RNA viruses (NSRVs) from the Family Bunyaviridae (BVae) and some from the Rhabdoviridae (RVae) are associated with insects (as hosts or vectors), particularly those in the Order Diptera. The sigma virus (SV), an RVae found in the Drosophilidae encodes the nucleocapsid (N) protein, essential for viral replication and transcription, that is more similar to those of insect-associated than to those of non-insect-associated RVae but its relationship to those of the BVae is unknown. The family Drosophilidae is more closely related to Psychodidae than to Culicidae, Aphididae, and Fulgoridae. Since SV and all BVae encode the N protein and SV and most BVae members (BVaes) occur in dipterans, we hypothesized that the SV N protein is phylogenetically more related to those of dipteran-associated BVaes than to those of non-dipteran BVaes. Furthermore, the N proteins of SV and of those BVaes from dipteran families that are phylogenetically closest to the Drosophilidae are themselves, most closely related. We determined the percent similarity and the phylogenetic relationship among the N protein sequences of SV, RVae, and BVae. ClustalW alignment demonstrated that the N protein of SV is more similar to those of dipteran-associated RVae and BVae than to those of other RVae and BVae. Phylogenetic analysis using parsimony (PAUP) revealed that viruses with N proteins that are most closely related are associated with the same insect family. The N protein of SV is phylogenetically most related to those of BVaes associated with Psychodidae (sandflies). Within the family BVae, members with N proteins that are most closely related are associated with Culicidae (mosquitoes). Similarly, within the family RVae, most closely related members are associated with the same family of insects. The insects could be Culicidae (mosquitoes and culicoides midges), Aphididae (aphids) or Fulgoridae (planthoppers). The N proteins of SV (with its host in the Drosophilidae) and BVaes (with hosts in the Psycodidae) are more closely related than they are to members of their respective families, which are found in distantly related dipteran host families. This, therefore, supports the hypothesis that the insect host/vector probably, influenced the evolutionary convergence of the N proteins of SV and BVaes to a greater extent than did the virus families themselves.

The development of genetic transformation marker systems for transformation of housefly and honeybee using tryptophan oxygenase gene

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The orthologs of *Drosophila melanogaster vermilion*, a mutant of tryptophan oxygenase (*TO*), are known to exist in many insect species. Housefly *ge* and honeybee *snow* are the eye color mutants of *TO*, and they are considered as good candidates for transformation markers. To develop transformation selection systems using these eye color mutations using mutant rescue for housefly and honeybees, we performed a series of preliminary experiments. First, the *TO* gene from *Anopheles gambiae* was introduced into the green eye color mutant (*ge*) of the housefly. *TO* was transiently expressed in the housefly, and several variations in eye color were observed in 40% of surviving G_0 adults. Based on this result, a *mariner* transformation vector with this mosquito *TO* gene was constructed and introduced into *ge*. Two out of 5 G_0 survivors produced offspring with wild type eyes. In later generations, the eye color phenotypes were inherited in a Mendelian fashion. These results demonstrated that *TO* can serve as a transformation marker in the housefly. Using a degenerated PCR primers design based on the mosquito *TO* gene sequence, DNAs were amplified with housefly and honeybee genomic DNAs. These DNAs were confirmed as a part of *TO* genes of these insects.

Ecdysteroids regulate expression of the homophilic adhesion molecule *Manduca* Fasciclin II during metamorphosis of the moth *Manduca sexta*.

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To investigate mechanisms underlying neuronal remodeling during metamorphosis, we have focused on an identifiable leg motoneuron (MN), the femoral depressor MN (FeDeMN), that undergoes dramatic morphological changes in response to fluctuating ecdysteroid titers. Changes in expression of the trans-membrane (TM) isoform of the homophilic adhesion molecule *Manduca* Fasciclin II (MFasII) by the FeDeMN also correspond to fluctuations in hormone titers. To test the hypothesis that rising ecdysteroid titers during prepupal stages underlie upregulation of TM-MFasII, we performed hormonal and surgical manipulations during the last larval stage and, subsequently, used confocal microscopy to detect anti-TM-MFasII-immunoreactivity in the FeDeMN soma. Manipulations that eliminated the source of ecdy-steroids at different times suggest that low levels of prepupal ecdysteroids are sufficient to upregulate TM-MFasII. Transection of the leg nerve also led to enhanced expression of TM-MFasII in the MN, suggesting that peripheral injury or loss of contact with target muscle also upregulates TM-MFasII. However, manipulations that interfered with central but not peripheral ecdysteroid actions indicate that changes in MN-muscle interactions associated with muscle degeneration are not sufficient to upregulate TM-MFasII. These results suggest that TM-MFas II expression by the FeDeMN may be increased by two mechanisms: central ecdysteroid action and nerve injury. Thus, upregulation of TM-MFasII might be a common mechanism by which ecdysteroids or injury influence postembryonic neuronal growth.

Genetic engineering of the blood meal activated anti-pathogen immunity in the yellow fever mosquito, *Aedes aegypti*

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In anautogenous mosquitoes, a blood meal is required for activation of genes encoding yolk protein precursors (YPP) by the fat body. Vitellogenin (Vg), the major YPP gene, is transcribed at a very high level following blood meal activation. Regulatory regions of Vg gene can be used to express anti-pathogen effector molecules in engineered vectors in a precise temporal and spatial manner, which would be secreted into the hemolymph and could affect a pathogen. To accomplish this goal, we established an efficient transformation system of *Aedes aegypti* utilizing the *piggyBac* transposable vector pBac [3xP3-Egfp afm], which allows utilization of the wild type mosquito strain. We have engineered several stable transformant lines of *Aedes aegypti* mosquito, in which the regulatory region of Vg gene activates the high level fat body-specific expression of potent anti-bacterial factors, defensin. We report here creation of similar transgenic mosquitoes expressing cecropin in response to a blood meal. In addition, we are exploring mosquito IMD-mediated regulatory cascade for designing the blood activation of systemic immunity, which would result in simultaneous production of multiple immune factors to test their synergistic effect.

Morphogenesis of a symbiotic entomopoxvirus from a parasitic wasp and the phylogenetic relationship of selected viral orfs to those of other poxviruses.

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An entomopoxvirus (EPV) occurs in the poison gland apparatus of female *Diachasmimorpha longicaudata* (Dl), a parasitic wasp of tephritid fruit fly larvae (host). DlEPV has a unipartite DNA genome of ~300 kb and its replication and assembly occur in the accessory (poison) gland filaments of the female wasp. The mature virions are stored in the muscular poison gland reservoir from which they are released into the median oviduct. During parasitism, the female wasp injects the virus along with her egg into the host's hemolymph from which the virus enters the hemocytes. The objectives of this study were to (1) document the morphogenesis of DlEPV within host hemocytes, (2) analyze the nucleotide and derived protein sequences of selected open reading frames of clones from DlEPV EcoRI and EcoRV genomic libraries, and (3) propose an appropriate category for placement of this novel virus within the Subfamily Entomopoxvirinae based on sequence comparisons with known insect poxvirus (Entomopoxvirinae) homologs. Transmission electron microscopy revealed that like other Poxviridae, DlEPV has a cytoplasmic site of assembly. The virions appear within 48 h of parasitism and the virogenic stroma occupies approx. 25-30% of the cytoplasm. Spherical particles characteristic of immature

poxviruses, have a central electron dense nucleoid and lack lateral bodies. Within 72 h post-parasitism, >50% of host hemocytes are infected and virions, each with an irregularly shaped central core, bud into the host's hemolymph where they differentiate into the mature extracellular enveloped virus (EEV). Virus morphogenesis is not synchronized and by 88 h post-infection, virus particles at different stages of maturation and with various nucleoid morphologies occur in the hemolymph. By 96-100 h post-infection, many particles in the hemolymph seem to have biconcave or irregular cores while virions of intracellular particles have only spherical nucleoids. Blastp and ClustalW alignment of partial open reading frames of a DlEPV putative NAD^+-dependent DNA ligase and DNA-dependent RNA polymerase with those of *Melanoplus sanguinipes* EPV (MsEPV), yielded respective homologies of 72 and 78% to MsEPV. The RNA polymerase had ~65% and 70% homology respectively to those of *Amsacta moorei* EPV (AmEPV) and vaccinia virus (VV, a vertebrate poxvirus). While 25-35% of the AmEPV and MsEPV gene sequences are conserved within the DlEPV homologs, indicating a high level of relatedness, the majority of the sequences are not identical and indicate that DlEPV is different from these EPVs. Furthermore DlEPV, unlike AmEPV and MsEPV, does not express spheroidin, the occlusion body protein. Thus, the unusual non-pathogenicity of DlEPV in the wasp, but pathogenicity in the fly host, along with the absence of expressed occlusion bodies, justify the placement of this virus in a new EPV Group D or in a subcategory of the dipteran (Group C) EPVs.

Molecular Mechanisms of Signal Perception and Inactivation in Pheromone-Oriented Navigation in Insects

Leal WS

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The remarkable fidelity of the insect olfactory system is unrivalled in nature, particularly its temporal precision during odour-oriented flights. While navigating *en route* to a pheromone source, a moth encounters intermittent chemical signals with the stimulus present in broken bursts and, consequently, the animal has to reset its detector in a millisecond timescale. Research in my lab is aimed at elucidating the molecular basis of pheromone perception and inactivation in insects. Recent studies unveiled some features of pheromone-binding proteins, which solubilize ligands and ferry them to their receptors. Upon interaction with negatively charged membranes, the C-terminal sequence, which is an unstructured conformation in the pheromone-PBP complex, rearranges to form a regular α-helix that occupies the pheromone-binding pocket, thus "ejecting" the pheromone to the receptor. On the other hand, the molecular basis of signal inactivation is still *terra incognita*. We are now testing the hypothesis that a fast step is achieved by rapid degradation of ligands by odorant-degrading enzymes.

Analysis of alternatively spliced *Manduca sexta* allatotropin mRNAs predicts the synthesis of additional bioactive peptides in a tissue and developmentally regulated manner

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The *Manduca sexta* allatotropin (Manse AT) gene is expressed as three alternatively spliced mRNAs predicted to encode distinct precursor proteins. Manse AT can be derived from each precursor, but two of the mRNAs can also encode a total of three additional allatotropin like peptides (Manse ATL). The biological activities of Manse ATL peptides overlap with those of Manse AT. One Manse ATL stimulates JH biosynthesis by adult female corpora allata in vitro, and each Manse ATL inhibits active ion transport across the larval midgut epithelium in vitro. Northern blot analysis using exon specific probes show that alternative splicing is regulated in a tissue specific and developmentally regulated manner. The level of one of these mRNAs is dramatically increased in the terminal abdominal ganglion in response to starvation, parasitism and the nonsteroidal ecdysteroid agonist RH 5992. Thus, alternative splicing might provide a mechanism to increase the potency of the overall physiological response mediated by the expression of a neuropeptide gene, and the increased levels of a specific Manse AT mRNA may be part of the complex response of larvae to nutrient deprivation. This work was supported by NSF IBN 9807907 to F.M.H and NSF IBN 9407313 to M.E.C.

Novel transmitters from immunoreactive octopaminergic neurons.

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Octopamine (OA) has become one of the most widely studied neuromediators in the arthropod CNS and aspects of its synthesis, release, action, and degradation are well known. Our recent studies however suggest that novel neurotransmitters may be contained in immunoreactive-OA (ir-OA) cells. We have focused on the molecular identification and expression of tyramine ß-hydroxylase (TßH), an enzyme required for OA synthesis. Degenerate PCR and 5' RACE were used to clone and sequence a 1.2 kb fragment that has highest similarity to *Drosophila melanogaster* TßH. RNA probes were synthesized and *in situ* hybridization methods were used to visualize TßH mRNA. We have observed in all abdominal ganglia of the fifth larval instar a pair of large midline neurons (50-60 µm) and a much smaller pair of midline cells (ca. 10-15 µm). Abdominal ganglia from pharate adults contained four midline cells that were closer in size (30-60 µm). Identical ir-OA neurons and their development have been previously described in *M. sexta* (Lehman et al., 2000 *J. Comp. Neurol*. 424:283-296). In contrast, ir-OA lateral neurons in *M. sexta* do not appear to contain TßH mRNA. We suggest that lateral ir-OA neurons do not contain OA, but contain tyramine (OA precursor) as a neurotransmitter. In addition, ir-OA and TßH mRNA containing midline neurons contain nitric oxide synthase (NOS), suggesting they produce nitric oxide (NO) (Zayas et al., 2000 *J. Comp. Neurol*. 419:422-438). Based on the ability of NO to modify specific amino acids, we have hypothesized that NO modifies OA.

We have treated acidified OA solutions with either NO or sodium nitrite and detected products with HPLC/EC detection. Within seconds, a single HPLC peak appears in treated samples. The appearance of this compound is dependent on the concentration of NO, time, and pH, and has a novel redox potential. This molecule appears to be stable for ca. 8-10 hours - NMR and MS/MS methods are being used to solve its structure. We suggest NO nitrates OA to form a putative neurotransmitter.

Role of a complement-like protein in the mosquito immune responses

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Mosquitoes, like other insects, rely on a combination of humoral and cellular responses to fight microbial infection. Humoral responses, mostly the expression of antimicrobial peptides, have been extensively explored. In contrast, molecular mechanisms of cellular responses, which include melanotic encapsulation and phagocytosis, remain less well understood. We have identified a molecule, aTEP-I, which provides a link between humoral and cellular immune responses in the malaria vector, *Anopheles gambiae*. It is a member of the thioester-containing protein family, which shows significant sequence and structural similarities to both the vertebrate complement factor C3 and to the universal protease inhibitor, α_2-macroglobulin. This hemocyte-specific acute phase glycoprotein is proteolytically processed in the mosquito hemolymph to an active form after septic injury. Although aTEP-I binds to both Gram-negative and Gram-positive bacteria in a thioester-depedent manner, it promotes phagocytosis only of Gram-negative bacteria. Of particular interest is the role of aTEP-I in parasite-mosquito interactions. The functional analysis of this protein during *Plasmodium berghei* infection will be discussed.

A plasmatocyte transmembrane protein of *Manduca sexta* involved in encapsulation is a member of the beta-integrin family

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Encapsulation is the immune response in which insect hemocytes attach to a large metazoan parasite, thus forming a cellular capsule around the foreign invader and rendering it inactive. In the moth *Manduca sexta*, the two hemocyte types involved in encapsulation are plasmatocytes and granulocytes. Monoclonal antibodies to *M. sexta* hemocytes were developed. Monoclonal antibody MS34 specific to plasmatocytes was found to inhibit encapsulation. Immunoaffinity purification with MS34 isolated a protein approximately 100 kDa in size. Edman degradation of trypsin fragments of this protein revealed short peptide sequences that were used to construct degenerate primers. RT-PCR with hemocyte mRNA produced an 803 bp DNA product. This DNA sequence encodes a protein highly similar to the beta-subunit of integrins which are transmembrane proteins mediating cell adhesion. These results suggest that an integrin expressed on the surfaces of plasmatocytes is involved in hemocyte adhesion during encapsulation.

Luteovirus-binding proteins associated with aphid transmission specificity.

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Aphid proteins specifically binding barley yellow dwarf luteovirus (BYDV) and potentially functioning in vector-specific transmission were identified using virus-overlay assays on blots containing proteins extracted from aphid heads and separated by 2-dimensional urea SDS-PAGE. Three proteins (MW/pI = 35/4.35, 49/4.56, 50/ 4.51) from the efficient vector, *Sitobion avenae*, reacted with BYDV-MAV and PAV; and a forth protein (50/ 4.41) reacted only to PAV. Three similar proteins (35/4.35, 50/4.51, 50/4.41) were identified in *Metopolophium dirhodum* (transmitting MAV, not PAV). Two proteins (33/4.26, 49/4.56) from *Rhopalosiphum padi* (transmitting PAV, not MAV) also bound MAV and PAV. No proteins from the non-vector, *R. maidis*, bound to either of these viruses. These proteins also were identified using antiidiotypic MAV-4 antibody that mimics MAV capsid epitopes. The presence of specific proteins corresponded to the ability to vector MAV or PAV. Results suggest that several aphid proteins are involved in movement of luteoviruses through vector tissues.

Regulation of juvenile hormone levels in mosquitoes

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Juvenile hormone (JH) titers must be modulated to permit the normal progress of development and reproduction in insects. In adult female *Aedes aegypti*, JH levels are elevated before feeding and low after a blood meal. JH titer is essentially determined by the rate at which the corpora allata (CA) synthesizes JH. Using an *in vitro* radiochemical assay the synthesis of juvenile hormone by the isolated corpora allata of *Ae. aegypti* adult female was studied. There is a dose-response relationship between the substrate concentration ([*methyl*- ^{3}H] methionine) and JH synthesis. The glands dissected from unfed females synthesized about 20-30 fmol/gland of JH III per hour. Corpora allata activity is significantly reduced in glands dissected 16 hours after feeding a protein meal. Our hypothesis is that the mosquito can detect "changes" after feeding and regulates the JH levels by affecting the activity of the corpora allata. This regulation might be mediated directly or indirectly by allatoregulatory peptides synthesized by the midgut, brain, ovaries

or other tissues. Midgut extracts from fed or unfed mosquitoes showed the presence of allatotropic activity. Midguts were homogenized in Bennett's mixture, and extracts were purified on a C-18-Sep-pak. Partially pre-purified midgut extracts showed a dose response stimulation of JH III synthesis. Allatotropins (AT) are peptides that stimulate the synthesis of JH in the CA. Recently, an allatotropin was isolated from *Ae. aegypti* (Veenstra and Costes, 1999). We assessed the ability of *Ae. aegypti* allatotropin to affect the biosynthesis of juvenile hormone III. Low concentration of peptide (0.1 fmol/µl x 10^{-10} M) showed a significant stimulation of JH biosynthesis ($P < 0.005$).

Isolation of a cDNA encoding a cathepsin-l like enzyme specific of midgut in *Rhodnius prolixus*

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Rhodnius prolixus is a triatomine that is the main vector of Chagas' disease in Central America and the Andean region. *Trypanosoma cruzi*, the etiological agent of Chagas' disease, interacts within the insect intestine with host enzymes and digestion products than modulate parasite development and infectivity. Identification of molecules related with parasite environment at the moment of its development and replication will contribute to the understanding of the vector-parasite relationship and this knowledge could also provide tools to design new methods for disease control. In triatomines various enzymes have been described that are related to blood digestion, mainly acid proteases cathepsin B and D. Since in *R. prolixus* no protein or DNA sequences of enzymes of these insects have been identified, the main objective of this study was to study one gene of a digestive enzyme of the insect midgut. The methodology included the generation of a cathepsin fragment (RpCat500) by RT-PCR from intestine RNA, with primers corresponding to the active site of cysteine proteases. After sequencing of RpCAT500 both ends of the cDNA were generated by RACE 5' y 3' strategies and of all these fragments were sequenced. As a result, a 1098-bp sequence (RpCAT) was obtained and analyzed. A peptide sequence was deduced with a 100-amino acid pro-peptide and an ERFNIN motif, diagnostic of a cathepsin L. A signal-peptide sequence was absent at N-terminus of the product but at the C-terminus, an exact sequence of 9 amino acids needed for secretion in other systems, is present. Sequence comparison grouped clearly RpCAT in L-cathepsin family. RpCat expression was specific of intestine and it was present in all insect stages except 5th instar, suggesting a hormonal regulation.

Isolation and characterization of the gene encoding lipophorin from the mosquito, *Anopheles gambiae.*

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Anopheles gambiae lipophorin was purified by KBr density gradient ultracentrifugation and shown to be composed of two subunits, Apolipophorin I (Mr 220,000) and Apolipophorin H (Mr 70,000). Endoproteinase Lys C digestion of purified lipophorin subunits was used to generate discrete peptide fragments for amino acid sequencing. A number of the peptides had primary sequences similar to those of other known lipophorins and these were used as basis for designing degenerate oligonucleotide primers for cDNA amplification by RT PCR. A 400 bp partial cDNA fragment was amplified and subsequently cloned and sequenced. The primary nucleotide and deduced amino acid sequences show a high percentage of identity and similarity to the *Aedes aegypti* lipophorin. This same cDNA was used as a probe to study the expression pattern of the *An. gambiae* lipophorin gene. Dot blot hybridization analyses showed expression of the gene during larval, pupal and adult stages. Genomic Southern blot analysis indicates that this is a single copy gene. A genomic clone has been isolated and sequenced partially to define putative cis acting promoter elements. This work was supported by a grant from the Burroughs Wellcome Fund.

Detection of the expressed sequence region on the chromosome of *Bombyx mori* by fluorescent in situ hybridization.

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The chromosomes in Lepidopteran insects are holocentric and difficult to distinguish from one another because large they are present in large numbers, are small and have and similar shapes. In *Bombyx mori*, many expressed sequences were cloned and genetically mapped. Based on two cDNA clones, 3L(*BmAntennapedia*) and m208, we otained four (GF3L) and three (GF208) kinds of genomic fragments (average size = 14Kb) respectively. These genomic fragments were labeled with biotin or digoxigenin using nick translation, and used as probes for FISH analysis to the chromosomal preparation from the testis on day 1 of the 5th instar larva. The two spot signals of GF208 were visible on the distal region of a chromosome. The specific signals of GF3L were also simultaneously detected on the 30-40% site apart from telomere signals by two-color FISH analysis. These signals were consistent with the RFLP maps by these cDNA clones.

Isolation, characterization, sequencing, cloning, and molecular modeling of JH diol kinase, a new enzyme of juvenile hormone metabolism.

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In 1993 we found that JH I diol phosphate (JHDP) is the major end product of metabolism of JH I *in vivo* in *Manduca sexta*. We now have isolated JH diol kinase (JHDK), the enzyme that catalyzes the formation of JHDP from JH diol, from Malpighian tubules of *M. sexta*. We purified it 1.5 million-fold to homogeneity, determined its enzymatic properties, and performed Edman sequence analysis on the intact enzyme and certain tryptic fragments. From protein sequence data, degenerate primers were designed and used to isolate a 489 bp RT-PCR product. This product was labeled and used to screen a cDNA library. The clone selected encodes a 20.0 kDa protein which has 59% sequence identity and >80 sequence similarity to *Drosophila melanogaster* sarcoplasmic calcium-binding protein (dSCP2). Characterization and partial purification of the *D. melanogaster* homologue of *M. sexta* JHDK from adult *D. melanogaster* gave material with JH diol kinase activity. This activity has an experimental pI and M_r that is nearly identical to that of dSCP2. Homology modeling and substrate docking of JHDK and dSCP2 has shown that three conserved nucleotide binding elements surround the putative substrate binding site and align with conserved sequence elements of Ras p21 and adenylate kinase. *D. melanogaster* dSCP2 is a homologue of *M. sexta* JHDK. These proteins constitute a novel kinase family that uses the scaffold of a sarcoplasmic calcium binding protein (2SCP).

Midgut gene expression in the potato tuber moth, *Phthoramiaea operculella*

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The potato tuber moth (PTM), *Phthorimaea operculella*, is widely distributed and causes serious damage to potatoes in storage, as well as plants in the field. The larval stages mine the foliage and infest the tubers. The number of PTM generations per year ranges from 2-12, depending on the environmental conditions. PTM is the most important potato pest in the Middle East, North and Central Africa, Mexico and South America. Chemical insecticides are the most common control strategy used to protect the potato crop. In an attempt to reduce dependence on chemical insecticides, alternative control strategies have been successfully used to help potato producers lower their insecticide usage. Insect-tolerant potato strains were produced by genetic breeding programs. Biopesticides such as *Bacillus thuringiensis* (Bt) and granulosis virus (GV) have been used in different regions of the world to control PTM, The PTM sex pheromone has also been used as a tool for controlling PTM populations, under field and storage conditions, reducing the amount of insecticide usage per season. Most of the research previously performed on PTM has focussed on the evaluation of these control strategies. Only a limited number of studies have concentrated on biological aspects of PTM to gain greater understanding of the insect's biology and to develop alternative control strategies. The insect midgut provides a rich target tissue for the identification of molecular targets that could be used to design a new control strategy. The main goal of this research is the identification of genes that are differentially expressed within the PTM midgut. The suppression subtractive hybridization (SSH) method was used to amplify differentially expressed cDNA fragments and simultaneously suppress highly abundant messages. The midgut cDNA population is subtracted from the carcass (except midgut) cDNA population through two rounds of hybridization. Results from these SSH procedures on the PTM midgut will be presented.

Molecular characterization of the bacterial symbionts of the glassy-winged sharpshooter and related insects

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Most sap-feeding insects possess maternally transmitted bacteria that are contained within specialized structures called bacteriomes. Here we present the first molecular characterization of symbionts of the largest group of sap-feeding insects, the leafhoppers (Insecta: Hemiptera: Cicadellidae), with focus on the sharpshooters (subfamily Cicadellinae). Based on 16S rDNA sequences, these symbionts form a well-defined clade within the γ-3 Proteobacteria. Our data and phylogenies for five symbionts and their host species are consistent with an ancient infection and strict vertical transmission. More extensive gene sequence information is reported for the symbiont of *Homalodisca coagulata*, which is a major vector of bacterial diseases of cultivated plants. The genome size, as determined by pulsed field gel electrophoresis, is approximately 670 kb, indicating that this symbiont has undergone massive genome reduction as is typical of other obligate insect symbionts. The sharpshooter bacteriome associates were given a new genus and species name, *Candidatus Baumannia cicadellinicola* (*sp. nov*.). with the symbiont of *H. coagulata* designated as the type strain.

Blocking transmission of a malaria parasite by anopheline mosquitoes: From transgenesis to fitness

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Interference with the mosquito vectorial capacity is an important strategy for malaria control that needs to be explored. One approach would be to introduce into the mosquito a gene whose product is harmful to the parasite but innocuous to the mosquito. Previously we demonstrated that the *Anopheles gambiae* carboxypeptidase (AgCP) promoter is strongly and rapidly induced in the midgut of transgenic mosquitoes upon blood ingestion (PNAS 97, 10895, 2000). To generate mosquitoes refractory to the parasites, the AgCP

promoter and its signal peptide were linked to the coding sequence of the bee venom phospholipase A2 PLA2) or of the SM1 tetramer, and transformed into *An. stephensi* using a *piggyBac* vector. SM1 is a dodecapeptide that binds to the *Anopheles* midgut epithelium and salivary gland (PNAS 98. 13278, 2001). Both PLA2 and SM1 inhibit ookinete midgut penetration and oocyst formation. Northern blot analysis indicated that both genes were abundantly expressed in the guts of transgenic mosquitoes. Expression was gut-specific and reached peak levels at 4 h after a blood meal, consistent with the temporal profile of AgCP expression. Immunofluorescence detected the recombinant proteins in midgut sheets of transgenic mosquitoes. *Plasmodium berghei* oocyst formation was inhibited by 68~99% in transgenic mosquitoes. Furthermore, transmission of the parasite from transgenic mosquitoes to naïve mice was dramatically reduced. Experiments in progress are measuring the fitness of the transgenic mosquitoes. Initial results suggest that the SM1 transgenic mosquitoes are as fit as wild type, as measured by longevity and egg production. Conversely, the PLA2 transgenics appear to have reduced fitness. These results are important for future strategies for malaria control.

Development of an AFLP-based genetic map for cotton bollworm *Helicoverpa armigera*, with ribosomal protein genes as anchor loci for comparative linkage mapping.

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We describe a linkage map consisting of 260 AFLP markers covering all 31 chromosomes of *Helicoverpa armigera*. Because AFLPs cannot readily be transferred to other species, we are adding anchor loci that can. Ribosomal protein (RP) genes are useful for comparative purposes, as they are generally single-copy, highly conserved, and randomly dispersed throughout the genome. We describe our approaches to map these using RFLP analysis and denaturing HPLC, and present linkage results for approximately 20 RP genes. Molecular and functional analysis of a differentially expressed IgE-dependent histamine release factor isolated from *Rickettsiae*-infected *Dermacentor variabilis* ovaries.

Is the cadherin gene associated with Bt resistance in pink bollworm?

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Transgenic crops expressing insecticidal toxins from *Bacillus thuringiensis* (Bt) are widely used for pest control. Although laboratory selection results show that many pests have the genetic potential to evolve resistance to Bt toxins, the molecular basis of resistance had remained elusive for many years. Recently, resistance to CrylAc in the cotton pest *Heliothis virescens* was associated with disruption of a cadherin superfamily gene. Here, we show the cadherin gene to be tightly linked to a high level of resistance to Cry lAc in a field derived strain of the pink bollworm *(Pectinophora gossypiella).* Resistance to CrylAc in the pink bollworm is recessive at high concentrations. We generated informative linkage families by crossing susceptible and resistant pink bollworm strains and backcrossing the F 1 generation to the resistant strain. The backcross progeny were exposed to a discriminating concentration of Cry lAc (causing a significant reduction in growth rate in hetrozygotes) and genotyped using polymorphic intron DNA markers in the cadherin gene. We found a perfect correlation between the DNA markers and the weight class of the insects after 11 days. All light individuals had DNA markers similar to the susceptible strain while all heavy individuals showed the marker patterns of the resistant strain. To our surprise, three different intron marker patterns were identified in the resistant strain. This polymorphism was found to be related to different field collections used to establish the Arizona pooled resistant strain (AZP R) in 1997. Evidence showing that mutations in the cadherin gene cause Cry lAc resistance in two species suggests that this mechanism may be important in other Lepidoptera as well. The molecular knowledge will be used to develop an efficient DNAbased screening method for detecting early phases of Bt resistance in the field.

Structure-function studies of the nitric oxide-insensitive soluble guanylyl cyclase MsGC-β3

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Soluble guanylyl cyclases (GCs) from a wide variety of species are known to be obligate heterodimers (comprising an α and a β subunit) that are stimulated by nitric oxide (NO). We have identified a novel soluble GC in *Manduca sexta*, named MsGC-β3, that does not need the presence of additional subunits for activity and is insensitive to NO (Nighorn et al., 1999; *J. Biol. Chem.* 274:2525-2531). We have begun a series of studies that are aimed at understanding the structural basis for these unusual properties. One notable structural feature of MsGC β3 is the presence of a C-terminal 338 residue domain that is not present in any other known GC. Removing this domain to generate the deletion mutant MsGC-β3ΔC338 yields a GC that is also active in the absence of additional subunits and is also NO-insensitive. A more detailed analysis of the enzyme kinetics revealed that in the presence of magnesium while both MsGC-β3 and MsGC-β3ΔC338 had similar values of V_{max}, MsGC β3ΔC338 had a significantly lower K_m for GTP; 0.4 mM compared to 2.6 mM for MsGC-β3. Models of the catalytic domain of GCs predict that all GCs need to form dimers for activity. Gel filtration experiments confirm that MsGC β3 forms homodimers both in vivo and when

expressed in heterologous cells. Using N-terminal tagged versions of MsGC-β3 and the two NO-sensitive subunits from *Manduca*, MsGC-α1 and MsGC-β1, reveal that MsGC-β3 is capable of forming heterodimers with both of these subunits. Pull-downs and site-directed mutagenesis studies further suggest that both of the heterodimers are inactive. This finding is surprising as models of the catalytic domain suggest that both heterodimers should contain all the residues necessary to bind GTP and catalyze the formation of cGMP. Nevertheless, consistent with the observed formation of inactive heterodimers, co-expression of all three subunits in COS-7 cells reveals that MsGC-β3 can act in a dominant negative manner, significantly reducing the level of NO stimulation of GC activity.

Insecticide-metabolizing cytochrome P450 (CYP6A1) system from the house fly *Musca domestica*: Coupling stoichiometries and protein-protein interactions.

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CYP6A1, which is overproduced in insecticide-resistant strains, cytochrome P450 reductase, and cytochrome b_5 have been cloned from the house fly, *Musca domestica*, and expressed in *Escherichia coli*. The interactions between these proteins and the stoichiometry of CYP6A1 catalysis with two different substrates, heptachlor and testosterone, were analyzed in the reconstituted systems with either P450 reductase or CYP6A1 present in excess, with or without holo- and apo-b_5 present. CYP6A1 activity is determined by a steady-state concentration of the P450 reductase-CYP6A1 complex, and the same concentration of the complex can be achieved in the presence of an excess of either P450 reductase or CYP6A1. Holo-b_5 stimulated heptachlor epoxidase and testosterone hydroxylase activities of CYP6A1 2.5-3 fold and 8-10 fold, respectively. Apo-b_5 also stimulated CYP6A1 activity but to a smaller extent than holo-b_5, suggesting that cytochrome b_5 has a dual effect on CYP6A1 function: the first is electron transfer to the P450, while the second is related to conformational interactions of the proteins. Coupling stoichiometries for heptachlor and testosterone oxidation were determined at [CYP6A1]=[P450 reductase], [CYP6A1]<[P450 reductase], and [CYP6A1]>[P450 reductase] with and without holo- or apo-b_5 present. Acceleration of CYP6A1-catalysis by holo- or apo-b_5 was accompanied by more efficient coupling. With both substrates, the slowest rates of NADPH and O_2 consumption, and H_2O_2 formation were observed at [CYP6A1]>[P450 reductase]. With holo-b_5 present and CYP6A1 present in excess, almost all reducing equivalents were utilized for testosterone hydroxylation, while with P450 reductase present in excess, almost 60% was used for H_2O_2 formation. NMR analysis of purified products of testosterone oxidation by CYP6A1 revealed that hydroxylation occurred at the 15β and 2β positions. Hydroxylation at the 15β position occurred 4-5 times faster than that at the 2β position. Another minor monohydroxylated product was identified as 12β hydroxytestosterone. Several dihydroxy products have also been identified. Two of these are 12β,15β–dihydroxy, and 2β,15β–dihydroxy derivatives.

Temporal expression of genes related to cell division of *Buchnera* in winged and wingless forms of the pea aphid, *Acyrthosiphon pisum*

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Parthenogenetic aphids exhibit two morphs, apterae or wingless forms and alatae or winged forms. Although both morphs harbor an obligatory endocellular symbiotic bacterium, *Buchnera*, in bacteriocytes, only alatae are able to recover the biomass of *Buchnera* after they decrease it. We suspect that this difference between the two morphs may give us clues to understand the mechanism of host aphids to control proliferation of *Buchnera*. As a first step to study the basics of cell division of *Buchnera*, we compared the two morphs with respect to the expression of thirteen genes of *Buchnera* that may be involved in cell division, using real-time quantitative RT-PCR technique. As a result, *Buchnera* in the two morphs showed significant differences in temporal expression of these genes.

Methyl-branched alkanes on the surface of male and female adults, and eggs of the Colorado potato beetle, *Leptinotarsa decemlineata*.

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The Colorado potato beetle, *Leptinotarsa decemlineata*, is the major defoliator of potato worldwide. It can also be a major pest of tomatoes and egg plant and can quickly develop resistance to insecticides. Host plant resistance is one method of potential control and a novel mechanism of resistance has been identified (Balbyshev and Lorenzen, 1997. Journal of Economic Entomology 90: 652-657). The leaves of a *Solanum* spp. hybrid are hypersensitive to the egg mass resulting in formation and subsequent detachment of a necrotic zone containing the egg mass, which falls to the ground, exposing the eggs and subsequent nymphs to ground dwelling predators. Material causing the necrosis was associated with the egg mass and remains unknown. The hydrocarbons of the eggs resembled those of the adults both qualitatively and in methyl-branch positions. Hydrocarbons are the major component of the cuticular lipids of adult males and females and show little sexual dimorphism in either the chromatographic profiles or in the structures of the methyl-branched components. Straight-chain alkanes are essentially absent from all stages but also are difficult to detect because unique methyl branching causes branched alkanes to elute with similar retention times to those of *n*alkanes. The beetle is unique from other

arthropods studied in that it has a preponderance of methylalkanes (mono-, di- and trimethyl-branched) with the first methyl branch on the second carbon. Each stage had two major components: one was a mixture of 2,10,16- and 2,10,18-trimethyltetratriacontanes (10-11%) males, females and eggs; the other was 2-methyltriacontane (11-12%) in males and females, but in the eggs was 2,6-dimethyltriacontane (11%). Tetramethylalkanes were trace components and one was identified, 13,17,21,25-tetramethylheptatriacontane. The major hydrocarbon class was the 2,x-dimethylalkanes, 30 and 40%, in females and eggs, respectively, while males had 23%. The major hydrocarbon class in males was the internally branched dimethylalkanes, 26%.

Genomics and proteomics of adult antennae and larval midgut of the tortricid moth, *Epiphyas postvittana*

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Genomics and proteomics approaches are being used to identify proteins expressed in adult antennae and larval midgut tissues of the leafroller moth, *Epiphyas postvittana* (Tortricidae, Lepidoptera). The goals are to identify genes involved in pheromone and odorant reception and signal transduction, and novel targets for insect control from the larval midgut. To date 4540 EST sequences have been collected including some from subtracted libraries. Construction of contigs reduces this number to 824 non-redundant sequences. A proteomics approach is also being undertaken in these tissues. 2D gel electrophoresis resolves over 200 spots from antennae. Gel comparison software shows differences in the relative amounts of several proteins when comparing proteins from male and female antennae. MALDI-TOF and electrospray MS-MS analysis of resolved spots is being used to identify proteins and relate them to proteins predicted from the EST sequence data. Comparisons to EST/protein datasets from similar tissues from *Bombyx mori* and *Manduca sexta* will be presented. Using this analysis we argue that genomic studies of the tortricid moth, *E. postvittana* will make a useful comparative model to identify important genes/proteins in the Lepidoptera and determine their function.

Guanylyl cyclase activating proteins are expressed in the olfactory system of *Manduca sexta*.

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Regulation of cGMP levels is important for long-term adaptation of olfactory receptor neurons. We are studying the regulation of cGMP in the olfactory system of the moth *Manduca sexta*. We previously cloned and characterized a novel guanylyl cyclase (MsGC-I), which is strongly expressed in the cell bodies and dendrites of olfactory receptor neurons. MsGC-I has a catalytic domain with sequence similarity to receptor guanylyl cyclases (rGCs), but contains no ligand-binding, transmembrane, or kinase-like domains. Guanylyl cyclase activating proteins (GCAPs) are small, calcium-binding proteins which regulate the activity of rGCs. To investigate whether the activity of MsGC-I could be regulated by GCAPs, we used degenerate oligonucleotide RT-PCR to identify fragments of two GCAP-like molecules from antennal cDNA. One fragment (MsGCAP-I) has 69% sequence identity with a neuronal calcium sensor protein (NCS-2) cloned from *Caenorhabditis elegans*, and the other fragment (MsGCAP-II) has 98% sequence identity with *Drosophila melanogaster* frequenin. The presence of GCAP-like molecules in the olfactory system suggests they might regulate the activity of MsGC-I or other rGCs in *Manduca sexta*.
Supported by National Institutes of Health grant DC04292.

Isolation and characterization of farnesyl diphosphate synthase from Coleoptera

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Farnesyl diphosphate synthase (FPPS) catalyzes the consecutive condensation of two molecules of isopentenyl diphosphate synthase with dimethylallyl diphosphate to form farnesyl diphosphate (FPP). In insects FPP is used for the synthesis of ubiquinones, dolicols, protein prenyl groups and juvenile hormone. Using degenerate primers designed from the conserved domains of FPPSs from other organisms, full-length cDNAs of FPPS were isolated from a male cotton boll weevil, *Anthonomus grandis* and a male Jeffrey pine beetle, *Dendoctonus jeffreyi* by PCR. The deduced amino acid sequences of the two coleopteran FPPSs were 75 % identical with each other and contained highly conserved regions found in other isoprenyl diphosphate synthases. They also had high similarity to two previously isolated insect FPPSs from *Agrotis ipsilon* and *Drosophila melanogaster*. The FPPS from *A. grandis* was further characterized in order to confirm the identity of the protein. Prenyltransferase activity was measured from purified recombinant protein overexpressed in *Escherichia coli*. Product analysis showed that the gene isolated *from A. grandis* encodes FPPS. Southern blot analysis indicated the presence of single copy gene in *A. grandis*. Using molecular modeling, the three-dimensional structure of coleopteran FPPS has also been identified and compared to x-ray crystal structure of avian FPPS.

A comparative study of o'nyong nyong and chikungunya viruses in cell culture and mosquitoes.

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O'nyong nyong (ONN) and chikungunya (CHIK) viruses (family Togaviridae, genus *Alphavirus*) have been shown to be closely related to each other (Powers *et al.*, 2000). ONN has at one point been considered a subtype of CHIK (Lee *et al.*, 1997). The ONN virus is unique with regards to its mosquito vector, being the only known virus transmitted by anopheline mosquitos rather than culicines. The unique vector phenotype displayed by this virus and its close genetic relationship to CHIK has led us to speculate on the molecular determinants of host range. Here we report a detailed comparison of the behavior of these viruses in cell culture and mosquitoes. We have tested three different strains of ONN (Gulu, SG650, and Igbo Ora) and one strain of CHIK (Ross) on their ability to infect *Ae. aegypti* (Rexville D strain) and *An gambiae* (G3 strain) mosquitoes. We have assayed the growth curves of these viruses in vertebrate (BHK and VERO) and invertebrate (C6/36 and Mos-55) cell lines. We have taken our analysis one step further in performing a multiple sequence alignment of the structural proteins in search of amino acid changes that could potentially be related to the phenotypes displayed by these viruses in the experiments described above. We have identified seven amino acid changes that could be related to the ability of some of the viruses to infect *Ae. aegypti* mosquitos, of these the most interesting mutation is a tyrosine to cysteine substitution in the ONN Gulu strain that infects *An. gambiae* mosquitos but not *Ae. aegypti*. The mutation mentioned above has been located to the E1 protein within the protein dimerization domain and vicinity of the fusion domain.

Establishment of protocols to study host-specific glycosylation of dengue-2 envelope glycoproteins.

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Dengue virus (DEN) is a flavivirus that is serologically divided into four different serotypes (1-4). Mosquitoes of the *Aedes* genus transmit this group of viruses in a cycle that alternates between the mosquito and the human host. DEN viruses are enveloped viruses composed of three structural proteins designated capsid (C), membrane (M), and envelope (E). The C protein surrounds a positive-strand RNA genome, while the outer shell of the virus is composed of the M and E glycoproteins attached to a host derived lipid bilayer. Alternating viral replication within cells of both evolutionarily distinct vertebrate and invertebrate host species poses interesting biochemical and evolutionary problems. The enzymes needed for protein synthesis and glycosylation are not coded in the viral genome and as a result the viruses are dependent on the host cell machinery for these processes. Consequently, these viruses will carry lipids and oligosaccharide structures that are characteristic of the host and cell type infected. Interaction of the viral envelope glycoproteins with the host cell surface is of fundamental importance for virus entry. In general, the oligosaccharides attached to proteins are rich in structural information. Their branched nature, with a number of possible monosaccharide types and glycosidic linkages presents a unique face, recognizable by the enzymes and receptors that interact with it. Our interest is to identify and characterize the host-specific N-linked oligosaccharides added *in vivo* to viral glycoproteins, as well as to investigate their role in virus/vector interactions. Here we present the establishment of the protocols required for this study, as well as preliminary characterization of oligosaccharides from Dengue-2 (strain 16681) infected mosquito (C6/36) and vertebrate (VERO) cell lines.

Biochemical characterization of transferrin from the tsetsefly, *Glossina morsitan centralis*

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Iron binding proteins such as transferrin play an important role in heamatophagous insects such as the tsetse fly. Midgut homogenate and heamolymph samples from *Glossing morsitan centratis* were screened for iron binding proteins by staining with Ferene S. SDS PAGE showed the presence of iron binding proteins(Mr 30 and 80 kDa) in the heamolymph. On native PAGE, iron binding proteins were observed at Mr 490 and 140 kDa. Hemolymph samples were subjected to KBr density gradient ultracentrifugation and the subphase used for transferrin isolation. The putative transferrin was electroeluted from a preparative non denaturing PAGE gel. Further purification was carried out by affinity chromatography on concanavalin A Sepharose column to which the protein bound. Homogeneity of the protein was established through SDS PAGE (M, 80 kDa) and two dimensional gel electrophoresis(pI 6.5). Transferrin retained its iron binding capacity with characteristic absorption spectrum under reducing and oxidizing conditions. There was also evidence of bloodmeal induced elevation of the protein titre.

Linkage mapping of a nonsusceptibility gene to densonucleosis virus in the silkworm, *Bombyx mori* by RFLP analysis

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Nonsusceptibility of *Bombyx mori* to densonucleosis virus type 2(DNV 2) is controlled by a recessive nonsusceptibility gene, *rcsd 2* (nonsusceptibility to DNV 2). For linkage analysis and mapping of *nsd 2*, BF, progeny from the crosses (C124 x No. 902) x No. 902 and No.902 x (C124 x No. 902) were analyzed, respectively. The parental strains C124 and No 902 were classified as being highly susceptible and nonsusceptible to DNV 2, respectively. BF, larvae were inoculated with DNV 2 virus at first and third instar stages and DNA was extracted from surviving fifth instar larvae. The DNA

was then analyzed through RFLP using probes specific to the twenty eight linkage groups of *B.mori.* Our results indicated that the *nsd 2* gene was linked to linkage group seventeen since all surviving larvae showed a homozygous profile in their genotype. The other linkage groups showed mixed heterozygous and homozygous profiles indicating independent segregation. Using the cDNA markers within linkage group seventeen, a linkage map of 30.6 cM was constructed with *nsd 2* mapped at 24.5 cM and four closely linked cDNA markers were identified.

RNA silencing of dengue viruses in mosquito cells by transcription of a hairpin RNA derived from the dengue-type 2 premembrane coding region.

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Double-stranded (ds)RNA initiates post-transcriptional gene silencing (PTGS) or RNA interference (RNAi) in a number of different organisms including plants, nematodes and fruit flies. Several groups have now designed eritable, DNA constructs that transcribe inverted repeat RNAs to induce RNAi and silence expression of targeted genes in transgenic plants and animals. We report here the testing of this strategy in mosquito cells to generate resistance to dengue viruses. Cultured *Aedes albopictus* mosquito cells (C6/36) were transformed with a plasmid (pIE1D2290IR) designed to express a hairpin transcript with an inverted repeat derived from the premembrane (prM) coding region of dengue virus type 2 (DEN-2; *Flavivirus*). Clonal cell lines were selected for hygromycin resistance and challenged with DEN-2 (Jamaica 1409). Cell lines were classified as either susceptible or resistant, based on the percentage of cells expressing DEN-2 envelope (E) antigen seven days after challenge. A DEN-2, resistant cell line, FB9.1, was selected for further studied. Southern blot analysis indicated that the cell line had pIE1D2290IR DNA integrated into mosquito cell DNA at multiple sites. Northern blot analysis showed transcription of both sense and antisense polarity as would be expected in cells transcribing the hairpin RNA. The mechanism of interference appeared to be RNA degradation in the cytoplasm of the cell, since DEN-2 virus genomic RNA failed to accumulate after virus challenge. Furthermore, Northern blot analysis of the FB9.1 detected small, DEN-2 specific, 21-23 nt RNAs that are similar to the small interfering RNAs (siRNAs) associated with RNAi. Finally, FB9.1 was highly resistant to other dengue virus serotypes that have >20% sequence divergence in the prM target site. This was unexpected due to the high sequence specificity thought to be required for an RNAi mechanism. However, our studies suggest that resistance may only require short regions of continuous sequence identity to produce resistance to the other DEN serotypes. This type of approach should be a powerful tool for generating virus resistance in transgenic *Aedes aegypti*.

DNA microarray analysis of wing discs in *Bombyx mori* during metamorphosis

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Wing discs of holometabolous insects undergo dramatic morphological changes during metamorphosis, and this process is controlled by actions of hundreds of gene products. Using the cDNA microarrays containing approximately 5000 cDNAs, we monitored gene expression profiles in wing discs of *Bombyx mori* at 13 time points during the larval-pupal transformation (Day-4 fifth instar larvae to Day-0 pupae). More than 4500 genes provided measurable signals. Of these genes, 1025 showed significant perturbation during the larval-pupal transformation. Expression of 180 genes including BHR3, E75, Urbain etc. increased during the earlier stage of the larval-pupal transformation. The functions of the 180 proteins were inferred mainly to be protein biosynthesis, cell cycle, transcription regulation, cell adhesion, protein transport and pre-mRNA splicing, based on the homology to known genes. During the later stage of the transformation, 422 genes were induced. They seemed to play a role in cuticle synthesis, pigmentation, ion transport and so on. This result suggests a programmed sequence of biological processes in the wing disc development during metamorphosis.

The evolution of arthropod body patterning and segmentation

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Genetic studies in model organisms such as *Drosophila melanogaster* have given us remarkable insights into the molecular mechanisms that generate pattern during embryonic development. It is clear, however, that insects, and arthropods in general, display a remarkable level of developmental diversity and there has been increasing interest in understanding how these differences arise. Comparative studies reveal that the overall hierarchy of maternal, gap, pair-rule, and segment polarity gene regulation is probably present throughout the insects, but there is extensive modification to the details of these networks which can help explain some of the obvious differences seen in morphology and responses to experimental manipulations. We have also begun to focus on the early patterning of the amphipod crustacean, *Parhyale hawaiensis*. Here we find a system of embryonic development that appears to be more closely tied to the pattern of cell lineage and division, yet nevertheless utilizes similar molecular components to those found in *D. melanogaster*. We also find that *Parhyale* embryogenesis is amenable to a variety of techniques that can be used to test gene function during development.

Comparison of wild-type and mutant *White Eye* alleles in the melon fly, *Bactrocera Cucurbitae*

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The DNA sequence of the coding region of the wild-type and mutant alleles of the *white eye* gene from the melon fly, *Bactrocera cucurbitae* (Diptera:Tephritidae), was obtained. The wild-type *white eye* allele sequence was 3.6 kb in length, including a 1.1 kb untranslated leader section and the polyAAA tail addition site. Intron 1 was not sequenced because of its large size. Seven exons (designated 1, 2, 3, 4a, 4b, 5 and 6) and six introns (1, 2, 3, 4a, 4b, and 5) were identified. The coding region of the wild-type *white eye* allele was 2.1 kb in length and encodes a putative protein of 679 amino acids. Three functional domains of the ATP-binding cassette and six transmembrane regions were identified in the putative *white eye* protein. The *B. cucurbitae white eye* promoter region did not contain an identifiable TATA-box, however, an Initiation Sequence (Inr) and a downstream promoter element (DPE) were identified. The Inr sequence, "TCAGTT", was located at -1107 to -1112 and is identical in sequence to that of *Drosophila melanogaster*. The placement at this position is consistent with initiation of the transcript to produce a 3.6 kb sequence. The DPE sequence "GTCG" was located at +39 to +42 bp relative to the Inr sequence. Three regions located within the untranslated leader section (upstream of the exon 1 start codon) have a very high similarity in DNA composition and location to three regions upstream of exon 1 in *Bactrocera tryoni*. One region is also similar to *Ceratitis capitata*. It is possible that these untranslated sections are a regulatory area and deserve further study in related organisms. The mutant *white eye* allele had a single base pair mutation at the 5' end of intron 4b in the RNA splice recognition site. The "A" at position 1505 disrupts the RNA splice recognition site so that intron 4b is not removed from the RNA transcript. The resulting mRNA transcript is 68 bp longer than that of the wild type. Because the intron sequence contains a stop codon, translation of such an mRNA would result in a protein truncated at amino acid 448. This putative truncated protein would not include the transmembrane regions that are essential for function of the *white eye* protein in transport of pigment precursors. As a result, transport of pigment precursors would be blocked, resulting in a lack of pigmentation deposition consistent with the known mutant phenotype.

Immunolocalization of sex-peptide pheromones on *Drosophila melanogaster* sperm.

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In *Drosophila melanogaster* mating elicits two characteristic alterations in female reproductive behavior: receptivity is reduced and oviposition is increased. Post mating responses last for about one week when sperm is present (= sperm effect). When sperm is absent, 1-2 days. Sex-peptide and DUP99B (the first two peptides characterized constituting the sex-peptide pheromone family) are sufficient to induce these post-mating responses. The two peptides share high homologies in their C-terminal parts. After a normal mating, the two peptides are transferred to the female. We have tested the hypothesis that the sperm effect may be based on binding of SP and/or DUP99B to sperm. Mature sperm dissected from the seminal vesicles or female genital tracts was incubated with SP or DUP99B, respectively. The binding of the peptides on sperm was visualised with polyclonal rabbit antibodies and an anti-rabbit IgG FITC conjugate. Immunolocalization with SP antibodies revealed a strong binding of SP to the head and tail of sperm. The N-terminal part of SP is responsible for binding. DUP99B binds to the tip of the sperm head (including the acrosome region). A region near the N-terminus of DUP99B is involved in binding. The binding regions of SP and DUP99B are not homologous in sequence. Furthermore, the binding patterns of the two peptides vary in space and time. These results support the above-mentioned hypothesis. Recent experiments show that sex-peptide is also expressed in the testes. This latter result suggests that sex-peptide may have a function in the male itself.

Evolution of host associations between *Drosophila melanogaster* and their parasitic nematodes: A worldwide phylogenetic shuffle.

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Mushroom feeding *Drosophila* flies (Drosophilidae), particularly in the *quinaria* and *testacea* species groups, are commonly infected by nematode parasites of the genus *Howardula* (Tylenchida: Allantonematidae). We used DNA sequence data to infer the phylogenetic relationships of both host *Drosophila* (mtDNA: COI, II, III) and their *Howardula* parasites (rDNA: 18S, ITS 1; mtDNA: COI). Molecular analysis revealed eight new *Howardula* species. Host and parasite phylogenies are not congruent with each other, with host associations characterized by frequent and sometimes rapid host colonizations and losses. Parasitic *Howardula* of *Drosophila* are not monophyletic, and host switches have occurred between *Drosophila* and distantly related mycophagous sphaerocerid flies. We also found evidence for some phylogenetic association between parasites and hosts, with some nematode glades associated with certain host lineages. Thus, host associations in this group are highly dynamic, and appear to be driven by a combination of repeated opportunities for host colonization due to shared mushroom breeding sites, and broad potential host ranges of nematodes.

Cloning and expression of a storage protein receptor from *Helicoverpa zea* fat body

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In Noctuids, storage proteins are taken up into fat body by receptor mediated endocytosis. These include arylphorin and a second, structurally unrelated very high density lipoproteins (VHDL). Previously, we have isolated a single storage protein receptor from fat body membranes of the corn earworm, *Helicoverpa zea*, which binds both arylphorin and VHDL. The receptor protein is a basic, 80 kDa protein that is N terminally blocked. Microsequencing of proteolytic fragments of the isolated receptor protein revealed internal sequences that were used to clone the complete cDNA of the receptor by 3' and 5' RACE techniques. The receptor protein, when expressed in vitro via a suitable insect expression vector (pIB/Vis His), reacted with antibodies against the native storage protein receptor and bound strongly to its ligand VHDL, thus confirming that the cloned cDNA represents indeed the previously purified storage protein receptor. The receptor protein and a second, similar protein also found in the fat body membrane protein fraction show considerable homology to basic juvenile hormone suppressible proteins cloned previously from other Noctuid species. Sequence analysis revealed that the receptor is likely a peripheral membrane protein, and that another, integral membrane protein may be necessary for the uptake of VHDL.

Identification and mapping of the promoter for the gene encoding the ferritin heavy-chain homologue of the yellow fever mosquito *Aedes aegypti*

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In hematophagous insects, the bloodmeal is important in reproduction, playing a crucial role in the regulation of vitellogenesis. Although a high concentration of iron is toxic for most organisms, hematophagous insects seem unaffected by the iron overload contained in their bloodmeal. Initially, iron in the bloodmeal is tightly sequestered by heme proteins. However, once ingested, the heme proteins are rapidly degraded by digestive enzymes, releasing heme and eventually free iron. Both heme and iron can react with oxygen to produce radicals that cause serious biological damage. One means by which hematophagous insects handle the iron overload is perhaps through the iron storage protein ferritin as in vertebrates, where ferritin plays the role of a cytotoxic protector. This work examines the regulation of ferritin, an iron-binding protein, in the yellow fever mosquito, *Aedes aegypti*. We have identified and mapped the promoter from the *Aedes aegypti* ferritin heavy-chain homologue (HCH) gene. This work was supported by funds from NIH, NIGMC (grant # GM55866).

Hormone G protein-coupled receptors (GPCRs) from mosquito *Aedes aegypti* respiratory and excretory systems

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In insects, the control of diuresis, excretion and respiration is coordinated by a number of hormones, such as serotonin and neuropeptides. Several neuropeptides controlling these functions have been isolated and/or sequenced from insects, some specifically from mosquitoes. However, the corresponding receptors have not been identified or functionally characterized. Most recently the *Drosophila melanogaster* genome project has identified GPCRs but the function of many of them remain unknown. We have cloned the cDNAs for a kinin-like neuropeptide receptor and a serotonin receptor from female mosquito respiratory and excretory systems. Localization by *in situ* hybridization and/or histochemistry provides insight into the function of these receptors in the adult female: the serotonin receptor cloned appears to be involved in regulating respiration in tracheoblasts. In addition to the understanding of mosquito endocrinology, the long-term goal is to validate these receptors as novel sites for the development of mosquitocidal compounds.

Enriched and suppressed gene transcripts in the brain of *Periplanata americana* after learning.

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A major goal in neuroscience is to understand molecular events underlying learning, memory formation and memory storage. To this end we have developed a visual-olfactory learning paradigm using the American cockroach as a model organism. We are working to identify molecular changes in the brains of trained animals. *Periplanata americana* learns to associate a visual cue with an attractive odor and remembers it for up to three days. The subtractive hybridization technique was utilized to enrich the transcripts that are either overexpressed or suppressed in the brain of male *P. americana* that performed well in the visual olfactory learning paradigm. Two subtractive cDNA libraries have been generated. One library is enriched in clones overexpressed in the brain of trained roaches. The other represents clones that are enriched in the brain of control animals. Of these libraries 300 randomly selected colonies were analyzed by differential screening using forward and reverse subtracted cDNA pools as probes. The differentially expressed clones were submitted to sequence analysis. After these screens 9 clones representing overexpressed mRNAs and 14 clones representing suppressed mRNAs in the brain of trained roaches are being investigated. A sophisticated version of the visual-olfactory learning paradigm entails surgically split brains. Roaches are trained unilaterally, using one side of the brain, leaving the other side to serve as the control. The brains of such roaches will be used in *in situ* hybridization to confirm learning/memory specific expression of all candidate clones.

Intraspecific variability of *leuB* and *trpE* gene copies in *Buchnera* within the aphid *Uroleucon ambrosiae*

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Aphids (Homoptera: Aphididae) feed exclusively on plant phloem, which is deficient in many essential amino acids. Most aphids receive these nutrients from their endosymbiont, *Buchnera*. In the leucine and tryptophan biosynthetic pathways, the rate limiting genes *(IeuA* and *trpEG*, respectively) in *Buchnera* are generally on plasmids and thus often amplified relative to chromosomal genes. This gene amplification is a likely mechanism by which some *Buchnera* over produce leucine and tryptophan for their hosts. Because amino acids are energetically expensive to produce, aphid *Buchnera populations* may be under selection to have relative *ZeuA* and *trpEG* gene copies commensurate with their needs. We tested this hypothesis in *Uroleucon ambrosiae*, which, because of its wide distribution and diet breadth, is an ideal aphid species in which to assess *leuAltrpEG* variability. We used quantitative PCR to calculate the ratio of *trpE* and *IeuB* (which is in a linked to *IeuA)* genes to a single copy chromosome gene, *trpB*, in 86 aphids across 15 populations. There were significant *trpEarpB* ratio differences between embryos/eggs and adults m one population, although three other analyzed populations displayed no significant difference. In addition, aphids on the favored host plant, *Ambrosia trifida*, had significantly lower *trpEarpB* ratios than those on a less favorable host, A. *confertaflora*. There were also significant *leuBarpB* and *trpEarpB* ratio differences between several individual populations. These differences probably result from differential selection between populations, possibly mediated through the aphids' host plant.

Functional characterization of *Manduca sexta* NSF homologue: A hexameric ATPase implicated in membrane fusion.

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The hexameric ATPase, NSF is implicated in mediating fusion between intra-cellular membranes. Due to the conservation of proteins in constitutive and regulated membrane fusion reactions, NSF and its downstream targets have been predicted to participate also in fusion reactions underlying endocrine function. Here we have characterized the NSF orthologue (MsNSF) from the endocrine model for development, *Manduca sexta*. MsNSF is developmentally regulated in endocrine organs of the protocerebral complex. Enrichment of MsNSF in corpora cardiaca (CC) and not in corpora allata (CA) indicates that it might play a preferential role in releasing hormones produced in CC. Endocrine/paracrine cells of the enteric system in *M. sexta* exhibit selective MsNSF enrichment. We further characterized the *in vivo* role of MsNSF by heterologous expression. In contrast to vertebrate NSF, MsNSF is functional in yeast membrane fusion in vivo. MsNSF rectifies defects in *SEC*18 (yeast NSF homologue) at nearly all discernible steps where Sec18p has been implicated in the biosynthetic route. This underscores the utility of our approach to delineate functional roles for proteins from systems that are not currently amenable to *in vitro* reconstitution.

Testing the feasibility of a simple genetic screen for active DNA transposons.

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The on-going genome revolution has produced an immense amount of sequence data from which diverse DNA transposons can be identified. If active, these transposons can be used as powerful transgenic and mutagenesis tools. Unfortunately, the vast majority of transposons have accumulated inactivating mutations during evolution, rendering the identification of active transposons a task of finding needles in the haystack. A genetic screen based on a bacterial transposon system has been developed to identify hyperactive copies of an insect *mariner* transposon among randomly mutated copies (Lampe *et al*., 1999, Proceedings of the National Academy of Sciences-USA, 96:11428). This approach can potentially be used to screen for active copies of transposons that do not require specific host factors. Here we report our initial effort to develop a simple alternative approach for the rapid screening of active DNA transposons. Our method is based on the design that the excision of the terminal inverted-repeats (and the internal region) by an active transposase allows the expression of the down stream reporter genes in a plasmid vector. All necessary constructs have been made which worked very well. An active mariner transposase and its TIRs are being used as the positive control to validate the approach. In addition, we are using this method to test the activity of a potentially active DNA transposon isolated from the African malaria mosquito, *Anopheles gambiae*.

Transcriptional control of long-term memory: Insights from a *Drosophila melanogaster* synapse.

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The mechanism of cortical plasticity is one of the major unresolved issues in Biology. Although eventual understanding requires integration of intracellular processes in the context of brain circuitry and biochemistry, many fundamental underpinnings of plasticity are rooted in cell biology. Patterned neuronal activity, a cell's perception of experience, induces signaling via cAMP, Ca^{++} and MAPKinases to induce transcription factors like CREB and the immediate early proteins (IEGs) Fos, Jun (that together form AP-1), c/EBP and Zif268. These proteins and their transcriptional targets are believed

to be required for persistent synaptic changes that underlie long-term memory (LTM). Remarkably, the specific functions of these transcription factors, and the identity and functions of their transcriptional targets remain largely unknown. Using genetics, genomics and celll biological methods, we have begun to analyze the identity, function and relationships of intracellular signaling pathways, transcription factors and effector proteins that mediate neuronal plasticity. We have: a) demonstrated the conservation of signaling pathways that lead to IEG-regulation by activity in *Drosophila melanogaster*; b) analyzed biological functions of AP1 in long-term synapse plasticity; and c) begun computational and microarray-based screens to identify activity-regulated effectors of long-term plasticity. Our data show that bursts of activity can induce ERK signaling, and transcriptional induction of Fos, Jun and c/EBP homologs in the *D. melanogaster* brain. AP1, modulated by JNK signaling, regulates both structural and functional plasticity in *D. melanogaster*; remarkably our observations indicate that AP1 functions upstream of CREB in long-term plasticity displayed at the *D. melanogaster* larval motor synapse. The ability to induce neural activity, and to assay intermediate stages in pathways that mediate biological plasticity processes has allowed us to initiate cell biological analyses of a large number of *D. melanogaster* mutants in long term memory recently isolated from a genetic screen performed in Cold Spring Harbor. Progress in these areas of research will be outlined.

Interaction of cuticular proteins and chitin.

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More than half of those arthropod cuticular proteins for which sequence data is available have a conserved consensus domain of G-x(7)-[DEN]-G-x(6)-[FY]-x-A-[DGN]-x(2,3)-G-[FY]-x-[AP], known as the R&R consensus. This consensus was first reported by Rebers and Riddiford in 1988 (J. Mol. Biol. 203:411), based on seven cuticular protein sequences, and has since been shown in a wide range of insect cuticular proteins, as well as in cuticular proteins from other arthropods. Because of the extensive conservation of this region, several workers have suggested that this region binds chitin. Using fusion proteins expressed in *Escherichia coli*, we have shown that the R&R consensus is necessary and sufficient for chitin binding (Rebers and Willis, Insect Biochem. Molec. Biol. 31:1083). Fusion proteins including the R&R consensus from proteins from either hard or soft cuticles fused to glutathione-S-transferase (GST) bind to chitin, although initial analysis indicates that a soft cuticular protein/GST fusion binds more weakly to chitin than a hard cuticular protein/GST fusion. Site-directed mutagenesis of conserved residues in the R&R consensus is being used to determine which amino acids are most critical for chitin binding. Mutating either a pair of tyrosine and phenylalanine residues within the strict R&R consensus or mutating a pair of threonine and aspartate residues within the N-terminal region conserved in hard cuticular proteins disrupts chitin binding. Experiments are in progress to determine the effect altering single residues in the R&R consensus has upon chitin binding.

Regulation of pigment gene expression in developing butterfly wing discs.

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In many insects ommochromes are utilized for eye pigmentation and tryptophan metabolism, but butterflies have evolved the ability to synthesize ommochromes in wing scales in order to generate colorful and spatially complex wing patterns. In order to study how ommochrome development is regulated in butterfly wings, we have cloned genes that encode ommochrome synthesis enzymes (*cinnabar* and *vermilion*), ommochrome precursor transporters (*white* and *scarlet*), and, for comparison, melanin synthesis enzymes (*pale* and *DDC*), from several nymphalid butterflies including *Vanessa cardui, Heliconius melpomene*, and *Heliconius erato*. We have made progress in determining the expression patterns of many of these genes in the developing wing discs of *V. cardui*. We present a model of wing pattern development in light of apparent relationships between the expression of ommochrome genes and patterning genes such as *engrailed* and *Distalless*. Work is underway to further test models of ommochrome regulation and to determine how the regulation of specific pigment synthesis genes is modulated to produce wing pattern variation in the mimetic butterflies *H. melpomene* and *H. erato*.

The peritrophic membrane plays an additional role by protecting lepidopteran insects from *Bacillus thuringiensis* Cry δ-endotoxins.

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The peritrophic membrane (PM) in lepidoptera has two proposed functions: to protect the midgut from abrasion by food particles and secondly, to protect the insect from invasion by micro-organisms and parasites (Tellam R. 1999. Insect Biochemistry and Molecular Biology 29: 87-101). When Cry δ-endotoxins from *Bacillus thuringiensis* species enter the gut, the activated toxins are believed to pass freely through the PM, based on the pore sizes observed in *Trichoplusia ni* larvae (Adang MJ and Spence KA 1983. Comparative Biochemistry and Physiology 75: 233-238). A combination of *in vitro* and *in vivo* techniques has been used on a range of Cry1 toxin susceptible and resistant insects to study the interaction of different toxins with the PM and midgut membranes. So far results have shown that activated Cry1Ac, Cry1Ba and Cry1Ca toxins remain bound to the PM in the resistant larvae 20 hours after ingestion and never reach the target receptors on the midgut membrane. In susceptible insects no toxin remains associated with the PM after 3 hours and some is found to pass through to the midgut membrane where its binding results in the lysis of epithelial cells. Several PM proteins have been found to bind toxins and have been

identified as insect intestinal mucins, previously reported in other lepidopteran species (Wang P and Granados R. 1997. Journal of Biological Chemistry 272: 16663-16669).

Juvenile hormone, broad, and the evolution of diverse metamorphic strategies in moths and flies

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Juvenile hormone (JH) prevents metamorphosis in most insect species but in the higher Diptera, it only affects the pupal-adult transformation of certain tissues. In the epidermis of both the tobacco hornworm, *Manduca sexta*, and *Drosophila melanogaster*, the ecdysone-induced transcription factor Broad (BR) first appears at the onset of metamorphosis and is necessary for the larval-pupal transformation. Normally BR disappears at the onset of the pupal-adult transformation, but the presence of JH causes its re-expression during the adult molt in the entire epidermis of *M. sexta* but only in the abdominal epidermis of *D. melanogaster*. Expression of the various isoforms of BR under the control of a heat shock promoter during adult development of *D. melanogaster* showed that early exposure to BR prevented bristle and hair differentiation. Later exposure at the onset of cuticle formation, particularly of BR-Z1, caused re-expression of pupal cuticle genes and suppression of an adult cuticle gene leading to reformation of a pupal cuticle in the head and thorax as well as in the abdomen. Precocious expression of BR-Z1 during the 2nd larval molt caused premature pupal cuticle formation and death at ecdysis. Thus, BR specifies pupal development of the epidermis. Consequently, JH exerts its "status quo" effect at metamorphosis by preventing the turning on of BR during the larval-pupal transformation in moths and preventing its turning off during the pupal-adult transformation of both flies and moths. The loss of sensitivity to JH of the imaginal discs, but not of the abdominal histoblasts, in flies may be coupled to the necessity of the former to proliferate during larval life when JH is high. The molecular basis of this loss of sensitivity to JH is unknown. Supported by NIH.

Characterization of the steroidogenic signaling cascade in the mosquito *Aedes aegypti*

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In female *Aedes aegypti* mosquitoes, a neuropeptide, ovary ecdysteroidogenic hormone (OEH) is released from neurosecretory cells shortly after a blood meal and stimulates ovaries to secrete cdysteroids. The rising titer of ecdysteroids activates the fat body to synthesize yolk proteins, which are stored in eggs and used during post embryonic development. Both OEH and bovine insulin stimulate ovary steroidogenesis *in vitro* through the activation of a receptor tyrosine kinase (RTK) and the phosphotidylinositol 3 kinase (P13 K)/protein kinase B (PKB) pathway, as indicated by the effects of selective inhibitors and activators. A mosquito RTK related to the insulin receptor has been characterized and localized on ovary follicle cells, and is tyrosine phosphorylated in the presence of bovine insulin. The receptor is also expressed in the follicle cells prior to a bloodmeal and during steroidogenesis, not after the cells switch to chorion production. This receptor likely is involved in the binding of radiolabeled insulin to ovaries and its displacement by both unlabeled OEH and insulin. The complete sequence of the gene encoding the mosquito PKB (MPKB) has been determined and closely resembles *Drosophila melanogaster* and human PKB. The expression of MPKB during development and a reproductive cycle has been characterized. The function and signal transduction of OEH, with its limited similarity to the insulin superfamily, support the concept developed from recent studies of mammals and invertebrates that insulins and the RTK/P13 K/PKB pathway are an important nexus for the regulation of reproduction, nutrient stores, growth, and immunity. Supported by a grant (AI33108) from NIH to MR Brown

Analysis of heat shock protein function during papal diapause of the flesh fly using RNAi

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Previously, our laboratory reported the upregulation of select heat shock proteins (hsps) during the papal diapause of the flesh fly *Sarcophaga crarsipalpis*. As this fly enters diapause, hsp23 and hsp70 are upregulated in the absence of stress, with levels remaining elevated throughout diapause. Conversely, hsp90 and hsc70 expression was unchanged by diapause. Although the expression of lisps during diapause has been well described, their function remains unclear. To resolve this issue, we have used the RNA interference technique to suppress the expression of select hsps either alone or in concert during diapause. When injected with hsp70 dsRNA prior to entering diapause, neither the incidence nor length of diapause appear to be affected, but injection of hsp70 dsRNA leads to a dramatic reduction in cold tolerance, a key component of the diapause state. Injection of lisp dsRNA can affect stress tolerance in nondiapausing individuals as well. Both hsp23 and hsp70 RNAi led to reduced survival of heat shock and a reduced ability to establish induced thermotolerance. Conversely, rapid cold hardening was not substantially affected by RNAi, suggesting molecular differences in cold tolerance conferred by diapause and that conferred by rapid cold hardening in nondiapausing individuals.

Long-PCR as a tool for detecting rearrangements of arthropod mtDNA

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Rearrangements of the mitochondrial DNA gene order have been used to help define the pattern of evolutionary divergence in arthropod taxa. We have employed a combination of highly conserved insect-based PCR primers with long-PCR to survey fourteen non-insect arthropods for mitochondrial gene rearrangements. The size of the amplified fragments was used to order the primer containing genes. Five chelicerates exhibit amplicons that are consistent with the insect mtDNA gene order. These five species comprise two soft ticks, two prostriate hard ticks and a harvestman. Six other chelicerates, all metastriate hard ticks, have a different arrangement that has been previously detailed in a complete mtDNA sequence. Three new major gene realignments of major coding regions were found. They were obtained from a terrestrial crustacean (Isopoda, sowbug) and two myriapods (Chilopoda, centipede; Diplopoda, millipede). In addition we found two possible new positions for $tRNA^{Met}$ in arthropods. A crustacean isopod and a myriapod diplopod have $tRNA^{Met}$ positioned between the 12S and 16S rRNAs on the downstream side of 12S. A myriapod chilopod appears to have $tRNA^{Met}$ located between CytB and ND4. The long-PCR approach affords an opportunity to screen larger numbers of divergent taxa for major rearrangements. Efficiency of the method would likely be improved with the use of primers derived from taxa more closely related to those being investigated and not relying entirely on insect primers. Detailed sequencing of gene boundaries or entire mt genomes can be reserved for those taxa that exhibit significant new patterns of mtDNA organization.

Two different *Wolbachia* types create a breeding barrier between populations of northern corn rootworm (D*iabrotica barberi*) in Illinois.

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Wolbachia sp. are endosymbiotic bacteria that are widespread in arthropods and are often associated with reproductive incompatibilities between infected and uninfected individuals. 16S rDNA primers detected *Wolbachia* in northern corn rootworms (NCR) from the eastern portion of their range with a *Wolbachia* +/- boundary in central Illinois. Portions of the *Wolbachia ftsZ* and *wsp* genes have been sequenced from several geographic locations. The 1058 bp *ftsZ* sequences from NCR are Type A *Wolbachia* and fall into two groups within that category. NCR Type I *Wolbachia* from eastern Illinois to Pennsylvania are nearly identical to *Wolbachia* from western corn rootworm (WCR)(<0.3%). NCR Type II *Wolbachia* from central Illinois differ by 3.1% from Type I and are nearly identical to a *Wolbachia* found in the European raspberry beetle (0.1%). The ~600 bp *wsp* sequences are also dramatically different. NCR Type I is identical to that from WCR, while NCR type II differs by 63 substitutions (>10%) and 15 indels. The boundary between these two very distinct strains of *Wolbachia* in adjacent geographical populations of NCR correlates with a previously observed mtDNA genetic boundary in eastern Illinois indicating that the two *Wolbachia* are incompatible and little if any introgression occurs between the two populations. There is currently no evidence that this boundary is being pushed in either direction. The boundary between the NCR Type II *Wolbachia* infected and uninfected insects appears to be less distinct and leaves open the possibility that *Wolbachia* infection is expanding westward in NCR. Also unresolved is the question of whether the eastern population of NCR might have acquired *Wolbachia* via horizontal transfer from WCR.

European and Asian corn borer data support asymmetric tracking hypothesis for evolution of sex pheromones

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Diverse pheromone structures are used in the Lepidoptera for long-distant mating signals, but the signal/response channel appears to be narrow for each species. The conundrum is how signal divergence has occurred in the face of strong selection pressures against small adaptive changes. Data from several corn borer (*Ostrinia sp.*) populations support the asymmetric tracking hypothesis in which a large mutational effect in female production can subsequently be tracked by male response. A nonfunctional ˆ14-desaturase gene in the European corn borer sex pheromone gland, possibly generated from gene duplication of the common ˆ11-desaturase in an ancestral population of *Ostrinia*, evidently was turned on in some stochastic event to initiate evolutionary changes that generated a species with new pheromone components. Additional support comes from behavioral studies that revealed rare European corn borer males responding to the new pheromone components, and from data that show other key genes in pheromone detection and production of the new species have not changed in their preference to the former pheromone components.

Immunohistological expression of human Insulin-Like Growth Factor I (hIGF-I) and human Growth Hormone (hGH) in the larvae of the mosquito *Culex pipiens*: Probable involvement in water and mineral balance.

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Most mosquito larvae develop in small aquatic environments. These habitats undergo sudden changes of salinity concentration due to evaporation. Fast and effective physiological changes are necessary to maintain **water and mineral balances adjusted to** changes of the environmental conditions. The presence of genes coding for proteins with high homology for several growth factors have been described in insects. IGF-I and GH are well known vertebrate peptidic hormones. They have a basic role as regulators of cell growth

and differentiation. They also have been described as regulators of water and inorganic ions balance in mammals. In the present work we analyze the presence of immunoreactive material against hIGF-I and hGH antisera in tissues of the larvae of *Culex pipiens*. We detected the presence of immunoreactivity in muscle, nervous system, and fat body, suggesting the presence of molecules with some grade of homology to these vertebrate hormones. In addition, we found positive reactions in Malpighian tubes (microvilli), midgut (exocytic vesicles) and hindgut. The presence of immunoreactivity in these tissues suggests an involvement of these peptides in the regulation of diuresis in insects. As a first approach to address this hypothesis, we have used an ELISA to analyze the effect of an osmotic shock on the levels of IGF-I. The immunoreactivity for hIGF-I after exposing *C. pipiens* larvae for 10 min to distilled water was significantly lower that the levels found in control animals (larvae raised in breed water). Mosquitoes exposed to distilled water for 10 min and then placed in breed water for 90 and 180 min showed a tendency to increment IGF-I levels. In summary, we propose that hIGF-I and hGH-like molecules might play a role as regulators of water and inorganic ions balance in mosquito larvae.

A genomic perspective on serine proteinases and their homologs from *Drosophila melanogaster*

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Serine proteinases (SPs) and serine proteinase homologs (SPHs) constitute the second largest family of genes in the *Drosophila melanogaster* genome. Their protein products are implicated in various biological processes ranging from digestion, embryonic development, to immune responses. Attempting to understand the evolutionary history of this gene family and provide guidance for future functional analysis, we have carried out a preliminary analysis of the *D. melanogaster* SP and SPH sequences retrieved from Genbank. Among the 147 SPs and 57 SPHs studied, 24 SPs and 13 SPHs contain at least one regulatory clip domain. Eighty-four SPs comprise less than 300 amino acid residues, and a significant portion of them are probably digestive enzymes. Some larger SPs contain one or more regions that may be important for protein-protein interactions, including clip domains, low-density lipoprotein receptor class A repeats and scavenger receptor cysteine-rich domains. We identified thirty-seven clusters of SP or SPH genes, which probably evolved from relatively recent gene duplication and sequence divergence. Gene movement along or among the chromosomes has spread SP-related genes over the genome. A majority of the SPs may have trypsin-like specificity and are probably activated by cleavage after a specific arginine or lysine residue. A multiple sequence alignment of the clip domains provided further information on structural conservation of these regulatory modules. Detailed sequence comparison led to an improved classification system for SPs containing clip domains. These analyses have established a framework of information about evolutionary relationships among the *D. melanogaster* SPs and SPHs, which may facilitate research on these proteins as well as homologous molecules from other invertebrate species.

Subtractive cdna libraries from the tick, *Ixodes ricinus*: Differential expression of genes induced by blood meal and pathogen infection

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Ixodes ricinus ticks are the major European vector of *Borrelia burgdorferi sensu lato* spirochetes, the causative agent of Lyme disease. In the Czech Republic, Lyme disease is a serious threat to public health. In ticks, a blood meal signals the start of the vitellogenic phase of the reproductive cycle. The tick bite is part of an interactive system in which both vector and host components are exchanged. The pharmacology and immunology of the host response to blood feeding have been the subject of many recent investigations. Conversely, ticks mount an immune response against pathogens, which they may encounter within ingested host blood. To date, very few of the genes that play a role in tick immunity and reproduction have been characterized. To investigate the identity and role of a wide range of differentially expressed genes that are modulated by blood feeding and/or exposure to pathogen infection, three subtractive-hybridization whole-body cDNA libraries were prepared from the tick *Ixodes ricinus*. The first library is enriched in cDNA corresponding to transcripts that are induced by a blood meal. It was constructed by "subtracting" cDNA prepared from non-blood-fed ticks (driver 1) from a sample of cDNA prepared from ticks that were given an uninfected blood meal (tester 1). The second library is enriched in cDNA corresponding to transcripts that are induced by a *B. burgdorferi* infected blood meal. It was constructed by "subtracting" cDNA prepared from non-blood-fed ticks (driver 1) from a sample of cDNA prepared from ticks that were fed with an infective blood meal (tester 2). The third library contains cDNA species that are differentially expressed between ticks that were fed a non-infected vs. an infected blood meal. It was constructed by "subtracting" cDNA prepared from blood-fed ticks (driver 2) from a sample of cDNA prepared from ticks that were fed with an infective blood meal (tester 2). Differential screening of all subtracted libraries confirmed the set of genes which were up regulated or down regulated by blood feeding and/or infection. Randomly selected clones were sequenced and compared with available databases.

The distributions and transmission modes of aphid secondary endosymbionts

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Many insects are known to associate with vertically transmitted (from parent to offspring) bacteria in facultative symbiotic relationships. Through the use of molecular and phylogenetic analyses, several studies have suggested that bacterial symbionts also undergo occasional horizontal transmission (tranmission between unrelated individuals) between distinct host taxa. Here, we explore the distributions of three facultative bacterial symbionts, provisionally named the R, T, and U types, across a variety of aphid and psyllid hosts through the use of diagnostic molecular screening techniques. Our results suggest that these bacteria have undergone extensive horizontal transfer between host species. However, several lines of evidence support the possibility of long-term persistence within single aphid lineages.

Prothoracicotropic hormone stimulated extracellular signal-activated receptor kinase (ERK) activity: The roles of Ca^{2+}- and cAMP-dependent mechanisms.

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The synthesis of ecdysteroids by the prothoracic gland of Lepidoptera is regulated by the brain via the neuropeptide hormone prothoracicotropic hormone (PTTH). PTTH is believed to bind to receptors on prothoracic glands, progressively initiating Ca^{2+} influx, Ca^{2+}-dependent cAMP generation, activation of protein kinase A and S6 kinase, phosphorylation of the S6 ribosomal protein, protein synthesis and finally protein synthesis-dependent ecdysteroidogenesis. Recent work in prothoracic glands of the tobacco hornworm *Manduca sexta* has shown that PTTH activates an additional phosphorylation pathway (Rybczynski *et al.*, Molecular and Cellular Endocrinology 184: 1-11, 2001), i.e. an extracellular signal-activated receptor kinase (ERK). Inhibition of the ERK pathway can inhibit both PTTH-stimulated and basal ecdysteroid synthesis *in vitro*. The path by which PTTH stimulates ERK phosphorylation was investigated using prothoracic glands challenged *in vitro* with either a Ca^{2+} ionophore or a cell-permeable cAMP analog at concentrations that maximally stimulate ecdysteroid synthesis *in vitro*. The results indicate that Ca^{2+} influx-dependent processes, other than cAMP generation, probably play the major role in causing ERK phosphorylation in larval prothoracic glands, while cAMP-dependent events become more important during pupal stages. However, simultaneous treatment of larval prothoracic glands with the cAMP analog and a sub-maximal dose of Ca^{2+} ionophore results in ERK phosphorylation much higher than when these two reagents are administered singly, i.e. the observed increase in ERK phosphorylation indicates that cAMP and Ca^{2+}-dependent processes may act synergistically *in vivo* to activate the ERK pathway.

Use of cDNA microarrays to examine midgut gene expression changes in response to a blood meal in the mosquito, *Aedes aegypti*

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The midgut plays an important role in the processing of a blood meal and is the primary site of infection by transmittable pathogens. Processes that occur in the midgut in response to a blood meal have been studied but are not yet fully understood. The use of microarrays in order to examine global gene expression is a very useful way to unravel these processes. We are currently developing a cDNA microarray consisting of clones obtained from an expressed sequence tag (EST) project. Individual clones are amplified by polymerase chain reaction (PCR) and printed onto glass slides. These microarrays are being used in order to study the effects of a blood meal on midgut gene expression of *Aedes aegypti*. Five time points are being examined: day 1 post emergence, and 3, 12, 24, and 72 hours post blood meal. A number of genes involved in ion regulation have been determined to up or down regulated in response to a blood meal based on preliminary microarray data. Specifically genes encoding V-ATPase subunits are upregulated within one day after a blood meal.

Proline can be utilized as an energy substrate during flight of *Aedes aegypti* females

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The activities of enzymes involved in the metabolism of amino acids were assayed in homogenates of thorax (flight muscles), abdomen (fat body) and midgut of *Aedes aegypti* females. The highest activities of alanine aminotransferase (AAT, EC 2.6.1.2), malate dehydrogenase (MDH, EC 1.1.1.37), NAD-linked malic enzyme (ME-NAD, EC 1.1.1.39) and proline dehydrogenase (PDH, EC 1.5.99.8) were found in the thorax. Glutamate dehydrogenase (GDH, EC 1.4.1.2) showed the same activity in thorax and midgut. Aspartate aminotransferase (AspAT, EC 2.6.1.1) activity was lower in thorax than in the other tissues. The activities of AlaAT and MDH were of the same order of magnitude in abdomen and midgut. ME-NAD, GDH, AspAT and PDH activities were lower in abdomen than in midgut. The concentration of amino acids was monitored at 0, 30 and 60 min after flight in hemolymph and thorax; in hemolymph and thorax 2 hours after a sucrose meal; and in thorax 24, 48 and 72 hours after a blood meal. The concentration of amino acids was much higher in thorax than in hemolymph. Two hours after a sucrose meal, the most abundant amino acids in hemolymph were: Pro (39%), Ala (19%), Thr (11%), Glu (7%), Ser (4%), Gln (4%), His (4%) and Arg (4%), whereas in thorax the most abundant amino acid were: Ala (23%), Thr (23 %), Pro (15%), His (11%), Arg (10%), Glu (6%) and Ser (4%). In thorax, 24, 48 and 72 after a blood meal, the results were similar to those after a sucrose meal. After flight, proline and

in only one case alanine changed its concentrations. After a sucrose meal, proline from hemolymph decreased 48% and 19% after 30 and 60 min of flight, whereas proline from thorax decreased 23% and 42% after 30 and 60 min of flight, compared with pre-flight levels. In both cases, the changes of concentration of alanine after flight were not statistically different. In thorax 24 hours after a blood meal, proline showed decreases of 40% and 69% after 30 and 60 min of flight. Alanine increased its concentration after 30 min of flight then it decreased. In thorax, 48 hours after a blood meal and after 30 min and 60 min of flight, the level of proline fell 65% and 51%, respectively, from control levels. The concentration of alanine did not change significantly after flight. In thorax 72 hours after a blood meal, no significant change in proline and alanine concentrations was observed during flight. The levels of enzyme activities observed in the thorax, and the marked decreased of proline during flight in the hemolymph as well as in the thorax after a sucrose meal and (in the thorax) 24 and 48 hours after a blood meal, support the suggestion that proline can be used as an energy substrate by flight muscles of *A. aegypti* females.

Structural diversity of prothoracicotropins and bombyxins in Lepidoptera

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Amino acid sequences of prothoracicotropins (PTTH) and bombyxins have been elucidated in just a few species representing a couple of lepidopteran families. Related antigens have been detected immunohistochemically in various insect orders, indicating that the hapten regions are preserved. They probably correspond to conserved amino acid sequences that can be identified by comparing known homologues of the hormones. The conserved parts of the PTTH are located around the cysteine residues. An QxIxDPPCxC sequence is close to the N-terminus, NCxxxQxxCxxPYxCKE in the central region, and SVxCTRDY close to the C-terminus. Surprisingly, a long stretch of conserved sequence DYxNMxxNDVxLLDNSxETRTRKRG, which includes the cleavage site (underlined) and initial glycine of the PTTH, occurs at the end of the intercalated peptide that is assumed to have no function. The conserved regions of bombyxins are also found around the cysteines. The core of A-chain (total length 25-28 residues) has the sequence GxxxECCxxxCxxxxLxxYC and that of B-chain (total length 20-23 residues) YCGRxLxxxxxxxC. The C-chain, which is discarded during prohormone processing, is of different length but always includes a tetrapeptide WxWL (x = P, R or K) in its central part and a cleavage and arginine-amidating sequence RGKR at its carboxy-terminus. It must be emphasized that "x" does not indicate a random variation of amino acid residues. Residues alternating in most of the positions are of similar properties. For example, K may be replaced with R, V with I, S with T, D with E, etc. Another conserved feature is the distance between individual cysteine residues.

Dachshund's role in the development of mufti branched limbs

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In *Drosophila melanogaster* and the mouse, *dachshund (dac)* encodes a nuclear protein that participates in proximal distal limb patterning. *D. melanogaster dac* mutations have a shortened leg phenotype, characterized by the absence of intermediate limb structures. During our investigation of the evolution of the gene regulatory network that patterns arthropod legs, we characterized the spatial expression pattern and function of *dac* in Triops longicaudatus, an arthropod with multiramous limbs. *Triops dac is* expressed in three discrete leg domains (termed domain 1, 2 aid 3). One expression domain covers most of the proximal gnathobase and extends in a stripe across the proximal leg (domain 1), another lies at the base of the endopod (domain 2). A final domain appears in the dorsal proximal region of each ventral limb branch (domain 3). Triops dac is not restricted to an intermediate region in any of the branches of the Triops limb, with the possible exception of the first branch that develops, which lies adjacent to domain 2. None of the branches, with the exception of the first to develop, grows to any great proximal distal extent and may not require the intermediate positional values provided by dac in *D. melanogaster* limb development. We tested the functional role of *dac* in Triops limb development using RNAi. Our preliminary results suggest that removing *dac* function during Triops multi branched limb development results in loss of the most proximal branch and alterations in morphology of most other branches. This suggests a novel role for dac in proximal limb patterning in multiramous limb development.

Transposons with a unique DD37E catalytic motif: Discovery, diversity, and possibly frequent horizontal transfer among mosquitoes

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A novel transposon, named *ITmD37E*, was discovered in mosquito species belonging to four genera including *Aedes*, *Anopheles*, *Armigeres* and *Toxorhrynchites*. The *ITmD37E* elements contain a unique DD37E catalytic triad which differs from those of previously described elements with DD34E (or D) triad in eukaryotic organisms. According to our analyses, the *ITmD37E* form an independent family distinct from the *Tc1* (DD34E), *mariner* (DD34D) and *pogo* (DDxD) families in the *IS630-Tc1-mariner* superfamily. *ITmD37E* family is further divided into two subfamilies. Both subfamilies share the same DD37E catalytic triads, however, there are only 38-49% identities at amino acid level between these two subfamilies. Wide distribution and high level of divergence between these two

subfamilies suggest that *ITmD37E* elements have existed and evolved in their host genomes for a long evolutionary time. In one of these subfamilies, the phylogeny for *ITmD37E* elements is incongruent with that of host species. The divergence level for *ITmD37E* elements is significantly lower than that of host genes, which results not from selective constraints or codon bias. Our analyses suggest that horizontal transfer events appear to have occurred recently between distinct mosquito species. Three *ITmD37E* copies with uninterrupted open reading frames encoding transposases were found from three distinct species including *Aedes atropalpus*, *Anopheles gambiae* and *Toxorhynchites amboinensis*, respectively, which imply that *ITmD37E* elements might be active for transposition. The identification and evolutionary analyses of the widespread transpsons in mosquitoes may help development of novel mutagenesis and transformation tools for a wide range of the medically important mosquitoes.

Vitellin and reproductive response to diet in the predatory bug *Orius insidiosus*, assessed by a monoclonal antibody-based ELISA.

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As with female hematophagous insects such as mosquitoes and assassin bugs, entomophagous insect predators commonly require a protein meal to initiate egg production. Our work with the minute pirate bug, *Orius insidiosus*, suggests that the quality as well as quantity of dietary protein (or compounds associated with ingested protein) determines timing and magnitude of vitellogenic and ovipositional responses. To assess reproductive responses to diet quality and quantity, we have developed quantitative yolk protein ELISAs (YP-ELISA) that employ monoclonal antibodies (MAbs). *O. insidiosus* eggs consistently contained one major YP band, of 263k M_r on pore-limiting native PAGE. On SDS-PAGE, the two major polypeptides in *O. insidiosus* egg homogenates appeared at 180k and 39k M_r. MAbs were produced against these apo-vitellins (apoVn). The 39k apoVn readily stimulated production of monoclonal antibodies in hybridomas, while hybridomas had to be carefully selected for IgG reactivity against the 180k apoVn. In batch monoclonal antibody-affinity isolations, MAbs against either of the apoproteins bound native Vn that contained both 180k and 39k M_r apoproteins. However, a minor band that appeared in fresh egg extracts at 116k M_r was enriched during purification. Adding a protease inhibitor mixture upon homogenization did not inhibit formation of the band, and incubation of homogenate for 24 h, or aging of eggs at room temperature for 24 h, did not enhance its formation. To determine the rates of degradation of apoVn, developing eggs were collected within 2 h of oviposition and tested for YP content 0-72 h later. Depletion of the MAb-detected epitopes in 180k and 39k apoVn followed the same time-course. Half-lives were determined to be 32 and 34 h, respectively. Tests of the YP-ELISA to determine female response to various supplemented diets demonstrated its potential utility in insectary quality control and diet development.

Why might bees be dying: The effects of two picornalike viruses and Varroa mites upon honey bee health

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Viral diseases of honeybees are a major concern in apiculture, causing serious colony losses worldwide, particularly in combination with the mite *Varroa jacobsoni*. The biology of bee viral diseases, their relationship with mites and their effects on bees are poorly understood. To data, most of the research has been focused on virus surveys in natural infections using serological tests. It has been suggested that mites may be vectors and / or activators of several honey bee viruses, particularly deformed wing virus (DWV), Kashmir bee virus (KBV), acute bee paralysis virus (ABPV) and black queen cell virus (BQCV). To better understand the relationship among viruses, mites and colony decline, we are characterizing several of these viruses. Here we report our progress with Kashmir bee virus (KBV) and black queen cell virus (BQCV). We have sequenced 70% the genome of a North American KBV isolate. The KBV genome has two major open reading frames, each encoding a large polyprotein. To produce antibodies specific for KBV, we expressed two structural proteins in the pQE bacterial protein expression system and purified the recombinant proteins. BQCV was activated by injecting bee pupae with buffer (insect Ringer), and artificially transmitted BQCV to other bee pupae. We purified BQCV and characterized it by transmission electron microscope, ELISA, and partial sequencing of its genome. Additionally, we investigated the prevalence of these two viruses throughout 2000 and 2001, in honey bee populations and related the disease incidence to the levels of mite infestations. In the future we will test the role of mites in activating and transmitting these viruses in the bees.

The *Bombyx* genome as a model of lepidopteran genomes.

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The *Bombyx* genome is approximately 530 Mbp in size, and is divided into 28 chromosomes. We, and another laboratory, are making BAC contigs on selected chromosomes and sequencing them. The EST database of *Bombyx* has collected approximately 30,000 ESTs, which are classified to 10,000 nonredundant cDNAs. The first goal of the project will be to determine the whole nucleotide sequence in the genome. The final goal is to explain the Lepidoptera-specific biological functions on the genomic basis and to utilize the genome information for the agricultural pest control and insect

industries including sericulture. So far we can point out two characteristics in the *Bombyx* genome. (1) Biased distribution of retrotransposons. The W chromosome seems to consist of only retrotransposons. Most of the retrotransposons are full-length. Functional genes have not yet been found on the W chromosome. On the other hand, the repetitive sequences on the Z chromosome and autosomes are mainly degenerative short fragments of retrotransposons. This contrast suggests that the W chromosome lacks a mechanism to removes the transposable elements. (2) Lepidoptera-specific genes. The comparative EST/cDNA analyses demonstrated the presence of *Bombyx*- or Leidoptera-specific genes. Several genes were more similar to bacterial genes than to those of *Drosophila melanogaster* and other eukaryotes. Because their orthologs are not found in other metazoan genomes, a possible explanation is horizontal gene transfers from bacteria to Lepidoptera. We also surveyed some gene families in *Bombyx*, and found that they contained *Bombyx*-specific members or subfamilies, which can be explained by recent gene duplications in Lepidoptera or gene losses in other organisms. The work on the *Bombyx mori* genome project was support by MAFF, BRAIN, and MEXT/JSPS, Japan.

Characterization of three alternatively spliced isoforms of the Rel/NF-B transcription factor Relish from the mosquito *Aedes aegypti*

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Drosophila melanogaster Rel/NF-κB transcription factor Relish performs a central role in the acute-phase response to microbial challenge by activating immune antibacterial peptides. We cloned and molecularly characterized the gene homologous to *D. melanogaster* Relish from the mosquito *Aedes aegypti*. Unlike the *D. melanogaster* Relish, *Ae. aegypti* Relish had three alternatively spliced transcripts encoding different proteins. The predominant *Ae. aegypti* Relish transcript of 3.9-kb contained both the Rel-homology domains (RHD and IPT) and the IκB-like domain, being similar to *D. melanogaster* Relish as well as to the mammalian p105 and p100 Rel/NF-κB transcription factors. The second *Ae. aegypti* Relish transcript contained Rel-homology domains identical to those of the major transcript but completely lackeing the IκB-like domain-coding region, which was replaced by a unique 3'-UTR sequence. In third transcript, there was a deletion in place of most of the N-terminal sequence and Rel homology domains, however, the IκB-like domain was intact. All three *Ae. aegypti* Relish transcripts were induced by bacterial injection but not by blood feeding. *In vitro* translated protein from the Rel-only construct specifically bound to κB motif from *D. melanogaster* cecropin A1 and *Ae. aegypti* defensin genes. PCR and Southern hybridization analyses showed that these three transcripts originated from the same large inducible mRNA encoded by a single Relish gene.

Cloning and sequence analysis of cDNA encoding juvenile hormone metabolising enzymes from the silkworm, *Bombyx mori*

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The titer of juvenile hormones (JH) in insects is controlled by at least two steps, biosynthesis and degradation. For the degradation of JH, two metabolizing enzymes, JH esterase (JHE) and JH epoxide hydrolase (JHEH), are known in lepidopteran insects. Based on the conserved regions in insect JHEs and in microsomal epoxide hydrolases, we designed degenerate primers. These primers were used in RT-PCR to amplify a fragment of cDNAs of JHE and JHEH from *Bombyx mori*. Full cDNA sequences of the enzymes were determined by 5'- and 3'-RACE. The deduced amino acid sequences of the JHE and JHEH protein were predicted as 576 and 461 amino acids residues, respectively. Recombinant proteins expressed in baculovirus system showed the appropriate enzymatic activity. According to the results of developmental changes in mRNA expression and enzymatic activity of JHE and JHEH, JH in the larval body is cleared from the mid-final instar hemolymph JHE and JH. The titers of JHE and JH in tissues are regulated by cellular JHE and JHEH before the clearance of JH.

Studies of nitrophorin: an NO-release protein from the saliva of the blood-sucking insect, *Rhodnius prolixus*.

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Nitrophorins, NP, are ferriheme proteins having a β-barrel fold that are found in the saliva of a blood-sucking insect from the Amazon river basin, *Rhodnius prolixus*. In the salivary glands, these proteins have nitric oxide (NO) bound to the ferriheme center, and transport NO to the victim where NO is released and histamine is bound in its place. This assists the insect in obtaining a blood meal. There are four Nitrophorins, NP1 4, whose amino acid sequences share overall 34% identity. A number of mutants have also been prepared of these nitrophorins. NMR spectroscopic investigations of the low-spin Fe(III) complexes of the nitrophorin 1 & 4 show chemical exchange crosspeaks for some of the heme resonances in the WEFT-NOESY spectra that are indicative of a protein-localized dynamic process. Estimates of the chemical exchange that results from the two protein conformational isomers can be measured. Spectroelectrochemistry can also be employed to measure the reduction potentials for these proteins in the absence and presence of NO and other ligands. We describe the NMR and spectroelectrochemistry studies of nitrophorin and some NP2 mutants, studying the affects of the amino acid changes in the mutants, in the presence of various ligands bound to the ferriheme center.

Comparisons of mushroom body structure using antibodies against taurine, glutamate and aspartate in cockroach, fly, and bee.

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A wide range of experimental evidence suggests that insect mushroom bodies support a variety of higher functions: olfactory discrimination, olfactory learning and memory, place memory, and contextual sensory discrimination. These attributes have been determined from a variety of insects and may not necessarily all be present in any one. Nevertheless, as shown in many recent neuroanatomical studies, mushroom bodies of different insects comprise similar types of efferent, afferent, and intrinsic neurons. The question that such studies raise is whether the internal organization of mushroom bodies across species suggest common functional properties. In our studies we found that Kenyon cells of cockroach *(Periplaneta americana,* Blattaria), bee (Apis *mellifera*, Hymenoptera) and fly *(Phaenicia sericata* and *Drosophila melanogaster,* Diptera) express glutamate, aspartate and taurine like immunoreactivity. In all three species there is similar chemical anatomy and developmental sequence of classes of mushroom body neurons. For example, across taxa, the last born neurons contain glutamate like immunoreactivity and do not contain taurine and aspartate. The conservation of the immunoreactivity patterns in these three widely separated species supports the conservation of structural and functional subdivisions amongst Kenyon cells. Supported by Human Frontier Science program RG0143/2001 B.

Plasmodium-vector interactions *in vivo* and *in vitro*: implications for the design of rational intervention strategies

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Analysis of the survival of *Plasmodium* in mosquitoes previously considered to be either 'vector' and 'non-vector', reveals that incompatibilities exist at many levels. These include the triggering of gametogenesis within the gut, during traversal of the midgut wall by the ookinete, and during migration of the sporozoite from the oocyst to the salivary glands. Progress in our analysis of *Plasmodium* ookinete development will be reviewed in the light of new developments in the analysis of protein expression. We have used techniques such as SSH, proteomic and gene knockout analyses in combination with *in vivo* and *in vitro* approaches to examine the biological roles of numerous important vaccine candidates (e.g. P28; P25; CTRP; chitinase) and other novel molecules. We have developed an improved technique for the serial culture of all the sporogonic stages of *Plasmodium in vitro* with which we have shown sporozoite infectivity to the vertebrate host will develop normally without a period of residence within the salivary glands.

Desaturases involved in the sex pheromone biosynthesis of New Zealand leafroller moths

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Among insects, sex pheromones form the basis of an extremely sensitive and precise mate recognition system where male moths respond only to pheromone produced by a con-specific female. We are using tortricid moths of the genera *Ctenopseustis* and *Planotortrix* to investigate how specific sex pheromones are being biosynthesised in closely related species. *Ctenopseustis* and *Planotortrix* moths are closely related, as defined by morphology, behaviour and pheromone use. Indeed, a number of species within these genera have been classified as 'pheromonal' species, as female moths of these genera produce fourteen carbon acetate sex pheromones that differ only in the position of a single double bond. We hypothesise that the enzymes responsible for double bond formation, the desaturase enzymes, play a central role in determining the specificity of the sex pheromones. We have isolated pheromone gland desaturases from five species within the two genera. These desaturases can be divided into two broad classes based on sequence similarity. We have designed a novel insect cell expression system to characterise the desaturases based on substrate preference and specificity of double bond formation. This model system allows us to investigate the role of desaturases in pheromone specificity, and the role they may play in the speciation of these New Zealand tortricid moths.

PTTH-stimulated ecdysteroid secretion, and cAMP synthesis, are dependent upon tyrosine phosphorylation in the prothoracic glands of *Manduca sexta*

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Prothoracicotropic hormone (PTTH) stimulates ecdysteroid secretion by the insect prothoracic glands. In *M. sexta*, PTTH has been found to activate cAMP synthesis in a calcium-calmodulin-dependent manner, leading in turn to the activation of cAMP-dependent protein kinase, phosphorylation of ribosomal protein S6, protein synthesis and ecdysteroid synthesis. We have recently found that PTTH also stimulates a rapid increase in tyrosine phosphorylation of at least five glandular proteins, as seen on Western blots of glandular lysates probed with anti-phosphotyrosine antibody. The pattern of phosphotyrosine-containing proteins stimulated by PTTH is mimicked at least in part by the calcium ionophore A23187, but not by cyclic AMP analogs. Inhibition of tyrosine phosphorylation with PP1, a specific inhibitor of Src-family tyrosine kinases, blocks PTTH-stimulated ecdysone secretion. PP1 also blocks PTTH-stimulated cAMP synthesis as determined by cAMP radioimmunoassay. PP1 has no effect on the steroidogenic effects

of the adenylyl cyclase activator forskolin, cAMP analogs, or the calcium ionophore A23187. These findings point to an important role for a Src-family tyrosine kinase on a PTTH-stimulated event that precedes the actions of cAMP and calcium, possibly at the level of a calcium channel. Supported by NIH R01 DK53992 to WAS, and NU-CEA Undergraduate Research Awards to JM and RP.

Identification of 20E response proteins in the salivary glands of the ecdysone-deficient *woc^{rgl}* mutant of *Drosophila melanogaster* using proteomic approaches

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The ecdysteroid deficient mutant *without children^{rgl}* (*woc^{rgl}*) of *Drosophila melanogaster* has a mutant allele located in the polytene chromosomal region 97F and the larvae homozygous for the recessive lethal allele *woc* fails to puparitate. The distinguishing phenotype associated with the homozygous *woc* larvae and the ability of the homozygous larvae to puparitate when treated with 20-hydroxyecdysone (20HE) make the *woc* mutant an excellent model system for study of the developmental gene network controlled by 20HE. Protein expression patterns in salivary glands from the third instar larvae of both the wildtype and the mutant were analyzed using 2-D PAGE and compared. Several *woc* mutant specific proteins were identified and their expression could be suppressed by 20HE incubation in a dose-dependent manner, suggesting that 20E negatively regulates the expression of these proteins. The mutant specific, 20HE-suppressed proteins were then isolated from 2-D gels, trypsinated and subjected to MALDI-TOF analysis. The data generated from MALDI-TOF analysis were used to search for the match up in the SWISS-PROT database using ProFound software. The matched proteins were verified using Western blot and immunocytochemistry with available antibodies. The unmatched proteins that could not be verified by Western Blots were subjected to partial amino acid analysis for identification.

Analysis of mosquito midgut responses to the developing *Plasmodium* oocyst.

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For transmission to occur, the malaria parasite has to complete a complex developmental cycle in the mosquito. After penetrating the midgut epithelium the ookinete remains beneath the basal lamina where it differentiates into an immotile oocyst. Knowledge of the mosquito defenses to the presence of the parasite is incomplete. We have constructed a subtraction cDNA library (*Plasmodium berghei*-infected *Anopheles stephensi* midguts minus non-infected midguts) enriched for genes up-regulated by parasite infection. The sequences of 692 ESTs were searched against databases. One clone (AsFLP) with similarity to fibrinogen-domain-containing proteins was selected for further study. The full-length cDNA was cloned by 5' and 3' RACE. The 921bp AsFLP cDNA codes for a predicted protein of 306 amino acids, including a 19-amino acid signal (secretion) sequence at the N-terminus and a 200-amino acid fibrinogen-domain at the C-terminus. Semi-quantitative RT-PCR and northern analysis indicate that the gene is induced by an infected, but not by a non-infected, blood meal. The similarity of this gene with horse-shoe crab tachylectin suggests that it could act as a broad-specificity immune molecule through interaction with glycoprotein acetyl groups. Moreover, because fibrinogen can interact with thrombospondin I (TSP) domains, the possibility arises that the AsFLP protein recognizes TSP domains of *Plasmodium* surface proteins, such as CS and TRAP.

Immunological inactivation of elongation factor-1 alpha in Sf21 cell lysates

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Elongation factor-1 alpha (EF-1a) is responsible for the binding of aminoacyl tRNA to the acceptor A site on the ribosome during eukaryotic protein chain elongation. Because EF-1a is essential for protein synthesis, the enzyme offers an excellent target for the disruption of insect metabolism, but agents known to interfere with EF-1a activity are toxic to humans. We have circumvented this problem by producing monoclonal antibodies (Mabs) that can disrupt insect EF-1a activity without cross-reacting with the human enzyme. Mabs were generated to EF-1a derived from Sf21 cells by immunizing mice with EF-1a eluted from SDS-PAGE gels. The Mabs were screened for cross-reactivity to human EF-1a by immunoblotting against human cells. The Mabs were further screened for the ability to immunoprecipitate EF-1a in its native conformation from insect cell lysates. Mabs that recognized the native enzyme in insects, but not humans, were added to lysates of Sf21 cells to determine whether the antibodies could inhibit incorporation of [^{35}S]-methionine into newly synthesized in vitro translation products. Of the four EF-1a-specific Mabs tested, three significantly inhibited protein synthesis when added to Sf21 lysates at antibody-to-enzyme molar ratios of 0.5:1, 1:1, and 2:1 ($P<0.05$).

Molecular cloning of cDNAs for three abundant cuticular proteins involved in the sclerotization of pharate pupal cuticle of *Manduca sexta*

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The insect cuticle is a dynamic and complex structure that functions

as both an exoskeleton and an interface and physical barrier to the environment. The chemical and physical properties of sclerotized cuticle are determined in part by the interactions of quinone metabolites of N-acylcatecholamines with cuticular proteins. It is hypothesized that these catechol derivatives are oxidized to quinones and quinone methides that readily react with nucleophilic imidazole nitrogens of cuticular protein histidine residues, forming carbon-nitrogen adducts and cross-links. To investigate further the mechanisms of catechol-protein sclerotization reactions in cuticle, large quantities of proteins are required as substrates in model reaction experiments. As a first step, we have cloned cDNAs for three major cuticular proteins from the pharate pupal integument of the tobacco hornworm, *Manduca sexta*. Small amounts of these proteins were purified previously from pharate pupal cuticle and the amino acid sequences fully determined. Good agreement was found between the cDNAs and the amino acid sequences determined by Edman degradation. Protein extractability in chaotropic solvents decreased as sclerotization progressed, suggesting that these proteins were covalently bonded to catechols to produce a stabilized exoskeleton. In future research, we will express recombinant forms of these cuticular proteins and utilize them in *in vitro* model experiments involving oxidized catechol derivatives to elucidate cuticle sclerotization reactions.

Characterization of ecdysone-inducible early gene E74 during mosquito vitellogenesis

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In the mosquito, *Aedes aegypti*, expression of genes encoding yolk protein precursors is triggered by a blood meal via ecdysteroid gene regulatory hierarchy. The early E74 gene, which belongs to the Ets transcription factor super family and is characterized by binding to a DNA motif GGAA, is one of the essential components of this hierarchy. We have cloned two mosquito isoforms, AaE74A and AaE74B, which differ in their 5'-untranslated regions as well as in N-termini of coding regions. *In vitro* fat body culture demonstrated that the two isoforms were inducible by 20-hydroxyecdysone. Transcript expression profiles and transient transfection assays suggested AaE74B may function as an activator whereas AaE74A as a repressor during vitellogenesis. Using electrophoresis gel mobility shift assay (EMSA) and the E74 consensus sequence, a protein-DNA complex was detected from in nuclear extracts from fat bodies of females at 12-24 hr post blood meal, but those at pre-blood meal and at termination period not. Importantly, EMSA demonstrated that a region, called hormone enhancement unit on vitellogenin promoter, contains E74 binding sites, and the transient cell transfection assay showed that this region is indeed responsible for transactivation by AaE74B and repression by AaE74A.

Structures of circadian clock genes in two lepidopteran model systems *Antheraea* and *Bombyx*

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We have cloned circadian clock genes from well known lepidopteran model species, *Antheraea pemyi* and *Bombyx mori*, since the regulatory mechanism of circadian systems in lepidopterans seemed different from what was found in *Drosophila melanogaster*. The most prominent feature in lepidopteran systems is that the Period protein never enters the nucleus. In *B. mori*, we have obtained multiple transcripts for per, tim, and cyc which have sequence differences as well as clk and cry. The latter two are detected in the EST data base. In *A. pernyi*, we obtained multiple transcripts of clk and cyc. The implications of the presence of both complete and trancated products in the regulation of circadian system shall be discussed.

Identification of corazonin and its new physiological function in the silkworm, *Bombyx mori*.

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The insect neuropeptide, [Arg[7]]- corazonin was isolated from the larval brain of the silkworm, *Bombyx mori*, and its amino acid sequences was identified. To investigate its effects on development, [Arg[7]]- corazonin was injected into fourth- and fifth-instar *B. mori* larvae. A single injection of 50 pmol of corazonin into fourth- and fifth-instar larvae induced prolongation of spinning period in all experimental groups except for those injected on day 10 of the fifth-instar, while it had no effect on the timing of larval ecdysis, larval body color and the feeding period of fifth instar. The spinning period was significantly prolonged even at a low dose of 1 pmol. Both the cocoon production and the ecdysteroid increase in hemolymph during the spinning stage were suppressed by injection of corazonin. However, corazonin injection during days 5-7 of the fifth instar suppressed cocoon production without influencing the ecdysteroid level until the end of day 8, thereafter the ecdysteroid increase in hemolymph began to be suppressed. Therefore, it appears that the suppressed ecdysteroid level observed in corazonin-injected larvae was not a cause but a result of suppressed spinning activity. These effects were observed only when the peptide was injected in oil, and a huge dose was required to elicit a similar effect if it was dissolved in an aqueous solution. This study is the first published report for the corazonin effect on the behavior in insects.

Selective transcriptional activation mechanism of antibacterial peptide genes by Rel proteins in *Bombyx mori*

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Rel/NFκB family proteins are known to be transcriptional factors that bind to the κB-like motif and activate immune genes in *Drosophila melanogaster*. A κB-like motif is also located in the 5'upstream regions of *Bombyx mori* antibacterial peptide genes, suggesting an important role in gene activation. Electrophoresis mobility shift assays revealed that factors that bind to the κB-like motif of *B. mori* antibacterial peptide gene exist in the nuclear extract of fat bodies excised from *B. mori* larvae injected with lipopolisaccharide. Two full length cDNAs, BmRel-A and BmRel-B, which encode Rel homology domain were cloned as candidates of binding factors. BmRel-B cDNA had the same nucleotide sequence as BmRel-A except for a 239bp nucleotide deletion, which included an estimated translation start codon of BmRel-A. The BmRel-B protein has a shorter N-terminal region than the BmRel-A protein. Interestingly, both BmRel-A and BmRel-B had a leucine zipper motif, which is not present in the C-terminal region of *D. melanogaster* Rel family proteins. Transient luciferase assay indicated that the attacin gene is strongly activated by BmRel-B, but very weakly by BmRel-A. On the other hand, lebocin 4 gene is activated more strongly by BmRel-A than BmRel-B. Immune gene activation by these factors was κB-like motif dependent. These results suggest that BmRels selectively activate antibacterial peptide genes through a κB-like motif. Interestingly, other insect, *Allomyrina dichotoma*, also was also found to possess two Rel proteins, whose N terminal region is different. Only one of these proteins showed to enhance coreoptericin A gene expression. These results suggests that the selective transcriptional activation mechanism by Rel proteins is common feature in insects.

Towards a phylogenetic framework for evolutionary studies of the arthropods.

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Understanding the evolution of the great diversity of animals is a major goal of biology. We would like to understand how evolution has happened in an historical sense - which characteristics arose in which lineages, when they arose and hopefully even an adaptive explanation of why they arose. All of these questions depend absolutely on mapping the characters of interest - be they developmental or any other heritable character - onto an accurate phylogeny of the animal kingdom.

Development and evolution of *Drosophila melanogaster* melanin patterns.

True J

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Insect melanin patterns evolve rapidly and are involved with many adaptations to the biotic and abiotic environments. I am studying the developmental genetics of melanin pattern formation in *Drosophila melanogaster* in order to gain insights into which genes may be involved in pattern evolution. Using genetic studies in *D. melanogaster* and comparative studies in other *D. melanogaster* species, we have shown that wing melanin patterns develop via a two stage process consisting of spatial prepatterning of melanin producing enzymes and co-factors, followed by elaboration of these prepatterns by diffusion of precursors from the vein hemolymph. We are also studying the reciprocal roles of the melanin promoting gene yellow and the melanin inhibiting gene ebony in body and wing pattern development. We have recently demonstrated that in the body, a balance of yellow and ebony expression is crucial to the final pigmentation state of the cuticle while in the rapidly evolving wing patterns of Asian *D. melanogaster* group species, yellow and ebony show complementary expression patterns. Investigations of the regulatory evolution of these two genes in *D. melanogaster*, as well as the roles of other melanin pathway genes, are underway. Despite considerable effort there is no final consensus regarding the position of the arthropods within the animal kingdom nor over the relationships between the main arthropod groups|: insects, crustaceans, myriapods and chelicerates. We will describe a variety of molecular approaches we have taken in an attempt to answer these questions regarding the phylogenetic relationships of the arthropods.

Juvenile hormone and the evolution of the insect larva.

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With the evolution of complete metamorphosis, the goal of embryonic development shifted from making a miniature version of the adult body plan to making a modified body plan, that of the holometabolous larva. Experimental studies on various types of insect embryos suggest that a shift in the timing of juvenile hormone (JH) appearance during embryogenesis was an important factor in this transformation. For example, JH applied to grasshopper embryos early in embryogenesis has two effects which together result in miniature, deformed embryos. These effects are best illustrated for the developing eye. After treatment with JH, the establishment of the eye primordium and the subsequent patterning of its ommatidia still occur on schedule but secondary growth of the primordium is suppressed, thereby resulting in a miniature eye. In addition, JH induces the precocious maturation of the patterned eye tissue, which occurs around katatrepsis and may involve an interaction with the early ecdysone peak that causes embryonic cuticle formation. Consequently, we see that early JH exposure has two actions that would have been needed for the evolution of a larval stage: it allows the establishment and maturation of the primary primordium, which can then be adapted to the needs of a modified larval stage. It also

suppresses the secondary growth of the primordium, setting aside embryonic tissue that could be used later to form the imaginal discs for the adult structure. Comparison of JH effects in grasshopper embryos with insects from more advanced (Lepidoptera: *Manduca*) and more basal (Thysanura: *Thermobia*) groups will illustrate how the timing of embryonic development in diverse groups may have facilitated this action of JH. Supported by the National Science Foundation.

Lipids transport in the *Bombyx mori* mutants of impaired carotenoid transport

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Carotenoid transport mutants offer unique research tools for studying lipid specific transporters in insects. We utilized the *YI*, *Y*+^{I}, +^{Y}*I* and +^{Y}+^{I} mutants to characterize lipid absorption and transport from midgut to silk glands via lipophorin. The carotenoid-binding protein (CBP), product of the *Y* gene, controls transport of carotenoid to midgut epithelial cells. The *I* gene product inhibits transfer of carotenoid from midgut epithelial cells to hemolymph lipophorin. Whether *Y* or *I* gene products also control lipid transport, such as diacylglycerol, cholesterol and hydrocarbon within the cell is not clear. When day 3 of 5th instar larvae of *Bombyx mori* mutants were fed [^{3}H] oleic acid, the free fatty acid was absorbed into the midgut epithelial cells and appeared in phospholipid, di-and triacylglycerols. Subsequently the radioactivity appeared in lipophorin exclusively in the diacylglycerol form. In all mutants, lipophorin diacylglycerol was rapidly cleared from the hemolymph, and most of the label appeared in fat body and silk gland as triacylglycerol. In contrast, the amount of carotenoids transported to the hemolymph was 100-fold greater in the *Y*+^{I} than in all other mutants. These results confirm the notion that aside from carotenoids, lipid absorption and mobilization in the carotenoid transport mutants of *B. mori* is normal. This further, substantiates the specific function of CBP encoded by the *Y* gene.

Mosquito transposable elements: diversity, evolution, genomic impact, and potential applications

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We have recently developed a suite of novel bioinformatic tools for rapid discovery and genome-scale analysis of different classes of transposable elements (TEs). Using these tools, we have discovered a number of novel TEs in the African malaria mosquito, *Anopheles gambiae* (e.g., Tu, 2001, PNAS, 98:1699; Tu, 2001, Gene, 263:247). In addition, we have isolated more than 30 families of TEs in the yellow fever mosquito, *Aedes aegypti*. Comparative genomic analyses of a number of Culicine and Anopheline mosquitoes suggest recent explosive amplifications of a few of these TEs in some Aedes species. The relative abundance and distribution of TEs may have contributed to the differences in size and organization of different mosquito genomes. We are currently exploring the potential applications of mosquito TEs from two different perspectives. First, we obtained preliminary evidence of active transcription and/or excision of a few DNA TEs in an *An. gambiae* cell line. Such TEs, if confirmed to be active, can be developed as transformation and mutagenesis tools. In addition, active endogenous TEs may serve as models to understand the behavior of mosquito TEs in natural populations. Such information will help evaluate the possibility of using transposons to drive transgenes into wild populations, which is otherwise impossible to directly study without the release of transgenic mosquitoes. Secondly, we have developed TE-display, a rapid genome-scan method to identify and isolate polymorphic TE insertions in both *An. gambiae* and *Ae. aegypti*. Such polymorphic TE insertions are powerful markers for the analysis of genetic variations in mosquito populations, which offer several significant advantages and complement current population genetic approaches. Supported by a NIH grant AI42121

Induced ectopic expression in transgenic *Bombyx mori*

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The newly pioneered germline transformation using the transposon *piggyBac* and the discovery of now opens ways for causal tests of gene functions also in non-drosophilid insects. One can interfere with the normal function of a gene by overexpressing its dominant-negative version or silence the gene via RNAi by expressing dsRNA. The two prerequisites for this approach are the ability (1) to transform the insect, and (2) to drive the ectopic expression of the transgene. We achieved stable germline transformation of the silkmoth *Bombyx mori* with the *piggyBac* transposon vector 3xP3-EGFP and showed that the *Drosophila melanogaster hsp70* promoter can be used for heat-inducible transgenic expression in live silkworms. We chose to overexpress the *B. mori* nuclear receptor Ftz-F1, an ecdysone response transcription factor required for metamorphosis in *D. melanogaster*. The *ftz-f1* coding sequence was cloned between the *hsp70* promoter and the terminator in the *piggyBac* vector. The transgenic *ftz-f1* mRNA was induced by 60-90 minute exposures of 2nd, 4th and 5th instar larvae to 42°C. Although basal expression at 25°C was detectable by RT-PCR, Northern blot hybridization showed only the heat dependent expression. Low levels of the mRNA were still present 6 hours after the heat treatment. Immunostaining of epidermis using anti-BmFtz-F1 antibody showed an increase of a nuclear signal 90 minutes after a heat shock. The above results suggested that the *hsp70* inducible system may be useful for a transgenic RNAi approach and encouraged us to design a *piggyBac*

vector for heat-inducible expression of the *ftz-f1* dsRNA. Supported by a FIRCA NIH grant R03 TWO1209-01 and Grant Agency of the Czech Republic.

Variability in and analysis of microarray data.

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Microarrays are becoming widely used to identify stress related changes in gene expression. In microarray experiments, the large number of genes sampled coupled with high experimental and biological variability create complex statistical issues that have not been resolved even for relatively simple experimental designs. These problems are magnified when the objective is to compare populations or species that may differ in their stress responses (e.g. magnitude or timing of expression changes, identities of genes involved). We are identifying and developing analytical techniques to model variability and gain accurate estimates of error in gene expression data from microarrays. We will examine variability and look at methods using a *Drosophila melanogaster* data set, and discuss the most effective methods of analyzing microarray data.

Gene silencing in *Manduca sexta*: Examining the role of the nicotinic subunit MARA1.

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Peripheral mechanosensory neurons in the caterpillar *Manduca sexta* release acetylcholine into the CNS to activate motoneurons and interneurons. A strong component of central cholinergic excitation is mediated by nicotinic acetylcholine receptors (nAChRs) but it has been difficult to assess the contribution of individual subunits to this response using heterologous expression. In these studies we show that RNA interference can effectively silence specific genes in fifth instar *M. sexta* larvae and that it can be used to determine the function of *M. sexta* acetylcholine aeceptor alpha 1 (MARA1) subunit and other signaling molecules on the nervous system. MARA1 can be detected in isolated cultured neurons by ISH, and its presence correlates with large nicotinic Ca^{++} responses. Downregulation of MARA1 significantly reduced both the number of cells responding and the amplitude of the remaining Ca^{++} response. To assess the efficacy of this approach in intact nervous systems we have developed an injection method to induce dsRNA into single ganglia. Preliminary experiments using the Nitric Oxide Synthase (NOS) as an easily visualized marker we find that NOS dsRNA injections appear to downregulate its expression and the effect spreads to adjacent ganglia. Ganglion injections of intact animals show that animals heal their wounds and that they show normal behaviors in the next 24 hrs post-injection. We are looking at the longer effects of downregulating NOS and MARA1 on segment specific behaviors and motor responses. Supported by NSF grant IBN 0077812

Universal assay system for binary gene expression techniques in insects

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Recent interest in functional genomics has rapidly increased also for non-model organisms. The vast amount of genetic sequence information, however, needs to be linked to biological function. This can be reached by insertional mutagenesis based on transposable elements. With this approach, mutating and tagging of a gene is possible at the same time. In addition, the genome can be scanned for enhancer sequences. For such multi-component genetic systems our lab has developed broad range transposons and separable transformation markers based on EGFP and its variants or Ds-Red. Our enhancer trap approaches are designed as binary ectopic expression systems, so that cloned genes can be driven by the trapped enhancer and their function can be tested in various tissues. In order to test different binary expression systems in non-model organisms, we employ the reliable eye-specific promoter (3xP3), which was originally developed in our lab for universal transformation systems. This promoter has been shown to be functional in dipteran, lepidopteran and coleopteran species. Under 3xP3 control, we place *Gal4*, *Gal4Δ*, *Gal4VP16*, *tTA*, or *lexAGAD* and test if they could mediate eye specific expression of *UAST lacZ*, *UASP lacZ*, *TRE lacZ*, or *LL lacZ* responder genes, respectively. Due to the broad range applicability of the transposons and markers used, this assay system should be transferable to many different insect species and help us to identify which binary expression system works best.

Effects of insect hormones and growth regulators on the expression of larval specific genes of mosquitoes *Aedes aegypti*

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The goal of this project is to isolate developmentally specific genes from *Aedes aegypti* mosquitoes extracted from a subtracted cDNA library. The subtracted cDNA library was from early 4th instar and pupal stages of mosquitoes, where metamorphosis occurs. More than 30 genes were identified, and several of them were selected for further study. Two genes were studied: cuticle protein gene (Ae4-7) and unknown gene (Ae4-54). These genes were analyzed by dot and Northern Blots and showed stage specific expression. Transcription of these genes was affected by methoprene, an insect growth regulator. Tissues cultures with JH, 20E and both JH/20E showed that expression of these genes were regulated by insect hormones in a specific way as well.

Investigations of sensory projections into the subesophageal ganglion of larval *Manduca sexta* over developmental time.

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During the course of larval development, larval *Manduca sexta* increase in weight by a factor of about 10^4 in the span of a few weeks before undergoing pupation. In the pupae, it is well known that the central nervous system (CNS) of holometabolous insects undergo a radical restructuring, but the changes in the CNS during the larval period have not been as well-investigated. In this study, we looked at the changes in gross size of the subesophageal ganglion (SEG) through the five larval instars, as well as the sensory projections of neurons into the three embryonic neuromeres (maxillary, mandibular, labial) using anterograde dye fills of the maxillary, mandibular, and labial nerves. Our findings indicate that the size of the SEG grows much less dramatically over the larval instars than the larval weight. In contrast, the sensory projections over the five larval instars from the three nerves are very consistent, growing in rough proportion to the linear dimensions of the SEG while maintaining their stereotypic projection patterns in the different neuropiles within the SEG. Supported by NIH Grant R15-DC01939-01 and the Whitehall Foundation F96-11.

Molecular cloning of two cDNAs from the salivary glands of the stable fly, *Stomoxys calcitrans*

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Salivary glands of blood sucking insects such as the stable fly *Stomoxys calcitrans* (Diptera: Muscidae) secrete proteins with anticoagulant effects to facilitate blood feeding. We have dissected salivary glands from male and female stable flies for RNA extraction and cDNA library construction. cDNA clones from the library were randomly sequenced. Two clones with potential function in blood feeding were identified. One sequence encodes a protein similar to thrombostasin. The complete cDNA sequence is 1,073 bp, containing an open reading frame of 927 bp, which encodes a protein of 309 amino acid residues. The protein sequence includes a putative 18-residue signal peptide and a 291-residue mature protein. The calculated molecular weight of this protein is 33,617 Da and the isoelectric point is 5.27. The deduced amino acid sequence of this mature protein shows 34% identity to thrombostasin from the horn fly (*Haematobia irritans*). The other sequence encodes a protein most similar to antigen 5 precursor from the salivary glands of the tsetse fly (*Glossina morsitans morsitans*), and its complete cDNA sequence is 888 bp. This cDNA contains an open reading frame of 780bp, encoding a 260-residue protein. The protein sequence includes a putative 20-residue signal peptide and a 240-residue mature protein. The calculated molecular weight and pI for this protein are 26,833 Da and 8.75, respectively. The deduced amino acid sequence of the mature protein shows 57% identity to antigen 5 precursor of the tsetse fly, 56% identity to Ag5r2 gene product of fruit fly (*Drosophila melanogaster*), 43% identity to putative secreted protein from salivary glands of *Aedes aegypti*, 41% identity to salivary allergen 2 of cat flea (*Ctenocephalides felis*), 39% identity to salivary antigen 5-related 2 protein of *Anopheles gambiae*, 36% identity to venom allergen 5 of common paper wasp (*Polistes fuscatus*), and 35% identity to venom allergen of red imported fire ant (*Solenopsis invicta*), black fire ant (*Solenopsis richteri*) and bald-faced hornet (*Dolichovespula maculata*). Characterization of the two proteins and their expression in stable flies is underway.

Prophenoloxidase-activating proteinase-2 from hemolymph of *Manduca sexta*

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Proteolytic activation of prophenoloxidase in insects is a component of the host defense system against invading pathogens and parasites. This process is mediated by a specific enzyme named prophenoloxidase-activating proteinase (PAP), but detailed molecular mechanisms of prophenoloxidase activation remain unclear. We have purified from hemolymph of the tobacco hornworm, *Manduca sexta*, a new serine proteinase that cleaves prophenoloxidase. This enzyme, designated PAP-2, differs from another PAP, previously isolated from integuments of the same insect (PAP-1). PAP-2 is composed of two clip domains at its amino terminus and a catalytic domain at its carboxyl terminus, whereas PAP-1 contains only one clip domain. Purified PAP-2 cleaved prophenoloxidase at Arg^{51} and yielded a product that does not have much phenoloxidase activity. However, in the presence of a mixture of two serine proteinase homologs, active phenoloxidase was generated at a much higher level and it formed high molecular weight polymers that are covalently linked. Since these proteinase-like molecules also associate with a bacteria-binding lectin in *M. sexta* hemolymph, they may be important for ensuring that the activation of prophenoloxidase only occurs in the vicinity of invading microorganisms. PAP-2 mRNA was not detected in naïve larval fat body or hemocytes, but became very abundant after the insects had been injected with bacterial cells. We are determining the nucleotide sequence of the PAP-2 gene to elucidate its exon-intron structure and regulatory elements.

Molecular and biochemical characterization of two P450 enzymes in the ecdysteroidogenic pathway of *Drosophila melanogaster*

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As many as seven different enzymatic activities, catalyzed by both microsomal and mitochondrial cytochrome P450 monooxygenases (CYP), have been hypothesized to be implicated in the biosynthesis of ecdysone, although none have been characterized completely, either at the biochemical or molecular levels. The present data show that the wild type genes mutated in two members of the Halloween Family of cuticular embryonic lethals, *disembodied (dib)* and *shadow (sad)*, code for CYP enzymes that catalyze the last two hydroxylation reactions in the ecdysteroidogenic pathway in *Drosophila melanogaster*, namely the C_{22}- and C_2-hydroxylases, respectively. Although the site of molting hormone biosynthesis in the embryo is unknown, during early embryogenesis when the ecdysteroid titers peak, these genes are expressed primarily in the epidermis. Subsequently, their expression during late embryogenesis, larval and adult stages is restricted to known ecdysteroid producing tissues. When transfected into *D. melnogaster* S2 cells, *sad* (CYP315A1) metabolizes 2-deoxyecdysone (2dE) to ecdysone (E), while *dib* (CYP302A1) converts the ^{3}H-ketotriol (2, 22-dideoxyecdysone) to ^{3}H-2dE. These data were confirmed when cells transfected with both *dib* and *sad* were shown to transform the ^{3}H-ketotriol to ^{3}H-E in high yield. Consistent with prior partial biochemical characterizations, these enzymes appear to be targeted to the mitochondria, based on their charged and hydrophilic N-terminal residues as well as their sequence similarities to other known mitochondrial cytochromes in both insects and humans. We are presently investigating the involvement in *D. melanogaster* ecdysteroidogenesis of three other CYP300 level enzymes shown to be the targets of mutations in the related Halloween mutants *spook, phantom* and *shade*. However, unlike *dib* and *sad*, these cytochromes appear to be microsomal.

Genomes and malaria parasite transmission.

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Recent advances in technology and genome sequencing have allowed new approaches to be taken to the investigation of transmission of malaria parasites by Anopheline mosquitoes. Comparative genomics has shown that the genome of the model rodent parasite, *Plasmodium berghei*, is remarkably similar to that of the major human malaria pathogen, *Plasmodium falciparum*. This relationship allied to the tractability of the rodent parasite and its biological similarity to human malaria parasites makes *P. berghei* an ideal model system. Bioinformatics has allowed the definition of a conserved gene family, P48/45 that encodes cysteine rich proteins that are involved in gamete fertilisation and perhaps other aspects of transmission. Parasite genetic manipulation allows gene disruption, replacement and transgene expression and facilitates a direct investigation of protein function and parasite vector interactions. Examples of gene replacement and direct visualisation of *in vivo* parasite/vector interactions will be demonstrated. Finally the application of DNA microarrays to malaria parasites is now possible and we demonstrate the developmentally regulated transcriptional programmes of the model rodent malaria parasite.

Homology modeling shows dramatic differences in surface charge patterns between ligand-binding domains of mosquito vitellogenin and *D. melanogaster* yolk protein receptors

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Vitellogenin (Vg) is the major yolk protein that plays a vital part in the life cycle of disease carrying insects such as the yellow fever mosquito *Aedes aegypti*. It is secreted by the fat body, and is accumulated by developing oocytes via receptor-mediated endocytosis. The mosquito Vg receptor (AaVgR) is a member of the low-density lipoprotein receptor (LDLR) family. It differs from other LDL receptors by having two ligand-recognition domains with five and eight cysteine-rich 40 amino acid modules, respectively. AaVgR shares significant structural homology with the *Drosophila melanogaster* yolk protein receptor (DmYPR), despite a very different ligand for the latter. To understand potential differences between AaVgR and DmYPR underlying the mechanisms of their respective ligand specificities, we have created homology models of the 13 ligand-binding modules of AaVgR and DmYPR. Modeller 4 was used to calculate the homology models using structure-based sequence alignments as input and using six NMR and x-ray crystallography structures of known modules (PDB codes 1AJJ, 1CR8, 1F8Z, 1J8E, 1D2L, and 1LDL). Six distance restraints at the calcium binding site and three restraints at beta strand-like hydrogen bonds were used to insure tight packing. Our structural models of individual modules show good stereochemistry, contain the known calcium-binding site, form the three canonical disulfide bonds, and consist of a well-packed hydrophobic core. Significant differences in surface charge patterns exist between corresponding ligand binding domains in VgR and YPR. In particular, first and fifth modules in first ligand-recognition domain and fifth module in second ligand-recognition domain of AaVgR are highly negatively charged in contrast to those of DmYPR. The ramifications of these differences are currently being investigated. This analysis of putative ligand recognition domains has opened the door to aggressive pursuit of the question of determination of VgR epitopes responsible for specific recognition of Vg.

Estimating genomic coordination between host and symbiont:

Transcriptome analysis of the aphid bacterial endosymbiont, *Buchnera aphidicola*

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The obligate mutualism of aphids and their primary bacterial endosymbiont, *Buchnera aphidicola*, spans a 200 250 million year history. *B. aphidicola* synthesize essential amino acids to supplement the nitrogen poor sap diet of its aphid hosts. Over the course of an aphid generation, nutritional supply and demand varies with shifts between host plants, and among aphid life cycle stages. The capacity for *B. aphidicola* to adjust to varying host needs and environments is presumably critical to the stability of the aphid *B. aphidicola* symbiosis, but the *B. aphidicola* genome is among the smallest of any bacteria and lacks many recognizable regulatory elements controlling gene expression (and thereby host provisioning). We measured the response in *B. aphidicola* gene expression profiles to a variety of host manipulated scenarios using full genome microarrays. In particular, we evaluated shifts in the *B. aphidicola* transcriptome when aphid hosts were reared on plants differing in nutritional value and when aphid hosts were subjected to prolonged heat stress.

Cockroach milk: A multigene family

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The brood sac of the viviparous cockroach, *Diploptera punctata*, is the source of a secretion that is ingested by the embryos during gestation. Although the composition of this secretion was previously determined, the protein component of this secretion has not yet been characterized. Here we describe the isolation and analysis of complete cDNA sequences encoding the proteins of brood sac secretion (referred to as Milk proteins). SDS PAGE of secreted proteins resolved four bands. Sequencing of 20 amino acids from the N terminus revealed that 14 out of 20 positions are conserved between the peptides from the four bands. RT PCR, 5' RACE, and analysis of individual clones resolved 30 closely related cDNAs. All the cDNAs encode a 171 as protein that includes a 16 as signal sequence. These sequences are putatively related to lipocalins. Given that no other species of cockroach is known to be viviparous, we believe that the high abundance of *Milk* cDNAs reflects a recent expansion of the gene family. This expansion may be correlated with the evolution of a nutritional function of Milk proteins. However, given the number of genes in this family, other functions could also exist. The evolutionary relationship of Milk gene family members is discussed with respect to selection pressures and possible alternative functions of the different Milk genes.

Identification & purification of a hemolymph protein from bacteria injected *Manduca sexta* larvae and an immune related protein from the C7 10 cell line of the mosquito *Aedes albopictus.*

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In granular cells from naive *Manduca sexta* larvae, a monoclonal antibody that binds the MS39 protein binds to vesicles. In immune activated larvae the monoclonal antibody, instead, associates with cell membranes. On western blots of hemolymph proteins, it binds negligibly to hemolymph from naive larvae but strongly to a protein from immune activated (bacteria injected) larvae. The monoclonal antibody that recognizes this protein also recognizes a protein made and secreted by the *Aedes albopictus* C7 10 cell line when these cells are incubated with bacteriacontaining medium. Both the *Manduca* and *Aedes* proteins bind conA resin. The M. *sexta* protein was successfully purified and polyclonal antiserum made. This antiserum, at high dilution, binds the *Ae. albopictus* protein, suggesting that the binding of the monoclonal antibody to the *Ae. albopictus* protein is not mere structural coincidence in a particular epitope. We present immunofluorescence microscopy showing the difference in binding patterns of the antibody to hemocytes from naive versus bacteria injected larvae; western blots profiling the presence of the immune related protein in hemolymph and hemocytes from control and bacteriainjected *M. sexta* larvae and from the *Ae. albopictus C7 10* cells; and the purification scheme which involved ammonium sulfate precipitations, size exclusion chromatography, affinity chromatography (for the *Ae. albopictus* protein), and electrophoresis.

Identification and purification of a hemocyte protein released early in the immune response of tobacco hornworm *Manduca sexta* larvae.

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In naive *Manduca sexta* larvae, an immune related protein, MS 17, is present in granules of granular cells (a class of hemocytes). It is readily released by granular cells when they are suitably stimulated. We have purified the approximately 86 kDa protein by affinity chromatography using a monoclonal antibody. We show immunofluorescence microscopy indicating an early release of this protein from granular cells; a western blot of its presence in naive hemocytes; and the purification scheme based on affinity chromatography. The 86 kDa protein can be preferentially retained within the granules of the granular cells by treating the cells in vitro with relatively high concentrations of thermolysin.

Zinc and *Manduca sexta* hemocyte function *in vitro*

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What is the effect of zinc on insect immune function? We study the role of the tobacco hornworm *(Manduca sexta)* hemocytes (blood cells) in its immune response. Two metalloproteases (proteolytic enzymes that require a metal ion) have recently been linked to immune function in Lepidoptera (butterflies and moths). This suggests zinc is involved in insect immunity. We tested medium containing zinc with conventional medium that lacked zinc. In the presence of 100 ,VM zinc, plasmatocytes were substantially longer than they were in the absence of zinc. Because zinc made a difference, we tested two zinc metalloprotease inhibitors, phosphoramidon and bestatin, to see whether zinc dependent proteases may be responsible for the enhanced length seen in the presence of zinc. In the presence of either of the two inhibitors, plasmatocyte length was shorter and network formation different from the controls. This work suggests that zinc and at least two different zinc proteases are involved in *M. sexta* immune function.

Exploring neuronal and molecular mechanisms that mediate odorant stimulated nitric oxide production in the antennal lobe of *Manduca sexta.*

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The unique anatomy of olfactory glomeruli and the expression of nitric oxide (NO) synthase and NOsensitive proteins in the glomerular neuropil of many species suggests that NO signaling participates in olfactory information processing. Using immunocytochemistry m the moth *Manduca sexta*, we show that NO synthase is expressed in the axons of olfactory receptor neurons, and that soluble guanylyl cyclase is expressed in a subset of antennal lobe neurons including projection neurons, GABAergic local interneurons, and the single serotonergic neuron. Using the NO marker, diaminofluorescein diacetate (DAF 2DA), we show that stimulating the antenna with odorants causes NO production in all glomeruli. One model to explain this phenomenon is that odorant stimulated activity in a single glomerulus excites multiglomerular neurons including either GABAergic local interneurons or the serotonergic neuron, resulting in NO synthase activation in unstimulated olfactory receptor neurons. To begin to test this hypothesis, we used degenerate oligonucleotide RT PCR to identify fragments of three ionotropic GABAA receptors, one metabotropic GABAB receptor, and two serotonin receptors. The expression of these receptors on olfactory receptor neurons and their involvement in activating NO synthase will be examined. Supported by National Institutes of Health grant DC04292.

Insect transgenesis and functional insect genomics

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Genetic manipulation of insects constitutes an important tool for basic research in genetics, zoology, and ecology. In addition, it will allow for new and efficient strategies to control insect pests. One major obstacle has been the difficulty to obtain marker genes that allow easy and reliable identification of transgenic animals. Here, I will present a universal marker system that drives strong expression of enhanced green fluorescent protein (EGFP) variants in larval, pupal, and adult eyes of insects. By using this marker in combination with promiscuous transposable elements, transgenesis has been established for several species of the three insect orders Diptera, Lepidoptera, and Coleoptera. This demonstrates the wide range applicability of the system. Transformation vectors based on *Hermes*, *piggyBac*, *mariner*, and *Minos* transposons, carrying the spectral variants EGFP, ECFP, EYFP, or DsRed were constructed. Using specific filter sets, ECFP, EYFP, and DsRed represent distinguishable markers that we have used to develop genetic multi-component systems. Insertional mutagenesis based on transposons presents a fundamental tool for functional genomics. The principle of transposon mutagenesis relies on the mobilization of transposable elements that can insert into new genomic loci and destroy gene activities. A 'jumpstarter' element providing transposase activity is used to mobilize a visibly marked, non-autonomous 'mutator' element. While acting as a mutagen, the mutator serves as a molecular tag to easily clone the mutated gene and as a visible label for stock keeping of the mutant. If the mutator is equipped with an enhancer-sensitive reporter, gene activities can be also identified based on tissue-specific expression patterns ('enhancer trapping'). Moreover, by using a heterologous transactivator gene as a reporter, the insertion will become a tool for tissue-specific expression studies. In a pilot transposon mutagenesis screen in *Drosophila melanogaster*, *piggyBac* mutators were mobilized by a *Hermes*-based jumpstarter providing *piggyBac* transposase activity. As primary reporters in the *piggyBac* mutators, we employed the heterologous transactivators GAL4 or tTA. To identify larval or adult enhancer traps, lines carrying *UAS-eyfp* or *TRE-eyfp* as secondary reporter genes were used. The wide range applicability of our system will permit the study of biologically relevant questions in many different insect species, and not only in established model organisms.

Love potions: Identities and functions of *Drosophila melanogaster* seminal proteins

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Insect seminal fluid contains polypeptides that stimulate ovulation and oogenesis, alter the reproductive behaviors of mated females, promote sperm storage in females and, at least in *D. melanogaster*, affect the lifespan of mated females. The male's accessory gland is the source of many of these proteins. In a comprehensive EST screen, Swanson *et al.* (PNAS 98, 7375 '01) identified 90% of the 83 predicted "Acp" (accessory gland protein) genes likely to encode *D. melanogaster* secreted seminal proteins. To determine the molecular identities of the new Acps identified in this screen, we are annotating their genes. [We find differences from Celera's

published gene predictions, similar to results reported by Karlin *et al.* (Nature 411, 259 '01)].
Among ~40 (so far) newly annotated or previously reported Acps, several have significant similarities to enzymes that carry out post-translational modifications. Eleven predicted Acps share sequence or structural homology with known proteases or protease inhibitors. This suggests that regulation of proteolysis is a major function of seminal fluid, possibly to regulate prohormone processing, semen coagulation and/or sperm viability. Six Acps have similarities to lipases, suggesting that lipid modification is likely to be of importance within the mated female. Nineteen annotated Acp genes encode putative secreted peptides (<100 amino acids). Very few of these include known structural motifs (though several contain stretches of low amino acid complexity). Thus the Acp peptides represent a potential source of novel protein motifs.
We are addressing the functions of these predicted proteins by knockout and ectopic expression approaches. As an example, the phenotype of a new mutation in Acp29AB will be presented.

A novel fatty acid response element controls the expression of locust flight muscle FABP.

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Fatty acids are the preferred fuel for migratory flight of locusts. Their cytosolic transport in locust flight muscle is mediated by a fatty acid binding protein (FABP) that also serves as a fatty acid buffer. The expression of the FABP gene is induced by exposure to elevated fatty acid levels by a hitherto unknown mechanism. We found that a 130 by promoter fragment of the locust gene, which includes a canonical TATA box and several GC boxes, is sufficient for the transcription of a reporter gene in mammalian L6 myoblasts. Two fold higher expression rates are observed when the promoter contains 280 by or more of upstream sequence. Treatment of myoblasts with various fatty acids leads to a marked increase of expression in the longer constructs, but not in the minimal promoter. We have identified a 19 by inverted repeat (162/ 180) as the element responsible for fatty acid mediated induction. Deletion of this element eliminates the fatty acid response, and gel shift analysis demonstrates specific binding to nuclear proteins from both L6 myoblasts and locust flight muscle cells. A similar palindrome was also found in the promoter of the *Drosophila melanogaster* muscle FABP gene, and in reverse orientation upstream of all mammalian heart FABP genes. Given the remarkable structural and functional conservation of muscle FABPs and their genes, it is possible that this fatty, acid response element also modulates the expression of the mammalian H FABP genes.

Construction and selection of recombinant single chain antibodies binding the circumsporozoite protein of *Plasmodium falciparum*.

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The widespread resistance of malaria parasites (*Plasmodium falciparum* and *P. vivax*) to common antimalarial drugs emphasizes the need to develop novel methods to control the disease. Altering vectorcompetence by creating transgenic mosquitoes producing an antiparasite gene is an approach being tested in our laboratory. Previously, it was shown that transgenic *Aedes aegypti* mosquitoes expressing single chain antibody against the circumsporozoite protein (CSP) of *P. gallinaceum* blocked invasion of the salivary glands by sporozoites. Following the avian malarial model system, a single chain effector molecule against the CSP of P. falciparum is being produced from a mouse hybridoma cell line, 2A10, that synthesizes monoclonal antibodies against the CSP of the sporozoite stage. The variable heavy and light chain cDNAs were assembled using a linker region consisting of (G1y4Ser)3 that reconstitutes the active configuration of the binding site of the variable region. ScFv was expressed in *Escherichia coli* and preliminary experiments with dot blot analyses show the binding of ScFv to the CS peptide repeat. Production of higher amounts of ScFv using a Sindbis virus expression system is underway. This work is supported by grant AI44800 from MAID.

Evidence supporting suppression of immunity in honeybees by parasitic *Varroa* mites

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Varroa mites are one of the major contributing factors to the recent serious honey bee loss in the US. The mites endanger honey production and pollination services valued annually over 15 billion dollars in the US. Our research addresses how the mites kill honeybees by investigating the impacts of *Varroa* mites on honeybee immunity and pathology. We hypothesize that *Varroa* mites depress the honey bee immune response to various pathogens. We used *Escherichia coli* as an immune challenger by injecting it into the bees to investigate their survivorship, which reflects the total ability of the immune system of the organism. A typical symptom of *Varroa* infestation is deformed wings. We compared three types of bees: normal bees without mites, bees with mites and normal wings, and bees with mites and deformed wings. After challenge with *Escherichia coli*, bees infested with mites but with normal wings lived a significantly shorter time than normal bees. This indicates that *Varroa* mites suppress the immune response of honeybees. The bees with deformed wings lived the shortest. The survivorship curves of the three groups of newly emerged bees are significantly different. A critical enzyme and marker of some cellular immune functions is phenoloxidase (PO). No PO activity was found in newly emerged and immuno-elicitor treated bees at 0 to 24 hrs, suggesting that they are immuno-incompetent at this developmental time point. It is during this immuno-incompetent period that the bees with mites are dying when either challenged with bacteria or with a putative deformed wing virus. We have developed a method to collect mite

saliva and are searching for the factors responsible for the immunosuppression through use of proteomic techniques.

Gene expression during nerve cord development in the tobacco hornworm, *Manduca sexta*.

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The molecular process by which the insect ventral nerve cord undergoes reconstruction during adult development is unclear. Programmed cell death (PCD) appears to be quite important during this period in the tobacco hornworm, *Manduca sexta*, as up to 50% of larval-specific neurons undergo PCD and apoptosis soon after pupal ecdysis. However, the underlying molecular mechanism(s) at this critical stage of development are relatively unknown. To address this question, PCR-based subtractive hybridization was performed on nerve cords to isolate differentially expressed transcripts at the larval/pupal boundary. The nerve cord subtraction yielded multiple PCR products that may represent wandering larvae-specific clones and early pupal-specific clones. The products were characterized by partial DNA sequencing and identity searches using GenBank (BLASTX). We also cloned *dalp* (Death-Associated Lim-only Protein) a gene that encodes a product that functions as a negative regulator of muscle differentiation during development in *M. sexta*. A 350bp PCR product of *dalp* cDNA was isolated, and RT-PCR results suggest that *dalp* is significantly upregulated as the hornworm larvae approach pupal ecdysis.

Cloning of three, desiccation regulated transcripts from diapausing Colorado potato beetle, *Leptinotarsa decemlineata*

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Clones of three desiccation up-regulated transcripts (LdDes-1, -2 & -3) were isolated from diapausing Colorado potato beetle using suppression subtractive hybridization. Northern blot analysis demonstrated that LdDes-1, -2 & -3 are highly up-regulated in diapausing beetles and in desiccated nondiapausing adults. Transcript sizes are 1.35 Kb (LdDes-1), 0.8 Kb (LdDes-2) and 0.54 Kb (LdDes-3). A BlastX search revealed that LdDes-1 & -3 have identity to no known genes. The highest score for LdDes-2 was to the 28 kDa desiccation protein of *Tenebrio molitor* at 31% identity and 48% positives (identity + chemically equivalent). LdDes-1 encodes for a deduced protein of 34 kDa in size and is enriched (>9% each) for the amino acids glutamic acid, leucine, methionine, arginine and asparagine. The complete open reading frame for LdDes-3 has yet to be determined.

Molecular cloning and characterization of a novel animal enzyme: Geranyl diphosphate synthase

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The male pine engraver beetle, *Ips pini*, uses the mevalonate pathway to synthesize the monoterpene pheromone component ipsdienol *de novo*. Geranyl diphosphate synthase specifically catalyzes the 1'-4 condensation reaction between the activated C5 isoprene unit, IPP, and C5 allylic diphosphate, DMAPP, to produce the monoterpenoid, geranyl diphosphate. This C10 product is the putative precursor to the aggregation pheromone component ipsdienol in male *I. pini*. Using standard PCR techniques, we isolated an isoprenyl diphosphate synthase that may be the first geranyl diphosphate synthase identified in an animal system. Coleopterans are one of the few metazoans that synthesize monoterpene compounds; therefore, they offer a unique opportunity to isolate geranyl diphosphate synthase. Northern analysis indicates expression of this transcript occurs in a JH-dose and -time dependent manner that corresponds to expression profiles of other known pheromone biosynthetic genes in this beetle. Preliminary structural modeling shows a similar, highly conserved helical fold found in the avian crystallized structure of farnesyl diphosphate synthase, an enzyme that synthesizes sequiterpenes (C15). There are some suggested differences in the binding pockets of the putative beetle geranyl diphosphate synthase and the avian farnesyl diphosphate synthase that may reflect their different products. We have expressed the putative geranyl diphosphate synthase in *Escherichia coli* and are performing functional assays to determine product specificity.

Hemolymph juvenile hormone binding protein allelic variation among three strains of *Manduca sexta*

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Juvenile Hormones (JH) are a class of acyclic sesquiterpenoids central to insect development and reproduction. In *Manduca sexta*, the hemolymph juvenile hormone binding protein (hJHBP) is essential for hormone transport and maintenance of JH titers. The structure of the wild type hJHBP gene consists of 5 exons spanning 6.6 kilobases. Sequencing of the Seattle wild type and *black (bl)* hJHBP gene and comparison with the Madison wild type hJHBP gene revealed nearly identical open reading frames; however, significant differences were found in the non coding regions. These differences include an insertion of a 354bp SINE (Short Integrated Nuclear Element) with 18bp direct repeats in intron 2 followed by a 211 bp deletion. Other deletions exist in introns 1, 3, and in a region 383bp upstream of the putative start site. A newly developed monoclonal based enzyme immunoassay demonstrated that Seattle wild type and BI M. sexta larvae display a 40 60% lower hJHBP titer than does the Madison wild types during certain times during the 4' stadium. A series of genetic crosses were performed that established a link between the different hJHBP alleles and hJHBP

levels. The FZ ratios from these crosses fit those expected from crosses involving a single gene. Previous data from our lab demonstrated that regulation of hJHBP gene expression is under the control of JH. The present results form the framework for the identification of the transcriptional elements involved in the regulation of the hJHBP gene.

Serine protease homologs in phenoloxidase activation of the tobacco hornworm, *Manduca sexta*

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In insects, the phenoloxidase activation system is a key defense mechanism against parasites and pathogens. Activation of phenoloxidase involves a serine protease cascade. Recognition of parasites or pathogens by pattern recognition receptors activates an upstream serine protease, which then triggers activation of the serine protease cascade, leading to activation of prophenoloxidase-activating protease (PAP). PAP converts inactive prophenoloxidase (proPO) to active phenoloxidase (PO), which then catalyzes oxidation of phenolic compounds to form melanin. Because quinonlic intermediates and melanin are toxic to both hosts and pathogens, activation of phenoloxidase must be tightly regulated and localized. We report here purification and cDNA cloning of serine protease homologs (SPHs) from the tobacco hornworm, *Manduca sexta*, which help PAP-1 to activate phenoloxidase. SPHs were co-purified with immulectin-2, a C-type lectin pattern recognition receptor, from hemolymph of *M. sexta* larvae. They contain an amino-terminal clip domain connected to a carboxyl-terminal serine protease-like domain. *M. sexta* SPHs are unlikely to have enzymatic activity, because the serine residue in the reactive center of the protease domain is changed to a glycine. Addition of SPHs helps recombinant PAP-1 to activate proPO, while PAP-1 alone can not activate proPO. Immulectin-2, proPO and PAP-1 in hemolymph bound to recombinant protease-like domain of SPH-1, indicating that a protein complex containing these proteins may exist in hemolymph. Since immulectin-2 is a pattern recognition receptor that recognizes and binds to surface carbohydrates of pathogens, such a protein complex may localize activation of phenoloxidase on the surface of pathogens, whereas SPHs function as mediators to recruit proPO and PAP to the site of infection.

Role of doublesex in the female-specific regulation of a mosquito hexamerin gene

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Fourth-instar larvae of the autogenous mosquito, *Aedes atropalpus*, synthesize one hexamerin or hexameric storage protein, Hexamerin 1.2 (AatHex-1.2), which is unique to females. *AatHex-1.2* mRNA accumulates only in female larvae and pupae and remains at low levels in adult female mosquitoes. This is the first observation of female-specific gene activity during pre-adult development of a dipteran insect. In order to investigate the molecular basis for this sex-specific expression, we have cloned and sequenced the *AatHex-1.2* gene, including over 1 kb of the 5'-flanking region. Putative binding sites for transcription factors such as GATA, C/EBP and doublesex, upstream from the coding region of the *AatHex-1.2* gene, suggest that mechanisms for sex-specific transcription in the fat body may be well conserved. To map conserved regulatory sequences in the *AatHex-1.2* gene, we generated germline *Drosophila melanogaster* transformants containing 0.7 kb of the 5'-flanking region, including two putative binding sites for the Doublesex (DSX) transcription factor, fused to a *luciferase* reporter gene. The *AatHex/luc* fusion gene was expressed in a tissue-specific (fat body-specific) and stage-specific manner. However, these same sequences were only able to partially repress luciferase expression in males. To demonstrate a role for the putative DSX binding sites, we crossed into one of the transformed fly lines a mutated *transformer* (*tra*) gene whose expression produces incorrect splicing of the *doublesex* gene transcript. Luciferase activity in genetic female/pseudo-male flies, that synthesize only the male form of the DSX factor due to the *tra1* mutation, was significantly reduced as compared to normal genetic females. Furthermore, luciferase levels of genetic female/pseudo-male flies were not significantly different from normal genetic males. These results suggest that the male form of DSX is able to repress *AatHex/luc* expression and that the putative DSX binding sites in the *AatHex* 5'-flanking region are functional in *D. melanogaster* transformants. Currently, we are generating transgenic mosquitoes to determine which *AatHex* regulatory elements can be used in common to generate sex-specific gene activity in both higher and lower dipteran larvae.

Expression cloning techniques for isolating membrane-bound lipid transporter cDNA's.

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Many species of insects such as *Manduca sexta* rely on lipids as their primary source of energy and for hormone production. The main purpose of the *M. sexta* larval stage is to feed and store sufficient nutrients to provide energy and resources for complete metamorphosis into adult moths and for egg production in females. During the *M. sexta* larval stage all dietary lipids are absorbed and used immediately or processed and stored for later use. Sterols are essential dietary lipids as insects lack the capacity to synthesize sterol *de* novo. In *M. sexta*, as in other insects, cholesterol is required for synthesis of molting hormones (ecdysones) and is an important component of plasma membranes. FAs are important dietary components as they are used for energy production (beta-oxidation), energy storage (in the form of triacylglycerol), and are essential components of phospholipid bilayers. Since lipid movement across

the plasma membrane is thermodynamically unfavorable, it has been proposed that plasma membrane proteins are necessary to facilitate the transport process into the aqueous cytoplasm. In this study an stably transfected monkey kidney cell line (COS-7) containing fat body and midgut cDNA expression libraries were incubated with fluorescent lipid analogues to show for the first time the putative existence of several proteins involved in membrane-bound lipid transport mechanisms. This novel functional protein screen provides a new and efficient tool for identification of potential transporter proteins.

A molt-associated chitinase cDNA from the spruce budworm, *Choristoneura fumiferana*

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A chitinase cDNA was cloned from the spruce budworm, *Choristoneura fumiferana*, by using RT-PCR with degenerate primers based on its *Manduca sexta* cDNA homolog. The cDNA was 2856 nucleotides long with an open reading frame of 1674 nucleotides potentially encoding a protein of 558 amino acids with a predicted molecular mass of 62kDa. The deduced amino acid sequence showed high similarities (76-79%) to chitinases of other Lepidoptera. The cDNA was expressed in bacterial and baculovirus systems into active recombinant proteins with an apparent molecular mass of 72kDa, while the native protein was 74kDa. The recombinant proteins reacted positively with the antiserum raised against the *M. sexta* chitinase. Developmental expression showed that *C. fumiferana* chitinase mRNA appeared prior to each molts from the second instar to the pupal stage, but disappeared immediately after the molts. Western blot analysis further revealed that the protein appeared 12hr prior to ecdysis. By 12hr after ecdysis the protein completely disappeared again. There are no transcripts detected during the intermolt periods. The ecdysone agonist, tebufenozide (RH5992), induced expression of CfChit in the early stage of 6th instar larvae and caused a precocious and incomplete molt into an extra larval stage. Western blot and immunohistochemistry showed that during the molt from the fifth to sixth instar stage, the enzyme was detected in the tissues that had an ectoderm origin and/or contained chitin such as the cuticle of integument, the epithelium of the midgut, tracheal system and Malpighian tubules. It was absent in the fat bodies and muscles. The recombinant protein expressed in *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) showed high levels of chitinolytic activity with an optimal pH range of 6-9. Glycosylation appeared to be necessary for the activity and secretion of the recombinant protein. The recombinant AcMNPV expressing chitinase had a lower LD_{50} value and killed the insects faster than control viruses when fourth instar *Trichoplusia ni* were injected with the viruses. This study was supported by the Canadian Biotechnology Strategy Funds.

Two RNA helicase cDNAs isolated from a lepidopteran species, the spruce budworm (*Choristoneura fumiferana)*

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RNA helicases are a family of enzymes that unwind nucleic acid duplexes, such as RNA/RNA and RNA/DNA, in the 3' to 5' direction to single-stranded polynucleotides. Their cellular functions may involve in the processes such as splicing, RNA editing, rRNA processing and translation initiation. Two lepidopteran cDNA clones encoding two different RNA helicases (*Cf*rHel-A and C*f*rHel-B) were isolated for the first time by screening a cDNA library of the spruce budworm, *Choristoneura fumiferana* with polyclonal antibodies produced against a methoprene-tolerant (*met*) gene from *Drosophila melanogaster*. The *Cf*rHel-A cDNA was 1997 bp long and coded a deduced protein of 555 amino acids with a molecular mass of 64kDa. The C*f*rHel-B cDNA was 3196bp long encoding a protein of 1012 amino acids with a molecular mass of 113kDa. The *Cf*rHel-A was similar to the *Candida albicans* translation initiation factor and the cold shock-induced RNA helicases of *Escherichia coli* and cyanobacterial *Anabaena sp*. The C*f*rHel-B was similar to the *D. melanogaster* vasa protein, human KIAA0801 protein and rat HEL117 protein. Both sequences contained DEAD (Asp-Glu-Ala-Asp) boxes and all the 8 functional motifs conserved in all members of the DEAD box RNA helicase family. The C*f*rHel-B contained RS and DERK domains at the N-terminal end of the deduced amino acid sequence, which may be involved in RNA binding. The *Cf*rHel-A did not contain these two domains. The *Cf*rHel-A cDNA was expressed in both bacterial and baculovirus systems and the generated protein had a molecular mass of 64kDa, as expected from its predicted amino acid sequence. Developmental expression of *Cf*rHel-A mRNA in *C. fumiferana* revealed that there were two transcripts detected when the full length *Cf*rHel-A cDNA was used as a probe. Embryos and the 1st instar larvae had a background level of mRNA expression, whereas high levels of expression can be detected through the second instar to the sixth instar stages. Higher levels of expression were detected in the fat body and midgut than in the epidermis of 6th instar larvae. Biological functions of these RNA helicases are under investigation. This study was supported in part by the Canadian Biotechnology Strategy Fund.

The temporal and spatial distribution of ^{14}C into the multiple metabolic pathways in the mosquito *Aedes aegypti* after ^{14}C-labeled protein meal and its biochemical and physiological significance

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Adult *Aedes aegypti* mosquitoes, which were 5 days old and had access to 10% sucrose solution *ad libitum*, were fed on Kogan's meal containing ^{14}C-labeled *Euglena gracilis* protein, after which no sugar and water were offered. The temporal distribution of ^{14}C from meal protein amino acids into body proteins, lipids (not phospholipids), glycogen, sugar (glucose, trahalose and sucrose) and amino acids; respired $^{14}CO_2$ and excretory waste; and the incorporation of ^{14}C into egg proteins and lipids post feeding were determined. Both glycogen and sugar quickly reached an equilibrated level, which accounted for 2% and 3~4% of the total ingested ^{14}C-Euglena protein, respectively, occurring within 48 h post feeding, and subsequently declined to 1% at 72 h. The distribution patterns of ^{14}C were similar for proteins and amino acids, which showed a successive decrease with time and displayed the highest average rate of decrease (7.5%/h and 1.7%/h, respectively) during the first 6 h post feeding. The level of lipids increased at the rate of 0.1~0.8%/h, reaching a peak of 13% at 36 h, and then declined at a slow rate (0.1%/h). Although the distribution of ^{14}C into the respired $^{14}CO_2$ and the excretory waste successively increased with time, the maximal distribution rate in $^{14}CO_2$ (1.5%/h) and waste (0.9%/h) occurred from 0 to 6 h and from 36 to 48 h, respectively. In general, at 72 h post feeding, up to 68% of meal protein were used in energy metabolism, and there were 18% body proteins (including egg proteins), 9% body lipids (including egg lipids), 1% glycogen, 1% sugar and 3% amino acids remaining. At 120 h post feeding, there was about 4% and 6% of ^{14}C incorporated into egg proteins and lipids, and the remaining maternal proteins and lipids were 8% and 4%, respectively. The biochemical and physiological significance of these distribution patterns in this mosquito strain are discussed. This is the first report that provides a dynamic overview of the metabolic fate of meal protein amino acids in mosquitoes by using a direct approach, and also lays a foundation for the further studies on the nutritional regulation of meal protein amino acid metabolism in mosquitoes. Supported by NIH grant AI 46541.

AaSvp, a mosquito homolog of COUP-TF is involved in termination of vitellogenesis by repressing the 20-hydroecdysone response

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In the mosquito *Aedes aegypti*, vitellogenesis is cyclic and activated via an ecdysteroid hormonal cascade initiated by a blood meal. The precise tuning of this hormonal response requires participation of both positive and negative transcriptional regulators. In *Drosophila melanogaster*, Svp, a homolog of Chicken Ovalbumin Upstream Promoter Transcription Factor (COUP-TF), inhibits ecdysone receptor complex-mediated transactivation *in vitro* and *in viv*o. Here we report the cloning and characterization of the Svp homolog in mosquito *Aedes aegypti*, AaSvp, which possesses a high degree of amino acid sequence similarity to the members of the COUP-TF/Svp subfamily. AaSvp transcripts and protein are present in the fat body at high levels from the state-of-arrest to about 60 h post blood meal. AaSvp binds strongly to a variety of direct repeats of the sequence AGGTCA, but weakly to *hsp27*EcRE. Moreover, transient transfection assays in *D. melanogaster* S2 cells showed that AaSvp was able to repress 20-hydroxyecdysone (20E)-dependant transactivation mediated by the mosquito ecdysteroid receptor complex. GST pulldown assays and mammalian two-hybrid assays indicated that the AaSvp protein was capable of interacting not only with AaUSP, but also with AaEcR and AHR38. Remarkably, AaSvp-AaUSP instead of AaEcR-AaUSP was detected in the nuclear extracts of fat body between 26 hours to 48 hours post blood meal, when expression of yolk protein genes is shut down rapidly. These data suggest that AaSvp negatively regulate the 20E signaling in the fat body during mosquito vitellogenesis. This work was supported by a grant from the National Institutes of Health (RO1 AI-36959).

cDNA and mRNA expression levels of cytochrome P450 monooxygenases CYP6X1v1 and CYP6X1v2 from pyrethroid-susceptible and -resistant strains of the tarnished plant bug *Lygus lineolaris*

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Cytochrome P450 monooxygenase cDNAs were cloned from both pyrethroid-susceptible (CYP6X1v1) and pyrethroid-resistant (CYP6X1v2) strains of *Lygus lineolaris*. These cDNAs contain a 1548-nucleotide open reading frame encoding a 516 amino acid residue protein. Predicted cytochrome P450 monooxygenases from cDNAs were highly similar to several insect CYP6 P450 monooxygenases responsible for reduced sensitivity to pyrethroid insecticides. The deduced protein had a molecular mass of 57.43 kDa and pI of 8.93, a 17-residue signal peptide, and a cysteine heme-iron ligand signature sequence FGEGPRNCIG. A total of 48 nucleotide substitutions were revealed between cDNAs of susceptible and resistant strains. Thirteen of them were observed on the coding region. Only one nucleotide substitution resulted in amino acid change from Ser^{487} in susceptible strain to Ala^{487} in the resistant strain. The gene modification was confirmed by polymerase chain reaction amplification of the specific allele of both RT-cDNA and genomic DNA prepared from individual insects. The resistant strain contained 2.1-fold higher P450 monooxygenase mRNA per microgram total RNA than the susceptible strain. Topical treatment with 10 ng permethrin elevated P450 monooxygenase mRNA levels by 2.2-fold for the susceptible strain and 1.6-fold for the resistant strain. Cytochrome P450 monooxygenase mRNA was expressed in tissues of the gut, head and thorax, and abdomen. No significant expression level was detected from the salivary gland complex.

Characterization of trypsin-like protease and molecular cloning of a trypsin-like precursor cdna in salivary glands of *Lygus lineolaris*

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Biochemical study and molecular cloning revealed that trypsin is the major proteinase for extra-oral digestion in *Lygus lineolaris* (Palisot de Beauvois). Trypsin-like enzymes from the salivary gland complex of *L. lineolaris* were partially-purified by preparative isoelectric focusing (IEF). Enzyme active against N-benzoyl-L-arginine-*p*-nitroanilide (Ba*p*NA) focused at approximately pH 10 during IEF. The trypsin-like enzymes from *L. lineolaris* had a pH optimum of 10. When analyzed on casein zymograms, the serine protease inhibitors, phenylmethylsulfonyl fluoride (PMSF) and lima bean trypsin inhibitor, completely inhibited the protease activity in the crude salivary gland extract as well as the IEF purified salivary gland extract. Chicken egg white trypsin inhibitor also inhibited protease activity of *L. lineolaris*, but it was not as effective as PMSF or trypsin inhibitor from lima beans. A cDNA coding for a trypsin-like protein in the salivary glands of *L. lineolaris* was cloned and sequenced. The 971bp cDNA contained an 873-nucleotide open reading frame encoding a 291-amino acid trypsin precursor.

Purification, characterization, molecular cloning, and inhibitory regulation of prophenoloxidase activating proteinase 3 from hemolymph of *Manduca sexta*

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Phenoloxidase (PO) is implicated in several defense responses in insects and crustaceans. It is converted from prophenoloxidase (proPO) through limited proteolysis by prophenoloxidase activating proteinase (PAP). We report here the purification and characterization of a new PAP from hemolymph of *Manduca sexta*. Similar to *M. sexta* PAP 2 and *Bombyx mori* PPAE, this enzyme (PAP 3) consists of two clip domains followed by a catalytic domain, whereas *M. sexta* PAP 1 contains only one clip domain at its amino terminus. Purified PAP 3 cleaved proPO at ArgS' and generated some PO activity. The amidase activity of PAP 3 was inhibited by the recombinant serpin 1J, which formed an SDS stable complex with the enzyme. Consistent with a prediction based on sequence alignment, the P1 residue of serpin 1J was determined to be Arg3'3 by MALDI TOF mass spectrometry. These suggest that *M. sexta* serpin 1J may contribute to the negative regulation of PAP 3. Immunoblot analysis indicated that proPAP 3 was not present in hemolymph from naive insects, but became much more abundant in larvae that had been injected with bacteria. This is consistent with the results from an RT PCR analysis, showing that transcription of PAP 3 gene was induced in fat body and hemocytes upon microbial infection. The PAP 3 gene, spanning over 8 kb, is composed of at least 7 exons and 6 introns. Along with our previous results on PAP 1 and PAP 2, these experiments demonstrated that the activation of proPO and synthesis of the PAP precursors are tightly regulated processes.

The organization of silk fibroin in pyralid moths

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The excellent mechanical properties of insect silk depend on the molecular arrangement of heavy-chain fibroin (H-fibroin). Regular iterations of short amino acid repeats in H-fibroin allow formation of β-sheets and their stacking into crystallites reinforcing the silk fiber. The fibroins of Bombyx silkworms contain GAGAGS repeats and those of *Antheraea* silkmoths contain stretches of 5-15 alanine residues. Very different fibroin organization was found in the moths Galleria mellonella and Anagasta kuehniella of family Pyralidae. The core of their fibroins consists of three long and highly ordered repeats that were designated A, B_1, and B_2. The repeat A (63 amino acid residues) alternates in *G. mellonella* with B_1 (43 residues) and B_2 (18 residues) in about 12 assemblies $AB_1AB_1AB_1AB_2(AB_2)AB_2$ that make up most of the 500 kDa fibroin. The core of A. kuehniella fibroin consists of homologous and similarly alternating repeats *A*, B_1 and B_2. The *A* and B_2 repeats are of similar length (58 and 17 residues, respectively) as the matching repeats A and B_2 of G. mellonella. The B_1 repeat is considerably longer (75 residues) than B_1 but sequence homologies between the two are obvious. Sequence similarities among all repeats suggest that they have evolved from a common ancestral sequence. Fibroin amino acid composition is dominated in both species by G, A, and S, and residues with long hydrophobic side chain (L, I and V). The H-fibroin of A. kuehniella is enriched in P, N, and Y that occur in some of the positions occupied by G, A, and L in G. mellonella. Pleated β-sheets and crystallites might be formed by alanine arrays such as SSAASAAAA that are present in both species or by the G-rich motifs such as GLGGLG present in G. mellonella. The fibroin of A. kuehniella is characterized by relatively high frequency of the triplets GPY, GPN and GLN. Conservation of the motif PVIVIED in the repeats A, *A*, and B_1 indicates the importance of hydrophobicity and electrostatic charge in H-fibroin cross-linking.