# TOWARD THE GENETIC MANIPULATION OF INSECTS

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**C3-001** DNA ORGANIZATION OF DIPTERAN TELOMERES AND THEIR ELONGATION BY SPECIFIC RETROTRANSPOSONS, Harald Biessmann[1], and James M. Mason[2]. [1]Developmental Biology Center, Univ. of California, Irvine, CA 92717, and [2]NIEHS, Research Triangle Park, NC 27709.

Telomeres are complex structures at the ends of linear chromosomes of eukaryotes that perform several cellular functions, at least two of which are vital: protection of the chromosome end from degradation and fusion ('capping') and complete replication of DNA sequences at chromosome ends. Telomeres in most eukaryotes contain short, simple repeats that are highly conserved. *Drosophila melanogaster*, on the other hand, does not have such sequences, but carries at its chromosome ends one or more LINE-like retrotransposable elements (HeT-A and TART). Instead of elongation by telomerase, occurring in organisms with simple telomeric repeats, incomplete DNA replication at the termini of *Drosophila* chromosomes is counterbalanced by transposition of these retroposons at high frequency specifically to the chromosome ends. These transposable elements are not responsible for distinguishing telomeric ends in *Drosophila* from broken chromosome ends. Proximal to the terminal array of transposable elements are regions of tandem repeats (minisatellites) that are structurally and probably functionally analogous to the subterminal regions in other eukaryotes.
In order to study the telomere structure of the malaria vector *Anopheles gambiae*, the telomeric region of chromosome 2L has been cloned by using a fortuitous integration event of a *D. melanogaster* pUChsneo P-element construct that occurred within 0.5 kb of the physical end of the left arm of the second chromosome in *A. gambiae*. By studying the DNA regions flanking the insert at the proximal and distal end, valuable information will be obtained that may be useful in future transformation attempts of *A. gambiae*. Studies in several eukaryotic organisms have shown that failure to elongate telomeres or altering telomere repeat sequences will result in cell senescence and death. Therefore, attempts to inhibit this vital cellular process in *A. gambiae* may have practical applications in vector control.

**C3-002** VERY OLD DNA FROM INSECTS, Rob DeSalle, American Musuem of Natural History, New York.

Recent developments in molecular biology have allowed for the amplification and characterization of DNA from very old insect specimens. These specimens include museum preserved insects in pinned and alcohol collections and mummified insects in amber. In general, DNA from these old specimens is highly degraded so special methods are discussed that describe the amplification of sequences from old tissue. The unique conditions required for preservation of insect DNA in amber are discussed and examined using molecular assays. The DNA sequences from old specimens can be used in conservation and phylogenetic studies. An example of the use of pinned insect collections in a conservation study is presented. The utility of fossil DNA sequences in systematics is discussed and the shortcomings of very old DNA in systematics are outlined.

**C3-003** EXPRESSION OF EXOGENOUS GENES IN MOSQUITOES, Anthony A. James, Alison C. Morris and Gregory Pott, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.

The genetic control of mosquitoes has been proposed as a means for decreasing the transmission of parasitic diseases and has been a focus of study for many years. Recently, successes in transgenic technologies in other organisms, principally *Drosophila melanogaster*, have fostered an increased interest in making the important mosquito species genetically tractable. The ultimate goal is to produce mosquitoes with reduced capabilities for vectoring parasites. In order to accomplish this goal, systems must exist for the stable and heritable transformation of mosquitoes by exogenous DNA. Work in this area is ongoing, but has yet to produce a usable system.

We have explored a number of techniques that would allow the assay of promoter-reporter constructs in mosquitoes in the absence of a stable transformation system. Using constitutively expressed promoter-reporter constructs, and tissue-specific promoter-reporter systems, we have expressed exogenous genes in the embryos and dissected salivary glands of the yellow fever mosquito, *Aedes aegypti*. In addition, some of these constructs were assayed in mosquito cell culture. Finally, a group of constructs containing putative promoter sequences from genes expressed specifically in the adult female salivary glands were introduced into *Drosophila melanogaster* by P-element mediated transposition.

Embryos proved to be a potentially powerful system for evaluating promoter function of genes that are constitutively expressed or expressed specifically in the embryonic stage. The expression of exogenous genes in dissected tissues was observed with strong promoters such as the *D. melanogaster* HSP70 promoter, but was weak with endogenous promoter reporter constructs. Some of the tissue-specific promoters were found to function in cultured cells but the nature of this expression in reflecting the proper functions of the promoter is in question. Expression of mosquito genes in *D. melanogaster* appears to be problematic in that the mosquito genes that we tried had altered patterns of expression. These techniques will serve the functional analysis of some genes from mosquitoes while efforts to develop a stable transformation system are ongoing.

*Population Genetics*

C3-004 POPULATION GENETICS WITH ARBITRARILY PRIMED PCR MARKERS, William C. Black IV, Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523

The Arbitrarily Primed Polymerase Chain Reaction (AP-PCR) amplifies many genomic regions simultaneously using a single primer of arbitrary sequence that is annealed to template DNA at a low temperatures. Many of the genomic regions amplified by AP-PCR are polymorphic in their presence among individuals in a species. Most of these polymorphisms segregate as dominant markers and this prevents their use in tests for Hardy-Weinberg proportions. However, they are still useful for analyzing genetic relationships among individuals and populations in nature (Black 1993). We demonstrate how AP-PCR markers were applied in a genetic fingerprinting study of oviposition behavior in the mosquito *Aedes aegypti* in Puerto Rico (Apostol et al. 1993; 1994). AP-PCR polymorphisms at 57 loci were also used to estimate effective migration rates (*Nm*) among *Ae. aegypti* populations using Wright's $F_{ST}$ or Weir and Cockerham's $\theta$ and assuming populations had reached a migration equilibrium. We demonstrate that estimates of *Nm* were down-wardly biased by small sample sizes and that *Nm* estimated with AP-PCR markers is twice the value estimated in an earlier isozyme study. Differences between allozymes and AP-PCR markers may be due to greater selection acting on allozymes or a greater mutation rate in AP-PCR markers. Linkage disequilibrium (LD) was estimated among AP-PCR loci in *Ae. aegypti*. Small sample sizes provided upwardly biased estimates of LD coefficients. No consistent LD was found between any locus pairs in 1,596 comparisons. Ohta's analysis of LD in subdivided populations was used to partition the variance in LD due to epistasis and genetic drift. Epistasis accounted for only 2.6% of the total variance in LD. Statistical methods and software developed for population genetic analyses of AP-PCR markers are described in detail.

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C3-005 POPULATION GENETICS OF INSECT DISEASE VECTORS: *CULICOIDES* AND BLUETONGUE DISEASE EPIDEMIOLOGY, Walter J. Tabachnick and Frederick R. Holbrook, Arthropod-borne Animal Diseases Research Laboratory, USDA-ARS, Laramie, WY 82071.

Genetic variation within and among vector populations is a major feature governing insect borne disease epidemiology. Factors influencing vector genetic variation influence variability controlling vector capacity and competence for disease causing pathogens. This concept is illustrated by the insect vector of the bluetongue viruses in North America, *Culicoides variipennis*. Bluetongue viruses cause disease in ruminant livestock. As a result of bluetongue, U.S. livestock and livestock germplasm trade is restricted with bluetongue free regions such as the European Union. Resulting annual losses to the U.S. livestock industry are estimated at $125 million. Cattle show high prevalence of bluetongue antibody in the south and southwest U.S. Cattle in the northeast U.S. show low seroprevalence, and bluetongue virus transmission has not been observed in this region. A population genetics survey U.S. of *C. variipennis* populations, analyzed genetic variation at several isozyme encoding loci. The results provided understanding of *C. variipennis* population structure, genetic variation, and systematics. *Culicoides variipennis* is composed of 3 subspecies. Evidence will be presented demonstrating these are distinct species. The only proven vector in the U.S. is *C. v. sonorensis*. The subspecies in the northeast U.S., *C. v. variipennis*, is not a vector of the bluetongue viruses, and this region may be considered bluetongue free for the purpose of trade in livestock and livestock germplasm. The absence of adult *C. variipennis*, during cold winters in particular regions of the U.S., influences genetic diversity. Studies of *C. variipennis* illustrate the importance of population genetics for understanding epidemiology and for developing new disease control strategies.

*Mechanisms of Immune Responses in Insects*

C3-006 INTERACTIONS OF INSECT PARASITOIDS AND POLYDNAVIRUSES WITH THE HOST INSECT IMMUNE SYSTEM. Nancy E. Beckage, Department of Entomology, 5419 Boyce Hall, University of California-Riverside, Riverside, CA 92421-0314.

Polydnaviruses represent unique mechanisms evolved by insect endoparasitoids to manipulate the immune system of their insect host. One of the most important effects of PDVs is that the viruses interfere with the cellular and humoral immune responses of the host and elicit host immunosuppression, allowing the parasitoid to develop to maturity in the hemocoel. The polydnavirus genome is integrated in the chromosomal DNA of male and female wasps, and extrachromosomal forms of the virus undergo replication and encapsidation in the ovarian cells of the female wasp, which are transferred to the host insect during parasitization and rapidly taken up by multiple tissues of the host including hemocytes, fat body, and other tissues localized in the hemocoel. The polydnavirus acts upon the immune, nervous, and endocrine systems of the host, affecting growth and development as well as immunity. Importantly, the virus causes immunosuppression via multiple mechanisms. For example, entry of PDV into hemocytes causes "rounding up" and inhibition of actin polymerization within the cytoplasm, reducing spreading behavior and attachment to foreign surfaces (i.e. parasitoid eggs); granulocytes and possibly other hemocyte classes undergo blebbing and other symptoms characteristic of apoptosis , and their lysis prevents these cells from participating in an active encapsulation response; PDV genes are rapidly expressed following parasitization, and several PDV gene products have been implicated as the molecular mediators of hemocyte dysfunction [1,2]. Immunosuppressive proteins have been isolated in parasitized insects, one of which bears signficant amino acid homology to the hemolymph storage proteins or arylphorins. Venom-associated proteins, as well as ovarian-secreted proteins, which are released into the lumen of the reproductive tract, also appear to act as mediators of host immunosuppression in some species; however, in tobacco hornworm larvae parasitized by *Cotesia congregata*, the PDV alone is sufficient to elicit the drastic apoptosis-like changes seen in hemocytes during the initial stages of infection [3,4]. Moreover, the host larvae as well as PDV-injected "surrogate hosts" are significantly less efficient in encapsulating abiotic targets such as sephadex beads compared to nonparasitized larvae (Lavine, 1995). The populations of granulocytes and plasmatocytes later recover as more prohemocytes undergo differentiation to form mature functional hemocytes. In the tobacco hornworm system, the factors inhibiting encapsulation of first and second instar parasitoids several days post-parasitization appear to differ from the molecular mediators and events associated with the earliest stages of parastism. In several host-parasitoid models, the level of phenoloxidase activity is also depressed.

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[3]Lavine, M.D. and Beckage, N.E. 1995. Parasitology Today (in press).
[4]Lavine, M.D. 1995. Temporal pattern of host immunosuppression in *Manduca sexta* larvae parasitized by *Cotesia congregata*, and evidence for viral intervention. M.A. Thesis, Biology Department, University of California-Riverside.

C3-007 MELANOTIC ENCAPSULATION REACTIONS OF MOSQUITOES AGAINST FILARIOID NEMATODES, Bruce M. Christensen, Department of Animal Health and Biomedical Sciences, 1655 Linden Drive, University of Wisconsin, Madison, WI 53706.

Lymphatic filariasis is a debilitating disease affecting nearly 100 million people in the tropics and subtropics of both hemispheres. Transmission of the causative agents, primarily *Wuchereria bancrofti* and *Brugia malayi*, is dependent on the availability of mosquito species that support the complete development of the parasite to the infective third-stage larva. The existence of mosquito species resistant to infection provides a potential avenue for novel control strategies in endemic areas. The mechanism for resistance in several mosquito species involves an active immune response whereby the invading parasites are sequestered in melanotic capsules formed as a result of both humoral and cellular processes. Although encapsulation of a parasite by hemocytes alone can occur, killing requires the production of melanotic materials through quinone-mediated protein cross-linking reactions. These are complex biochemical events that also are necessary for cuticular tanning, egg chorion tanning, and wound healing reactions, as well as melanotic encapsulation defense responses. There is an obvious biochemical relationship between these physiological events, and increasing our understanding of the mechanisms controlling these reactions in mosquito vectors is essential if new control strategies based on resistance mechanisms are to be realized. Following parasite penetration of the midgut and migration into the hemolymph in a resistant mosquito, biochemical events are initiated that activate phenol oxidase (PO) and make available the initial substrate, tyrosine. Tyrosine hydroxylation produces dopa that is either oxidized by PO to dopaquinone or decarboxylated through the action of dopa decarboxylase (DDC) to produce dopamine (DA). Although the acetylation of DA is an important event in cuticular tanning, melanotic encapsulation seems to involve the oxidation of DA to DA-quinone, which functions as a more effective quinone-mediated protein cross-linking molecule than dopaquinone. Dopaquinone likely undergoes cyclization and oxidation to produce dopachrome, which is converted to 5,6-dihydroxyindole (DI) through the activity of dopachrome conversion enzyme. DI is oxidized by PO to DI-quinone, a highly reactive molecule that can either function in polymerization reactions to produce melanin polymers or partake in protein cross-linking reactions. A number of regulatory points likely play a major role in these reactions, including the activation of PO, the production and availability of DDC, the production of other hemolymph peptides/polypeptides, and the events that make the initial precursor, tyrosine, available for hydroxylation. Understanding the genetic regulation of these biochemical events will be valuable in our attempts to clarify those effector mechanisms that destroy invading parasites.

C3-008 THE CONTROL OF THE COORDINATE EXPRESSION OF GENES ENCODING ANTIMICROBIAL PEPTIDES IN *DROSOPHILA*, Jules A. Hoffmann, Bruno Lemaitre and Jean-Marc Reichhart. Institute of Molecular and Cellular Biology, Strasbourg, France.

Bacterial challenge or septic injury induce within 30 min to 1 h the transcription in the fat body and in some blood cells of numerous genes encoding antibacterial and antifungal peptides/polypeptides. The combined use of several experimental approaches (DNase I footprint analysis, electrophoretic mobility shift assays, transfection of immunoresponsive tumorous blood cell lines and establishment of transgenic fly lines) has provided valuable information on the *cis*-regulatory sequences of some of these genes (1-4). This analysis has highlighted the role of several binding motifs which are conserved between insects and mammals. In particular, the role of κB-related sequences has been shown to be crucial for the expression of some (but not all) antimicrobial peptide genes. Two distinct Rel-proteins can bind to these sequences, dorsal, which is reexpressed during the immune response, and the dorsal-related immune factor Dif (5,6). Injury leads to the rapid translocation of both Rel-proteins from the cytoplasm to the nucleus. Data will be presented which strongly suggest that the nuclear translocation of dl during the immune response is controlled by the Toll signaling pathway. Other control mechanisms will be analysed in view of results indicating that in some mutants most of the antimicrobial peptide genes are not, or minimally induced by immune challenge. Finally the cooperativity between various transactivators in the expression of these genes will be discussed.

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C3-009 MECHANISMS OF IMMUNE RESPONSES IN INSECTS, Bengt Åsling, Mitchell S. Dushay, and Dan Hultmark, Department of Molecular Biology, Stockholm University, S-106 91 Stockholm, Sweden.

The immune reactions in insects are apparently activated without the help of immunoglobulins or T cell receptors. This response is of considerable theoretical and practical interest. It may serve as a useful model to study mechanisms of innate immunity, and the potential for direct applications is also considerable because of the great economical and medical importance of insects as pests and as vectors for human disease.

In an effort to understand how the immune response is activated in insects, we have chosen to work with the fruit fly, *Drosophila melanogaster*. Like in other insects, infections induce the synthesis of several bactericidal proteins and peptides in *Drosophila*. We have so far isolated the genes for fourteen antibacterial factors from this species. Best studied are the four cecropin genes, that are strongly induced after infection, and we now exploit these genes as convenient markers for the immune response. In tissue culture, the blood cell line mbn-2, originally isolated by Gateff from a *Drosophila* blood tumor mutant, reacts to the addition of microbial substances by the induction of the endogenous cecropin genes.

We are currently screening for genes that are induced in *Drosophila* during the immune response, using the PCR-based technique of differential display. Among the inducible molecules we expect to find not only further effector molecules, but possibly also molecules, such as receptors, signal molecules and transcription factors, that are involved in the activation of the response itself. Indeed, we have found a new inducible Rel protein that, similarly to mammalian p105, includes a domain that is related to the negative transcriptional regulator IκB. It has previously been shown that Rel proteins like *Dif* and *dorsal* can activate transcription of cecropins and other bactericidal peptides in insects, and we are investigating the possible involvement of the new Rel factor in cecropin transcription. We have also identified a *Drosophila* homolog of attacin, an antibacterial protein first found in the Cecropia moth.

*Vector Competence*

C3-010 GENETIC CONTROL OF SUSCEPTIBILITY OF MOSQUITOES TO THE PARASITES THEY TRANSMIT, David W. Severson, Department of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI 53706.

Mosquito vectors serve as obligate intermediate hosts for numerous diseases that collectively represent a major source of human morbidity and mortality worldwide. An alternate strategy for controlling these diseases would be the genetic manipulation of mosquito populations to reduce their inherent competence as disease vectors. The development of genetic markers, based on DNA sequence polymorphisms, has facilitated a renaissance in quantitative genetics, because these markers provide a mechanism to partition complex traits, such as vector competence, into discrete Mendelian components. We have constructed a genetic linkage map for the mosquito *Aedes aegypti*, using restriction fragment length polymorphism (RFLP) markers developed mainly from cDNA clones. This map presently consists of over 80 RFLP loci that cover 153 cM across the *Ae. aegypti* genome. We used these markers to identify two quantitative trait loci (QTL) affecting susceptibility of *Ae. aegypti* to the filarial parasite, *Brugia malayi*. One QTL, *fsb[1,LF178]*, exhibits a recessive effect with respect to susceptibility and maps to the general genome region containing the previously described *f*[a] locus on chromosome 1. A second QTL residing on chromosome 2, *fsb[2,LF98]*, exhibits an additive effect on susceptibility. We also identified two QTL affecting susceptibility of *Ae. aegypti* to the malarial parasite, *Plasmodium gallinaceum*. One QTL, *pgs[2,LF98]*, exhibits a partial dominance effect on susceptibility and maps to the general genome region containing the previously described *pls* locus on chromosome 2. A second QTL residing on chromosome 3, *pgs[3,Mall]*, exhibits an additive effect on susceptibility. Because *fsb[2,LF98]* and *pgs[2,LF98]* both implicate the same genome region, we conclude that either a tightly linked cluster of independent genes or a single locus affecting susceptibility to these parasites has evolved in the genome region near the *LF98* locus. We have initiated efforts for the map-based identification and cloning of the genes associated with these QTL. We have demonstrated the utility of using RFLP markers for *Ae. aegypti* to rapidly develop comparative linkage maps for other mosquito species, with *Ae. albopictus* as a model, and hypothesize that comparative linkage maps will provide the opportunity to examine the significance and function of orthologous QTL associated with mosquito vector competence for disease transmission.

C3-011 MOLECULAR ASPECTS OF *PLASMODIUM* RESISTANCE IN *ANOPHELES GAMBIAE*. Kenneth D. Vernick, National Institutes of Health, Bethesda, MD 20892.

The African mosquito, *Anopheles gambiae,* is the most efficient vector of human malaria in the world. Consequently, it is likely that reducing the vectorial capacity of this mosquito will be a necessary component of an effective strategy to control malaria transmission in Africa. One means to reduce vectorial capacity may be the genetic transformation of wild vector populations with genes conferring resistance to infection with *Plasmodium*. Before we can do this, it is crucial to understand the host-parasite biology of malaria in the mosquito host. To this end, we have identified a refractory mechanism of host-parasite incompatibility in which oocysts of *Plasmodium gallinaceum* fail to develop in *An. gambiae* as a result of ookinete death which occurs within 30h of midgut epithelial cell invasion. The mechanism of parasite killing does not involve a previously described refractory mechanism of parasite encapsulation. Genetic crossing of selected lines which are refractory and susceptible to midgut infection suggests that the refractory trait is controlled by a single dominant genetic locus. Intracellular ookinete killing appears to involve a previously unrecognized host defense mechanism against malaria parasites that involves direct destruction of the invading organism.

*Hormonal Control of Gene Expression*

C3-012 INSULIN RECEPTOR-LIKE TYROSINE KINASES IN MOSQUITOES, Rolf Graf[1], Paul Helbling[1], Urs Ackermann[1], Stefan Neuenschwander[1] and Mark R.Brown[2], [1] Zoologisches Institut, Universität Zürich, 8057 Zürich, Switzerland, [2] Department of Entomology, University of Georgia, Athens Ga.30602

After the female mosquito, *Aedes aegypti*, takes a blood meal, a cascade of hormonal events initiates and maintains vitellogenesis and oocyte maturation. The major hormone(s) with gonadotropic action, the ovarian ecdysteroidogenic hormone (OEH), induces ovarian ecdysteroidogenesis directly and vitellogenesis indirectly. In silkworms, ecdysteroidogenesis is stimulated by bombyxins, which are structurally related to insulin, and we have found that insulin stimulates this process in mosquito ovaries *in vitro*. Based on this provocative lead, we have proposed that insulin receptors belonging to the tyrosine kinase family transduce hormonal signals for ecdysteroidogenesis in the mosquito ovary. By sequence homology, we have designed PCR-primers to synthesize fragments coding for conserved subdomains of tyrosine kinases. Their deduced amino acid sequence was homologous to the tyrosine kinase subfamily. One such fragment was used to clone cDNAs from ovaries. An open reading frame coding for a protein of 1390 amino acids (mosquito insulin receptor, MIR) shows the typical landmarks of a receptor tyrosine kinase: a cysteine-rich extracellular domain, a processing site for precursor cleavage into an α- and a β-subunit, a transmembrane domain in the β-subunit followed by typical consensus sequences for tyrosine kinases. Expression analysis of the mRNA indicated a highly specific accumulation of MIR-mRNA in the ovaries. *In situ*-hybridization clearly demonstrated a high expression in the nurse cells and a low expression in the follicle cells before a blood meal. After a meal, follicle cells increased their MIR-mRNA level. Towards the end of the maturation process, a decrease of MIR-mRNA was observed along with the degeneration of follicle cells and the formation of the acellular chorion. Immunocytochemical detection of MIR, using an antibody against a portion of the extracellular domain, demonstrated a colocalization with MIR-mRNA.

To establish functional assays targeting the insulin receptor homolog, we have used porcine insulin as an agonist to test whether it can activate previtellogenic ovaries. *In vivo*, ovaries increase their protein synthesis rate several-fold after activation by a blood meal, making protein synthesis a simple indicator for the activation state of the ovary. In an *in vitro* protein synthesis assay we demonstrated that insulin can induce a two to three fold increase in previtellogenic ovaries. Thus, it is likely that an insulin receptor homolog, possibly MIR, is involved in the activation process that leads to a vitellogenic ovary.

Despite the advantage of readily available agonists it will be important to elucidate the structure of the hormones involved in activation and maintenance of the vitellogenic cycle. The key hormone, OEH, has been purified by one of us (MRB) and partially sequenced. Oligonucleotides were designed to perform PCR on genomic DNA. A fragment representing the expected sequence was used to identify cDNAs from a head library. Synthesis of the recombinent peptide and its use in functional assays will define its function and relationship to the MIR during the reproductive cycle in the mosquito.

C3-013 MOLECULAR BIOLOGY OF MOSQUITO VITELLOGENESIS, Alexander S. Raikhel, Department of Entomology, Michigan State University, East Lansing, MI 48824.

In anautogeneous mosquitoes, the processes of disease transmission and egg maturation are intimately tied together through the requirement of blood feeding. Therefore, elucidation of the molecular basis of egg maturation is of fundamental importance in the search for alternative strategies of mosquito control. Molecular studies of the yolk protein precursors in the female mosquito, *Aedes aegypti*, have changed dramatically our understanding of the fat body's role in vitellogenesis. We have discovered that together with the major yolk protein precursor, vitellogenin (Vg), the fat body of vitellogenic female mosquitoes produces two yolk protein precursors that are proenzymes deposited in developing oocytes and activated during embryogenesis. One of these is the previously described vitellogenic carboxypeptidase (VCP). In addition, we found that the vitellogenic fat body also produces and secretes a 44-kDa yolk protein precursor that is a cathepsin B-like thiol protease (VTP). Properties of the novel yolk protein will be discussed.

Our recent studies have set the stage for elucidating the molecular mechanism of regulation of gene expression by 20-hydroxyecdysone (20E) in the mosquito. We cloned a cDNA encoding ecdysteroid receptor (AaEcR) and characterized its expression during vitellogenesis. We also cloned a cDNA encoding the mosquito *Ultraspiracle* homolog (AaUSP) which is a heterodimeric partner of the ecdysteriod receptor. We further demonstrated that AaEcR and AaUSP, expressed from cloned cDNAs by *in vitro* transcription/translation, are capable of hormone and DNA binding as a functional heterodimer. One of the 20E-responsive yolk protein genes coding for mosquito VCP was also cloned. However, potential hormone response elements present in two allelic VCP genes differ from the consensus sequence for the ecdysone response element. Experiments utilizing cycloheximide, an inhibitor of protein synthesis, showed that 20E action on the mosquito yolk protein genes for Vg and VCP is indirect and likely mediated by a regulatory cascade similar to that in *Drosophila*.

The most critical aspect of vitellogenesis is specific internalization of yolk protein precursors by developing oocytes. As a significant step towards understanding the molecular basis for receptor-mediated accumulation of Vg, its receptor (VgR) was purified and partially microsequenced. Production of anti-VgR antibodies allowed us to demonstrate that the VgR is a noncovalent homodimer consisting of two 205-kDa subunits. Having obtained a partial VgR cDNA clone we are currently cloning cDNAs corresponding to the 7-kb VgR transcript. In addition, we cloned the gene encoding the mosquito clathrin heavy chain (CHC), which the major structural protein of coated vesicles. We found that the ovary has a specific CHC mRNA which is 1 kb shorter (6.5 kb) than the one present in somatic tissues.

C3-014 ECDYSTEROID REGULATION OF GENE EXPRESSION IN MOLTING AND METAMORPHOSIS, William A. Segraves, Department of Biology, Yale University, New Haven, CT 06520-8103.

The insect molting hormone ecdysone induces a small number of early ecdysone-inducible puffs in the third instar larval salivary gland of Drosophila melanogaster. The products of the corresponding early genes are believed to repress early gene expression and to control downstream regulatory hierarchies responsible for the developmental effects of ecdysone. The E75 early gene from the 75B puff includes three ecdysone-inducible transcription units, E75 A, E75 B and E75 C, which are expressed with distinct developmental and spatial profiles. The three corresponding proteins are members of the steroid receptor superfamily, with conserved regions corresponding to the DNA and hormone binding domains of known receptors.

Using a combination of classical genetic techniques and imprecise excision of marked P elements, we have identified mutations affecting each of the E75 transcription units, and demonstrate that they correspond to genetically separable functions. These functions are shown to be essential for embryonic development, larval molting, and pupal development.

Immunocytochemical studies of E75A protein localization in polytene chromosomes suggest a role for E75A in the regulation of early genes, such as E75, as well as late genes. We have examined the regulation of the E75A promoter by ecdysone and by the E75A protein, and have localized the specific DNA binding sites for these factors. These findings and the role of E75 and other ecdysone-regulated members of the steroid receptor superfamily will be discussed in the context of models to describe the regulation of ecdysone response.

C3-015 CYCLIC AMP-RESPONSIVE ELEMENTS AND TRANSCRIPTION FACTORS IN GENE REGULATION, Ronald E. Rose[1], Nicole Gallaher[1], Deborah Andrew[2], Richard H. Goodman[1] and Sarah M. Smolik[1,3]. [1]Vollum Institute, Oregon Health Sciences University, Portland, OR 97201, [2]Department of Cell Biology and Anatomy, Johns Hopkins School of Medicine,Baltimore, MD, [3]Department of Molecular and Medical Genetics, Oregon health Sciences University, Portland OR.

External signals can elicit a variety of intracellular responses through the action of second messengers such as cAMP. The responses involve a rapid phosphorylation of pre-existing proteins and the initiation of new patterns of gene expression. This process of signal transduction is important for cellular differentiation and development. A cAMP response element (CRE) is required for the transcription of many genes that are expressed in response to cAMP. We have previously described the cloning of the *Drosophila melanogaster* homologue to the mammalian cyclic AMP response-element binding protein, CREB. This protein, dCREB-A, is a member of the bZIP family of transcription factors, shows specific binding to the CRE and can activate transcription in cell culture (1). We now describe the gene structure for dCREB-A, the protein expression patterns throughout development and the necessary role for this gene in embryogenesis. The 4.478 kb transcript is encoded in six exons that are distributed over 21 kb of DNA. There are seven start sites and no strong promoter sequences. The dCREB-A protein is expressed in the nuclei of the embryonic salivary gland, proventriculus and stomadeum. Late in embryogenesis, the nuclei outlining each segment and lateral bands of nuclei show immunohistochemical staining with anti-dCREB-A antibodies. In the adult, dCREB-A is expressed in the migrating follicle cell nuclei of stage 9 egg chambers and in the stage 10 follicle cells that surround the oocyte. In stage 11 egg chambers, only a few follicle cell nuclei over the diminishing nurse cells express dCREB-A and by stage 12, no dCREB-A protein can be detected. Deletion mutations in the dCREB-A gene give rise to animals that no longer express dCREB-A protein and die late in embryogenesis prior to or at hatching. dCREB-A mutations have a haploinsufficient phenotype. Although embryos heterozygous for the dCREB-A null alleles and a wild-type chromosome survive, the lumen of the salivary gland is twisted and the filzkorper is rotated dorsally. The essential role of dCREB-A in Drosophila embryogenesis together with its structural similarity to mammalian CREB proteins makes it an ideal candidate to study the functional significance of bZIP protein interactions during development.

1. Smolik, S.M., Rose, R.E. and Goodman, R.H. (1992). A cyclic AMP-responsive element-binding transcriptional activator in *Drosophila melanogaster*, dCREB-A, is a member of the leucine zipper family. *Mol. Cell. Biol.* **12**, 4123-4131.

*Target Genes: Reproduction and Development*

C3-016 LONG-TERM REGULATORY CASCADES AND TERMINAL CELL DIFFERENTIATION DURING SILKMOTH OOGENESIS, Luc Swevers, Joel R. Drevet, Yasir A.W. Skeiky, and Kostas Iatrou, Department of Medical Biochemistry, The University of Calgary, Calgary, AB, Canada.

In the domesticated silkmoth *Bombyx mori*, oogenesis occurs over a period of 9 days during pharate adult development and is controlled by the hormone 20-hydroxy-ecdysone (20-HE). On day 5 of pupation, the cells of the epithelium that surrounds each follicle initiate a programme of terminal differentiation that involves the regulated activation of more than 100 genes whose products are the constituents of the eggshell or chorion, a structure that is responsible for the protection of the developing embryo.

The objective of our studies is to deduce how follicular cells become committed to their terminal differentiation pathway (choriogenesis), and how the programme of choriogenesis is executed. Results will be presented suggesting that (i) 20-HE acts on follicular cells by initiating a regulatory cascade that results in the appearance of a receptor protein for a ligand (choriotropic factor) that is present in the hemolymph 1.5 days prior to the onset of choriogenesis; (ii) the interaction between this receptor and its ligand is responsible for the establishment of the choriogenic potential in the cells of the follicular epithelium; and (iii) for chorion genes that are expressed during the final stages of choriogenesis, this potential is already implemented before the end of vitellogenesis.

For this class of chorion genes, the implementation of the choriogenic potential involves, among others, the activation of a GATA-type transcription factor-encoding gene whose transcripts are subject to temporally-regulated alternative splicing. The alternative splicing results in the generation of at least three mRNA isoforms, all of them encoding GATA factors required for activation of the relevant target chorion genes. In addition to alternative splicing, the implementation of the choriogenic programme during the 2.5-day period separating the initial activation of the gene encoding the GATA factors (end of vitellogenesis) and the appearance of these factors in the nuclei of follicular cells (late choriogenesis), also involves a post-translational mechanism of control of gene expression. Phosphorylation of the GATA factors results in their initial sequestration into the cytoplasm of follicular cells, while dephosphorylation during the late stages of choriogenesis appears to mediate their recruitment into the nuclei of these cells, where they exert their function through binding to the promoter regions of cognate chorion genes.

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*Molecular Basis of Insecticide Resistance*

C3-017 PROPERTIES OF *BACILLUS THURINGIENSIS* CryIAc and CryIC δ-ENDOTOXIN BINDING IN SUSCEPTIBLE AND RESISTANT INSECTS, Michael Adang, Ke Luo, Yang-jiang Lu, Sreedhara Sangadala and Stephen Garczynski. Dept. of Entomology, University of Georgia, Athens, GA 30602-2605

*Bacillus thuringiensis* δ-endotoxins attach to target molecules, i.e. receptors, located in the brush border membrane of susceptible insects. We compared the binding characteristics of two δ-endotoxins (CryIAc and CryIC) to brush border membranes from insect species that differ in their susceptibilities to these toxins. CryIAc and CryIC bound with high-affinity to binding sites in *Manduca sexta*, *Spodoptera exigua* and *Spodoptera frugiperda*. In each species CryIAc and CryIC did not compete for the same binding sites. Can toxins recognizing alternate binding sites be used as a tool in *Bt* resistance management? This approach is being successfully applied in the field for *P. xylostella* that have become resistant to CryIAc toxin. Fortunately, CryIA-resistant *P. xylostella* still bind and are killed by CryIC toxin. Also, reversal of CryIA resistance is reported under conditions when selection pressure was removed. The return of CryIA binding sites was correlated with the reversal of resistance. Our studies of toxin-binding in CryIC-resistant *S. exigua* (obtained from B. Moar, Auburn University) reveal a more complicated relationship between binding sites and toxicity. We found that CryIC binding was reduced to 50% of the normal levels in resistant *S. exigua* larvae. There was also a significant increase in non-specific, low-affinity binding to brush border membranes from resistant insects. Importantly, *S. exigua* resistant to CryIC toxin was cross-resistant to other Cry toxins. These results demonstrate the complexity of the toxin-cell membrane interaction. While toxin binding is a critical part of toxin action, there are obviously other important steps in the toxin process.

A second approach was to identify CryIAc and CryIC binding proteins in *M. sexta*, a species highly susceptible to both toxins. The major CryIAc binding protein in *M. sexta* is a 120-kDa protein, aminopeptidase-N. When reconstituted into membrane the 120-kDa protein enhances toxin-induced pore formation. The 120-kDa protein is anchored in the brush border membrane by a glycosyl-phosphatidylinositol moiety. Toxin binds to the aminopeptidase when cleaved from the lipid anchor. Incubation in the presence of N-acetylgalactosamine blocks toxin binding on ligand blots. Periodic acid treatment which removes carbohydrate residues also eliminates toxin binding. Our analyses of CryIC binding proteins in *M. sexta* show that CryIC toxin recognizes a different protein than CryIAc toxin in the brush border membrane. A 43-kDa protein was purified using a CryIC-affinity column. The CryIC binding protein in *M. sexta* is similar in molecular size to the *S. litoralis* CryIC binding protein. These results correlate the unique insecticidal properties of CryIAc and CryIC toxins with their ability to distinct sites and proteins in the midgut membrane.

C3-018 ESTERASES IN RESISTANT PEACH-POTATO APHIDS, *MYZUS PERSICAE* (SULZER), Alan L. Devonshire and Linda M. Field, IACR, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, UK.

The peach-potato aphid, *Myzus persicae*, is a major pest of many crops around the world, causing damage directly by feeding and indirectly by transmitting virus diseases. Widespread use of insecticides has led to the development of resistance and in all cases studied this has been associated with the increased production of an esterase that detoxifies the insecticidal esters (organosphosphorus, carbamate or pyrethroid) before they reach their target sites in the nervous system. The underlying mechanism is gene amplification, with resistant aphids having up to 60 copies of the esterase structural gene and devoting approximately 1% of their protein production to this one enzyme. A resistant aphid produces one of two very similar esterases, E4 or FE4, and this is linked with its karyotype, E4 in aphids with a particular chromosomal translocation and FE4 in those of normal karyotype.

These two forms of the amplified gene are highly homologous; each covers ca. 4.3 kb with 7 introns in identical positions and there are 21 point mutations between their coding sequences 12 of which are silent, and one of which deletes a stop codon resulting in the FE4 protein being 12 amino acid residues longer. Both restriction mapping and sequencing of PCR products encompassing intron and exon regions from 20 resistant aphid clones of wide geographic origin have shown that each gene appears invariant, and that they differ slightly from the unamplified gene of susceptible aphids which again appears invariant in the 12 clones examined.

Although *M. persicae* reproduces mainly asexually, many populations can overwinter sexually. This has enabled controlled crosses (with R.L. Blackman, Natural History Museum) which have shown that the amplified genes are inherited as a unit, although in some clones crossing data were consistent with their occurrence at 2 loci; this has now been confirmed by *in situ* hybridisation.

Besides reflecting variation in gene copy number, the amount of esterase produced can also be influenced by transcriptional control. This is apparent when some clonal populations spontaneously lose resistance and the elevated esterase despite retaining their amplified genes. This correlates closely with the presence of methylated cytosines in and around the gene; expressed genes have 5-methylcytosine at some sites identifiable by restriction analysis with *MspI/HpaII*, and this is lost progressively as esterase production falls. This opens the possibility of understanding the role of methylation in the expression of this gene in an intact higher organism.

C3-019 P450 MEDIATED RESISTANCE IN THE HOUSE FLY, René Feyereisen, Department of Entomology and Center for Insect Science, University of Arizona, Tucson, AZ 85721.

Cytochrome P450 monooxygenases are found in virtually all living organisms, and it is believed that the human genome has at least 50 P450 genes. Each P450 protein (typically 45-60 kDa) is the product of a distinct P450 gene. P450 proteins are heme-thiolate proteins that catalyze an incredible diversity of chemical reactions. They are best known for their monooxygenase role, catalyzing the transfer of one atom of molecular oxygen to a substrate and reducing the other to water. But P450s also show activity as reductases, desaturases, dehalogenases, allene oxide synthases, prostacyclin and thromboxane synthases, etc. Moreover, because of their complex catalytic mechanism, P450 enzymes often, perhaps always, generate superoxide or hydrogen peroxide from "unsuccessful", or uncoupled reactions, leading to oxidative stress in cells. There are soluble forms of P450 (in bacteria) and membrane-bound forms (in microsomes and mitochondria of eukaryotes). P450s are dependent on redox partners for their supply of reducing equivalents from NADH or NADPH (NADPH cyt P450 reductase and cyt b5 in microsomes; a ferredoxin and a ferredoxin reductase otherwise). Insect microsomal P450 enzymes are best known for their role in insecticide detoxification (and resistance) and in the metabolism of plant chemicals. Insect P450 enzymes also activate certain types of insecticides, for instance the conversion of phosphorothioates (P=S) to phosphates (P=O). This can result in an increased potency for inhibition of acetylcholinesterase by 3 or 4 orders of magnitude. P450s are also involved in the biosynthesis of ecdysone, juvenile hormone, and pheromone components.
We have cloned and sequenced 19 P450 genes or gene fragments in the house fly, *Musca domestica*; 17 in the mosquito *Anopheles albimanus*; and 10 in *Drosophila melanogaster*. Heterologous expression of P450 cDNAs in *E. coli* and reconstitution of activity with pure NADPH cyt P450 reductase has allowed the exploration of the substrate specificity of pure P450 proteins. The house fly CYP6A1 is highly active as epoxidase of cyclodiene insecticides. We have shown that constitutive expression of the house fly *CYP6A1* gene is at least 10 times higher in the (resistant) Rutgers strain than in the (susceptible) *sbo* strain. This high constitutive expression is not caused by an amplification of the *CYP6A1* gene in the Rutgers strain. Overexpression of the *CYP6A1* gene is under control of an incompletely dominant locus on chromosome II, both in larvae and in adults. Because *CYP6A1* is located on chromosome V, overexpression is therefore the result of a mutation affecting a *trans*-acting factor that regulates *CYP6A1* expression on chromosome V. We hypothesize that this factor negatively regulates expression of *CYP6A1*, and that a mutation lifts this inhibition. As a result *CYP6A1*, other P450 genes and detoxification enzymes are overexpressed, thus leading to broad metabolic resistance. A mutation in the *CYP6A1* gene is ruled out. Our results support the hypothesis of Plapp that a major resistance gene on chr. II is a regulatory gene. Similar evidence is accumulating for *CYP6A2*, *CYP6D1*, as well as for glutathione S-transferases. Supported by NIH grants R01 GM 39014 and P30 ES 06694.

C3-020 INSECTICIDE-RESISTANCE GENES AS POTENTIAL SELECTABLE MARKERS FOR THE GENETIC TRANSFORMATION OF MOSQUITOES, Richard ffrench-Constant[1], Frank Shotkoski[1], Nicola Anthony[1], Allison Morris[2] and Anthony James[2], [1]Department of Entomology, University of Wisconsin-Madison, Madison, WI 53706, [2]Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.

Our ability to transform mosquitoes is currently hindered by a lack of both suitable vectors and selectable markers. We are therefore interested in developing insecticide resistance genes as selectable markers, particularly for detecting low frequency non-vector mediated events. Here we report on our characterization of two resistance genes from *Aedes aegypti*, the cyclodiene resistance gene *Resistance to dieldrin* or *Rdl* and the acetylcholinesterase gene *Ace*. *Rdl* codes for a γ-aminobutyric acid (GABA) gated chloride ion channel and resistance is conferred by a single alanine > serine replacement in the predicted lining of the ion channel pore. We are currently investigating two methods of using this gene. Firstly, we are building a mini-gene by fusing the cDNA with appropriate 5' regulatory sequences. Data on the identification of the *Rdl* promoter using a series of genomic deletion constructs to drive a luciferase reporter gene following injection into *Aedes* embryos will be presented. Secondly, we are attempting to insert the resistance associated mutation into the mosquito genome via homologous recombination following embryo injection. We are therefore using a construct incorporating exon 7 of the gene, the region containing the resistance associated mutation, and sequence from the flanking introns to facilitate recombination. *Ace* codes for acetylcholinesterase which is the target site of organophosphorus and carbamate insecticides. We have cloned the *Aedes* homolog, using degenerate PCR, whose sequence will be reported. We are currently investigating if a number of mutations, known to confer resistance in other species, confer insensitivity to insecticide inhibition following functional expression of the mutated *Aedes Ace* gene in *Xenopus* oocytes.

*Target Genes: Physiological Systems*

C3-021 ION TRANSPORT IN THE MIDGUT AND MALPIGHIAN TUBULES, Sarjeet S. Gill, Patricia V. Pietrantonio, Linda S. Ross, Inderpal S. Randhawa and Daniela I. Oltean. Department of Entomology, and Environmental Toxicology Graduate Program, 5419 Boyce Hall, University of California, Riverside, CA 92521-0127

Insects utilize a variety of food sources and are found in nearly all environmental habitats. This diversity in adaptation has resulted in insects having evolved a number of mechanisms for ion regulation. For example, lepidopteran larvae encounter high K+ levels in plant material, female adult mosquitoes receive high Na+ levels during blood feeding, and mosquito larvae survive in aquatic environments with differing salinity. The mechanisms by which these, and other insects manage these high ionic loads can vary. In lepidopteran insects, the high dietary K+ concentration requires an efficient removal of K+ from the hemolymph. The presence of a coupled transport system, that is a unique plasma membrane V-type ATPase and a K+/nH+ antiporter in the midgut goblet cell cavity, facilitates this extrusion of K+ ions from the hemolymph and midgut. The presence of high K+ in the lepidopteran insect midgut also facilitates secondary processes, such as amino acid uptake. Additional channels transporting Cl- and K+ occur in the midgut. The V-type ATPase from insects resembles that from other eukaryotes, and consists of a number of subunits. A number of these subunits have been cloned and antibodies developed against two of these subunits are being used to localize the V-type ATPase in the midgut and Malpighian tubules.
Ion excretion, however, predominantly occurs via the Malpighian tubules. A number of regulatory processes have been demonstrated in this tissue. The V-type ATPase and a cation/proton exchanger are present in the apical cell membrane of Malpighian tubules. While Na+/K+ ATPase and a Na-K-Cl cotransport activities are present in Malpighian tubule basal membranes. Moreover hormonally regulated processes including those mediated by diuretic hormone and serotonin occur in insect Malpighian tubules.
The presentation will attempt to cover the physiological and molecular characterization of some of these proteins both in the midgut and Malpighian tubules.

C3-022 MOLECULAR ANALYSIS OF HEMOLYMPH PROTEINS: SERPIN GENES, Michael R. Kanost[1], Haobo Jiang[1], Hong Gan[1], and Amy B. Mulnix[2], [1] Department of Biochemistry, Kansas State University, Manhattan, Kansas 66506 and [2]Deparment of Biology, Earlham College, Richmond, IN. Serine proteinases are important regulators of many extracellular processes. The proteinases themselves are often regulated by serpins, which are protein inhibitors of serine proteinases. We have identified a family of serpin proteins in hemolymph of *Manduca sexta*. These proteins are 25-30% identical in amino acid sequence to mammalian serpins and are encoded in *Manduca* by at least two genes. *Manduca* serpin gene-1 encodes a family of more than 12 proteins which have a constant region consisting of the amino-terminal 336 amino acid residues, and a variable region consisting of the carboxyl-terminal 40-45 residues. The variable region encodes the reactive site sequence of the inhibitor, which interacts with the active site of a serine proteinase. Thus, the serpin variants produced from serpin gene-1 have the potential to inhibit different serine proteinases. The sequence of *Manduca* serpin gene-1 reveals that the reactive site-region variants are produced through mutually exclusive alternate exon use. The gene is composed of ten exons. The ninth exon is present in at least 12 versions arranged in tandem between exons 8 and 10. Hemocytes use all of the versions of exon 9 without apparent bias, but fat body selects predominantly one version of exon 9, indicating a tissue specificity in alternate exon use. The serpins produced from serpin gene-1 have been expressed in *E. coli*, and their enzyme inhibitory properties are being characterized. Some of the variants inhibit chymotrypsin, some inhibit elastase, and some inhibit trypsin. Site directed mutagenesis of the P1 residue in the reactive site, from alanine to lysine, can convert an elastase inhibitor to a trypsin inhibitor; mutation of alanine to phenylalanine converts the protein to a chymotrypsin inhibitor. We have recently identified a second serpin gene in *Manduca* (serpin gene-2). The sequence of a cDNA from this gene encodes a protein that is approximately 40% identical in amino acid sequence with the proteins encoded by gene-1. We have begun to search for endogenous serine proteinases that might be targets of the hemolymph serpins. We have used PCR and primers for a highly conserved region around the active site serine in serine proteinases to amplify fragments of cDNAs from fat body and hemocyte libraries. One of these fragments was used to probe a hemocyte cDNA library, and four different serine proteinase clones were isolated. Searches of sequence databases indicate that these proteins are most similar to enzymes involved in coagulation pathways and to antimicrobial proteinases from vertebrate leukocytes.

C3-023 MOSQUITO MIDGUT TRYPSINS: MOLECULAR BASIS OF REGULATION BY BLOOD-FEEDING, Fernando G. Noriega[1], Carolina V. Barillas-Mury[2], Xiao-Yu Wang[1], James E. Pennington[1], Qijiao Jiang[1], and Michael A. Wells[1], [1]University of Arizona, Tucson, AZ, 85721, and [2]European Molecular Biology Laboratory, Heidelberg, 6900, Germany.

Female mosquitoes require a blood-meal in order to obtain the nutrients necessary to produce eggs. However, at the moment the blood-meal is taken, the midgut is not competent to digest the proteins in the meal, because the midgut does not contain any active trypsin. The blood-meal induces the synthesis of trypsins in the midgut, as well as many other events related to secreting proteins into the midgut lumen and absorbing digestion products. We are studying the regulation of trypsin synthesis as a paradigm for the many events which occur within the midgut following a blood-meal Two types of trypsins have been identified in the midgut of *Aedes aegypti*, an EARLY TRYPSIN, which is produced within 2-4 hours after the blood-meal, and a LATE TRYPSIN, which is produced maximally 18 hours after the blood-meal. The synthesis of early trypsin is post-transcriptionally regulated. The mRNA for early trypsin appears in the midgut during the first 3 days following emergence from the pupal state, a period of midgut maturation during which the mosquito will not take a blood-meal. However, the mRNA is not translated until the blood-meal is taken - there is no detectable trypsinogen stored in the midgut. In the absence of a blood-meal, the mRNA for early trypsin is stable for several weeks. The activation of early trypsin translation is linked to the physical act of taking the blood-meal, either through osmotic effects or stretching of the midgut, and once translation is activated, the mRNA for early trypsin is rapidly degraded. Although made in only small amounts, the activity of early trypsin is essential for subsequent events which occur in the midgut (including synthesis of late trypsin) and, ultimately, egg production depends on the activity of early trypsin. Digestion products produced by the action of early trypsin on blood-meal proteins, perhaps in concert with amino- or carboxypeptidases, activate transcription of the late trypsin gene, persumably through activation or synthesis of a transcription factor - the activation of transcription of the late trypsin gene is inhibited by translation inhibitors. Late trypsin is produced in large amounts and is primarily responsible for the endoprotease activity which leads to digestion of the blood-meal proteins. The amount of late trypsin mRNA produced is dependent on the amount of protein in the blood-meal, suggesting that some mechanism exists which "measures" the amount of protein in the blood-meal. After the majority of the blood-meal proteins are digested and eggs have laid, the midgut returns to a quiescent state, during which the mRNA for early trypsin reappears and the mosquito is ready to take another blood-meal. The regulation of the synthesis of early and late trypsins in the mosquito midgut is a complex series of events regulated both transcriptionally and translationally. Supported by NIH grant AI 31591.

*Gene Transfer: Prospects and Perspectives*

**C3-024 VIRAL TRANSFORMATION VECTORS FOR INSECT VECTORS, Barry Beaty, Jonathan Carlson, Stephen Higgs, Kenneth Olson, Boris Afanasiev, Kurt Kamrud, and Ann Powers. Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Colorado State University, Fort Collins, CO 80523.**

We have developed RNA and DNA virus systems that permit introduction into, and expression of, genetic constructs in mosquitoes and mosquito cells. Sindbis virus ((SIN), genus Alphavirus, family Togaviridae)) and *Aedes* densonucleosis virus ((AdDNV), genus Densonucleosis virus, family Parvoviridae) infect mosquitoes naturally. We have exploited the biology of SIN and AeDNV as expression vectors.

SIN virus is an arbovirus maintained in nature in a cycle involving mosquitoes and birds. The SIN virus genome consists of a single molecule of single-stranded RNA, 11,703 nucleotides in length. Infectious cDNA clones of SIN have been developed and noninfectious and infectious expression vectors have been constructed by C. Rice and colleagues. The infectious SIN vectors have proven to be particularly useful. These vectors have two subgenomic mRNA promoters; one transcribes structural genes and the other transcribes heterologous genes. These double subgenomic SIN (dsSIN) vectors can infect and be transmitted to all cells. The dsSIN system has been used to express heterologous genes, such as CAT and scorpion toxin, in mosquito cells and in mosquitoes. The dsSIN systems has also been used to intracellularly immunize cells to infection with alternate arboviruses. In addition, a SIN replicon system has been developed to express large heterologous genes.

Densonucleosis viruses are parvoviruses of arthropods. Parvoviruses are small, icosahedral, nonenveloped viruses and have a linear single stranded DNA genome, 4,000 to 5,000 nucleotides in length. The AeDNV was originally isolated from a laboratory strain of *Ae. aegypti*. We have prepared a functional, infectious clone of the AeDNV genome and demonstrated that it could be used to transduce a recombinant genome containing the B-gal gene in mosquito cells. Almost 20% of the cells were stained blue, which resulted from packaging of the B-gal recombinant genome in AeDNV virions followed by particle mediated delivery of B-gal gene to the fresh cells - transduction. Thus, the cloned genome of AeDNV can serve as a vector for delivery and expression of foreign genes in mosquito cells and mosquitoes.

C3-025 TRANSFORMATION OF *DROSOPHILA MELANOGASTER* CELL LINES BY HOMOLOGOUS RECOMBINATION, Lucy Cherbas and Peter Cherbas, Department of Biology, Indiana University, Bloomington, IN 47405.

We have demonstrated that gene targeting (insertion of DNA sequences into the chromosome by homologous recombination) occurs at appreciable rates in a *Drosophila melanogaster* cell line. In our experiments Kc cells are transfected with long, linear fragments of Drosophila DNA interrupted by methotrexate-insensitive DHFR gene driven by a strong promoter. The Mtx-resistant clones that arise from these transformations include examples of both targeted and non-homologous insertions whose structures and frequencies have been diagnosed by Southern analysis. We will present data on targeting frequencies at two genes and discuss the utility of this technique for mutagenesis in cell lines as well as its implications for transformation of flies and other insects.

C3-026 GENETICS OF MOSQUITO CELLS IN CULTURE: APPLICATIONS TO TRANSFORMATION OF THE ORGANISM.
Ann M. Fallon, Department of Entomology, University of Minnesota, St. Paul, MN 55108

Control of mosquito-borne disease by genetic manipulation of vectors presents formidable technological challenges. Mosquito research lacks the extensive framework of classical genetics available for the model insect, Drosophila melanogaster. Moreover, the complex host-parasite interactions that take place in the mosquito have few if any counterparts in the model organisms that stand at the forefront of molecular research. Thus, an understanding of physiological and molecular bases of vector competence for the many viral, protozoan, and filarial parasites transmitted by mosquitoes rests on direct research with mosquitoes. For certain applications, the genetic manipulation of mosquito cells in culture provides advantages for analysis of processes that would be difficult to characterize in a small insect lacking the advantages available for Drosophila. Our research uses cultured mosquito cells to develop and test genetic strategies that can adapted to mosquito embryos, given suitable transposable elements.

Most of the mosquito cell lines available today were established to investigate the replication of mosquito-borne viruses. It was only in the late 1970's that a systematic application of the techniques and approaches of somatic cell genetics was applied to cells from the Aedes albopictus line of Singh. Techniques for isolation of clonal populations, mutagenesis, selection and characterization of mutants, somatic cell fusion, and both transient and stable gene transfer have been established and optimized for these cells. Mosquito cells are ideal for investigating genes that encode housekeeping functions expressed in all cells. Early efforts at embryo transformation will likely use these genes, since their expression can be expected in every cell of the transformed insect. These genes will serve as a basis for identifying promoters and regulatory elements that will restrict gene expression to the tissue or developmental stage of choice. Mosquito cell mutants resistant to methotrexate, which amplify the dihydrofolate reductase (*dhfr*) gene, facilitated the cloning of this gene and the subsequent development of gene transfer vectors that confer stable resistance to methotrexate when expressed in wild type cells. We have recently used vectors encoding the *dhfr* gene to recover populations of thymidine kinase-deficient ($TK^-$) cells that express the herpes simplex viral *tk* gene. These transformed cells are being used to evaluate differential effects of anti-herpetic drugs on TK activity encoded by the viral gene, relative to the endogenous mosquito gene. In subsequent applications, expression of the viral gene under the control of a tissue-specific promoter, followed by treatment with an appropriate drug, could be used to specifically ablate the function of the tissues expressing viral TK activity.

Besides housekeeping functions, mosquito cells express activities that may be useful in modulating vector-pathogen interactions. For example, a cecropin-like activity, and four larger proteins, are secreted by cells after challenge with heat-killed bacteria. Mosquito cells also express ecdysone-inducible proteins, whose in vitro analysis may provide insight into the effects of ecdysone on growth and cell cycle-specific events. The potential contributions of cell culture to molecular and biochemical analysis of mosquito physiology remain in early stages of development. As genes encoding receptors and regulatory functions are cloned, stably transfected cells provide a means of generating novel phenotypes that will provide a unique perspective on the biology of an important group of disease vectors.

*Transposable Elements*

C3-027 TRANSFORMATION OF BENEFICIAL ARTHROPODS BY MATERNAL MICROINJECTION, Marjorie A. Hoy, James K. Presnail, and A. Jeyaprakash, Department of Entomology and Nematology, PO Box 110620, University of Florida, Gainesville, FL 32611-0620.

Maternal microinjection involves injecting plasmid DNA into adult arthropod females in the vicinity of the ovaries[1]. Adult females of the predatory mites *Metaseiulus occidentalis* and *Amblyseius finlandicus* (Acari: Phytoseiidae) and of the parasitoid wasp *Cardiochiles diaphaniae* (Hymenoptera: Braconidae) transmit the injected plasmid DNA into multiple eggs by unknown mechanism(s)[2]. Plasmid DNA, injected without helper plasmid capable of producing transposase, stably integrated into the genome of both *M. occidentalis* and *C. diaphaniae* , arthropods important in the biological control of pests. Several stable lines of *M. occidentalis* were obtained. Four lines of *M. occidentalis*, containing a *lacZ* construct with a *Drosophila hsp70* promoter, have been maintained for more than 120 generations in the laboratory. All individuals from three of these lines are positive for the *lacZ* construct by PCR analysis. Southern blot analysis indicated that one copy is integrated into the nuclear genome in one line. All four lines are transcribing mRNA, and a lacZ assay indicated translation occurs as well. Targeted gene insertion was attempted by maternal microinjection of twenty *M. occidentalis* females using a plasmid containing the *hsp70-lacZ* construct plus a fragment of a *mariner* sequence cloned and sequenced from *M. occidentalis*[3]. Two stable lines were isolated from the progeny of these injected females, and Southern blot analysis indicated the *lacZ* construct is integrated in the nuclear genome of both. The disappearance of *mariner* bands in the Southern blots of the injected lines suggests that targeted insertion of the construct occurred. Maternal microinjection of a plasmid containing the parathion hydrolase gene *(opd)* regulated by a *Drosophila hsp70* promoter resulted in stable transformation of the braconid *C. diaphaniae* and modest differences in tolerance to parathion; Southern blot analysis confirmed integration into the nuclear genome in one line. In all cases, transformation was achieved without using a transposable element vector or adding active transposase. Genetic manipulation of arthropods by maternal microinjection appears to result in high rates of stable transformation. The use of maternal microinjection, instead of transposable element vectors, could reduce concerns about risks associated with releases of transgenic beneficial (or pest) arthropods into the environment[4].

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**C3-028** TRANSPOSITION OF HOBO AND HOBO-RELATED ELEMENTS IN INSECTS, David A. O'Brochta[1], William D. Warren[2], Kenneth J. Saville[1], Steven Whyard[3], Craig J. Coates[3], Peter W. Atkinson[3]. [1]Center for Agricultural Biotechnology, College Park, MD 20742, [2] Center for the Application of Molecular Biology to International Agriculture, Canberra, Australia, [3]Commonwealth Scientific and Industrial Research Organization, Canberra, Australia.

Insect trangenesis continues to be a major priority for those involved in insect molecular science. Developing non-drosophilid transgenesis systems based on transposable elements, like those currently employed to manipulate the genome of *D. melanogaster*, are desirable because of their proven utility. Two strategies were employed to achieve this goal. First, the ability of currently available elements to transpose in foreign insect hosts was tested. Second, new transposable element systems from non-drosophilid insects of interest, or their close relatives were developed into gene vectors.

*Hobo* is a short inverted repeat type element with limited sequence similarity to *Ac* from maize and *Tam3* from snapdragons. We tested directly the ability of *hobo* to act as a gene vector using two methods. First, we tested the ability of *hobo* to transpose between plasmids introduced into *D. melanogaster*, *Musca domestica*, *Bactrocera tryoni* and *Helicoverpa armigera* embryos. In all cases *hobo* transposition events were recovered which had the hallmarks of a transpositional recombination event, including transposase dependence and 8bp duplications of the insertion site. Second, we used *hobo* as a germline transformation vector in *B. tryoni* and successfully recovered transgenic individuals. The characterization of these chromosomal integration events will be reported.

We isolated transposable elements related to *hobo*, *Ac* and *Tam3* (*hAT* elements) from *M. domestica*, *M. vetustissima*, *B. tryoni*, and *Lucillia cuprina*. *Hermes*, the *hAT* element from *M. domestica*, is 2749bp and encodes for a protein 55% identical to *hobo* transposase. Comparisons of the terminal inverted repeats of *Hermes* with other *hAT* elements revealed conserved features. We tested the mobility of *Hermes* in two ways. First, we tested the ability of *Hermes* to transpose between plasmids introduced with a source of transposase into embryos of *M. domestica*. *Hermes* transposed and resulted in the precise movement of *Hermes* sequences, causing 8bp duplications of insertion sites. Therefore, *Hermes* is a functional *hAT* element in *M. domestica*. Second, we used *Hermes* as a gene vector in *D. melanogaster* where it transposed efficiently and at high rates. Transgenic *D. melanogaster*, resulting from *Hermes* transposition and integration will be described, as will results from other insects.

**C3-029** EVOLUTION AND HORIZONTAL TRANSFER OF *MARINER* TRANSPOSABLE ELEMENTS, Hugh M. Robertson and David J. Lampe, Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

The *mariner* family of transposons is widespread in insects and related arthropods and members have recently been found in other animal phyla (nematodes, platyhelminthes, cnidaria, and vertebrates). The details of the distribution of these transposons demonstrate that they have been transferred horizontally across orders of insects and phyla of animals. A PCR assay was used to detect *mariners* in approximately 15% of over 700 species examined. Sequences of these PCR fragments, comprising the central half of the transposase gene, from at least six clones from each of 70 species delimit twenty subfamilies on the basis of both sequence differentiation (25-35% amino acid identity) and length differences (1-5 amino acids). Five major subfamilies are found in insects (1, 2). Many species have several distinct types of *mariners*. Some sequences from a platyhelminth and a cnidarian, each of which have at least five distinct kinds of *mariners* in their genomes, fall phylogenetically within these five major subfamilies, and others define new subfamilies.

The relationships of these *mariners* within particular subfamilies are highly incongruent with those of their host species. Several instances of relatively recent horizontal transfer have been investigated in more detail, by cloning and sequencing full-length copies from genomic libraries. In one case the consensus sequences of the *mariner* transposase genes from a lacewing (order Neuroptera) and a horn fly (order Diptera) differ by just one amino acid among 349. Within particular species the copies of *mariners* generally evolve neutrally, however the consensus sequences from different species are more constrained. We propose that ability to transfer horizontally between sometimes distantly related hosts provides the primary selective constraint on these transposons. We also infer that the ultimate fate of these elements within a particular genome is to mutate to inactivity, so their persistence and wide distribution is primarily ascribed to repeated horizontal transfers to new hosts.

The *mariner* family of elements is related to the *Tc1* family found in nematodes, *Drosophila*, and fish (we have recently found members of this family to be numerous and widespread in most animal species). Comparisons of their transposases reveal conserved amino acids that are presumably crucial to their function. These elements therefore have an extended evolutionary history facilitated by horizontal transfer and their apparent ability to function in the nuclear environments of diverse hosts, perhaps independently of host factors. We therefore hope that they will serve as general transformation vectors for insects and perhaps other animals. Efforts are underway to establish activity of particular reconstructed *mariners*, and several other laboratories are characterizing *mariners* from particular insects and evaluating the original active element from *Drosophila mauritiana* as a possible vector for other insects.

1. Robertson, H. M. 1993. The *mariner* transposable element is widely distributed in insects. *Nature* 362, 241-245.
2. Robertson, H. M. and E. G. MacLeod 1993. Five major subfamilies of *mariner* transposable elements in insects, including the Mediterranean fruit fly, and related arthropods. *Insect Molecular Biology* 2, 125-139.

*Insect Genomics; Population Genetics*

**C3-100** RETROTRANSPOSON INSERTION SITE SPECIFICITY ASSESSED BY UNIVERSAL PCR, Richard W. Beeman, U. S. Grain Marketing Research Laboratory, USDA, ARS, Manhattan, KS 66502

We have shown that random insertion junctions of a specific transposon can be readily cloned from genomic DNA by "universal" PCR. This strategy uses nested, transposon-specific primers in conjunction with any of several very highly degenerate (universal) primers with specific 4-6 base anchor sequences at their 3' ends. If transposon copy number were low enough, all insertion junctions for a given transposon could, in principle, be cloned by this approach. We report progress towards the goal of cloning all insertion junctions of an active retrotransposable element, "*Woot*", from two unrelated strains of the red flour beetle, *Tribolium castaneum*. Evidence to date suggests that the two strains differ in genomic distribution of *Woot* copies. Sequence analysis of a large number of 3' *Woot* insertion junctions revealed the consensus target motif GTACT.

**C3-101** THE *WHITE* LOCUS OF *ANOPHELES GAMBIAE*: GENE STRUCTURE AND MUTANT ISOLATION, Nora J. Besansky, Mark Q. Benedict, Hans Chang, and Frank H. Collins, Centers for Disease Control, Division of Parasitic Diseases, Atlanta, GA 30333 and Emory University, Department of Biology, Atlanta, GA 30322

Toward the development of a reporter gene useful for genetic transformation of *Anopheles gambiae*, DNA from the *white* ($w^+$) gene of this mosquito was cloned by homology to *w* of *Drosophila melanogaster* and *Lucilia cuprina*. It mapped by *in situ* to division 2A of the X chromosome, within 3 cM of another eye color gene, *pink* ($p^+$), mutations of which can also confer a white or pink eye phenotype. Northern blotting of mRNA from $w^+$ adult males and females detected two transcripts of 3 kb and 3.5 kb. DNA sequence of 14 kb of the $w^+$ locus, compared to a 2.9 kb cDNA, revealed 5 exons, including a 410 bp untranslated leader interrupted by an intron. Amino acid identity to *D. melanogaster* $w^+$ was 68%. Wild-type alleles were polymorphic for the insertion of a small transposable element, Pegasus, in intron 3. Using a novel mass screening strategy, we have isolated 7 X-linked eye color mutations, one induced by EMS and the others by $\gamma$–irradiation. Five were allelic to spontaneous white and pink eye mutations of $p^+$, $p^w$ and $p$, respectively, and were $w^+$ by Southern blotting and PCR. The two others, $w^2$ and $w^5$, formed a complementation group distinct from $p^+$, differed from $w^+$ by Southern blotting and PCR, and had numerous pleiotropic effects reminiscent of *D. melanogaster w* mutants. Together, these data support the conclusion that we have cloned the *D. melanogaster* $w^+$ homolog and isolated mutants with lesions in the cloned gene.

**C3-102** USE OF INTRON SEQUENCES FOR MAPPING INSECTICIDE RESISTANCE GENES IN *DROSOPHILA SIMULANS*, Bruce J. Cochrane, Matthew Morrow, Gigi Perinchery, and Michael Windelspecht, Dept. of Biology, Univ. of South Florida, Tampa, FL 33620.

A key question with respect to the genetics of insecticide resistance is the number of genes that contribute to the resistant phenotype in field-selected populations. We have identified a site at which multiple insecticide resistance has evolved in *Drosophila simulans* in response to intense use of malathion resistance for mosquito control and appears to have a polyegenic basis. To identify the specific genes involved, we have created recombinant inbred lines, in order to look for cosegregation of resistance and molecular markers. Our strategy has been to identify PCR primers that will amplify introns of sequenced genes in *D. melanogaster*, assuming i) that these sequences will be likely to contain informative polymorphisms that can readily be detected by restriction analysis, and ii) that the degree of sequence identity between D. simulans and D. melanogaster is such that these primers can be used in either species. We focus on several candidate loci, among them acetylcholinesterase, mdr50 (multidrug resistance), and calmodulin (not itself a candidate but linked to the previously mapped resistance locus at 2-64 in *D. melanogaster*). In every case, we easily identified restriction site polymorphisms that distinguish between alleles from resistant and sensitive lines of flies. We are examining 60 recombinant inbred lines, started by crossing resistant lines with sensitive laboratory ones, for cosegregation of resistance to different classes of insecticides with these markers. These and similar experiments should help determine the number and nature of genes involved in resistance, as well as to shed light on the genetic basis of cross-resistance to different classes of insecticides.

**C3-103** ISOLATION OF NOVEL CYTOCHROME P450 GENES FROM ALKALOID-TOLERANT CACTOPHILIC *DROSOPHILA* AND THEIR PHYLOGENETIC RELATIVES, Phillip B. Danielson*, Ross J. MacIntyre† and James C. Fogleman*, *Department of Biological Sciences, University of Denver, Denver, CO 80208, †Department of Genetics and Development, Cornell University, Ithaca, NY 14853

In the Sonoran Desert *Drosophila*, the results of *in vitro* metabolism assays support a central role for cytochrome P450s in the metabolism of the toxic alkaloids found in the columnar cacti that serve as feeding and breeding substrates. The extensive background information on the natural history of the cactus-*Drosophila* model system make it an excellent context in which to investigate the molecular evolution of P450-mediated insect-host plant interactions. A 3'RACE approach using a degenerate primer directed to a conserved region of cytochrome P450s in families 3, 4 and 6 was employed to isolate novel P450 cDNA fragments from alkaloid-tolerant *Drosophila* and several of their phylogenetic relatives which do not utilize alkaloid-containing substrates. Partial sequence for over 90 novel P450 cDNAs have been identified in an initial screen of over 260 clones. These sequences fall primarily into families 4 and 6 (sequences in the latter family have been implicated in xenobiotic metabolism in other insect species). The family 6 partial sequences isolated from the desert and related species of *Drosophila* appear to fall into 7 classes. In one species, at least 7 different family 6 genes are expressed simultaneously. Northern analysis of mRNA from uninduced, alkaloid-induced and phenobarbital-induced *D. mettleri* (alkaloid tolerant) larvae has identified at least one family 6 P450 gene is alkaloid inducible. and several others that are strongly induced by phenobarbital. In contrast, none of the family 4 genes tested appear to be inducible by cactus alkaloids or phenobarbital.

C3-104 MOLECULAR MARKERS OF THE *ANOPHELES GAMBIAE* CHROMOSOMES.

Alessandra della Torre, Kostas Mathiopoulos, Guido Favia, Gabriele Mariotti and Mario Coluzzi, Istituto di Parassitologia, Fondazione Pasteur-Cenci Bolognetti, Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185 Roma, Italy

A large number of cDNA clones, cosmid clones and RAPD polymorphic fragments have been localized by *in situ* hybridization to the ovarian nurse cell polytene chromosomes of the malaria vector *Anopheles gambiae*. First, the cDNA clones were isolated from a random sample and their approximate map position was determined by hybridization with the microdissected divisional probes of the *An. gambiae* polytene chromosomes. Their exact localization was determined by *in situ* hybridization. Although a large degree of accordance was observed, a few discrepancies were also noted. For fourteen of the cDNA clones a partial nucleotide sequence has been obtained and is presented here along with sequence similarities observed in the GenBank. Second, the cosmid clones were principally the result of screening with a few of the aforementioned cDNA clones of particular interest (i.e., the ones that lied close to inversion breakpoints) as well as a small set of randomly isolated cosmid clones. In addition, for two of the cosmid clones (that are in divisions 3D and 22F) we performed chromosomal walks, thus obtaining overlapping clones that span about 200 kb (see poster by Mathiopoulos et al.). Third, the RAPD clones are a set of polymorphic fragments, diagnostic for the various chromosomal forms of *An. gambiae* s.s. that are currently being analysed (see poster by Favia et al.).

C3-105 MOLECULAR CHARACTERIZATION OF POLYMORPHISMS THAT DISTINGUISH AMONG DIFFERENT CHROMOSOMAL FORMS OF *ANOPHELES GAMBIAE*.

Guido Favia, Gabriele Mariotti, Alessandra della Torre, Kostas Mathiopoulos and Mario Coluzzi, Istituto di Parassitologia, Fondazione Pasteur-Cenci Bolognetti, Università "La Sapienza", P.le A. Moro 5, 00185 Roma, Italy.

Ecological and ethological heterogeneities in the desease vector taxon represent a major problem for epidemiology and control, in view of possible important variation in the vectorial capacity. *Anopheles gambiae* s.s., the most efficient malaria vector in sub-Saharan Africa, is divided into discrete supopulations, each carrying a unique set of chromosome inversions and apparently maintained by assortative mating. These chromosomal forms are presently distinguishable only by the analysis of the characteristic inversion polymorphisms shown in the polytene complement. This type of cytotaxonomic analysis, however, depends on chromosome polytenization which occurs only in specific tissues of particular mosquito life stages. In order to develop more widely applicable molecular markers we performed a RAPD analysis on single laboratory specimens of three chromosomal forms of *An. gambiae* known as Savanna, Mopti and Bamako. Out of sixty random primers used we have identified five that yield reproducible differences, i.e., fragments specific to one of the three chromosomal forms. The reliability of these primers was tested on karyotyped field collected mosquitoes from West Africa. Primers that appear to be diagnostic for either SAVANNA, MOPTI or BAMAKO, were selected for further molecular characterization. The diagnostic fragments are currently being cloned and mapped by *in situ* hybridization. Furthermore, the cloned fragments are being sequenced in order to investigate the origin of the observed difference and create more specific diagnostic PCR primers for the different chromosomal forms.

C3-106 CYTOPLASMIC INCOMPATIBILITY IN THE FLOUR BEETLE *TRIBOLIUM CONFUSUM*: A MULTI-STRAIN ASSAY TO SEARCH FOR BIDIRECTIONAL INCOMPATIBILITY,

Roberto F. Fialho and Lori Stevens, Department of Zoology, University of Vermont, Burlington, VT 05405

Numerous insect species have been shown to harbor a rickettsial symbiont (*Wolbachia* sp.) that causes cytoplasmic incompatibility (CI) in its hosts. Unidirectional incompatibility occurs in crosses between infected males and uninfected females and is expressed as a reduction in progeny or as complete inviability. Bidirectional CI is a more rarely observed phenomenon that has been described for only a few insect hosts. It occurs in crosses between different host strains that are both infected with the symbiont and is thought to be related to different genetic strains of the microorganism. Despite its widespread distribution, little is still known about the genetic diversity and incompatibility patterns of such bacteria, especially on the host intraspecific (interstrain) level.

Several strains of the confused flour beetle (*Tribolium confusum*) are infected with *Wolbachia* sp. and show complete unidirectional CI when crossed with uninfected or cured strains. A single-pair mating experiment was conducted using 8 naturally infected geographical strains (b+, bYI, bYK, b-McGill, b-Thailand, b-Pakistan, b-Georgia and b-Ebony) to search for bi-directional CI. Each strain was crossed with itself and with all others in a factorial design. Infection was diagnosed by amplification of *Wolbachia* DNA. Results suggest no evidence of bidirectional incompatibility among the strains tested. RFLP analysis is being used to characterize *Wolbachia* from these strains and to determine if infection is caused by one or more common cytotypes.

C3-107 AUTOCIDAL BIOLOGICAL CONTROL: A NEW GENETIC STRATEGY FOR INSECT CONTROL,

Karl J. Fryxell and Thomas A. Miller, Departments of Biology and Entomology, University of California, Riverside, CA 92521

We have recently shown that the cold-sensitive embryonic lethality of the *Drosophila* $Notch^{60g11}$ mutation is expressed in heterozygotes, provided that the temperature is low enough (<19°C). Lethality is also observed in heterozygotes with an extra copy of the normal allele ($N^{60g11}/ N^{+}/ N^{+}$). We have constructed a homozygous strain, which also carries the $N^{+}$ gene on another chromosome ($N^{60g11}/ N^{60g11};\ N^{+}/ N^{+}$), as a model of a transgenic mutant strain that could be produced in other species. This homozygous strain shows good viability and fertility at 26°C. Pupae of both sexes from this strain were raised at 26°C and then added to a normal *Drosophila* population at 18°C. The result was a major reduction in the fertility of the normal population in the first generation, and complete extinction of the normal population in three generations. $N^{60g11}$ flies raised at high temperature have normal longevity and normal success in competing for mates in a mixed population at low temperature. The *Notch* gene has been highly conserved in vertebrates and arthropods, and the $Notch^{60g11}$ mutation corresponds to a simple frameshift mutation. Thus, similar mutations will be generally applicable for the genetic control of insect pests.

C3-108 STUDIES ON THE GENOME ORGANISATION AND POPULATION GENETICS OF *CERATITIS CAPITATA* USING MOLECULAR AND BIOCHEMICAL MARKERS, G. Gasperi[1], L. Baruffi[1], L. Gomulski[1], C.R. Guglielmino[2], C. Bandi[3], G. Damiani[4] and A.R. Malacrida[1], [1]Dept. of Animal Biology, University of Pavia, Pavia, Italy, [2]Dept. of Genetics & Microbiology, University of Pavia, Pavia, Italy, [3]Inst. of Veterinary Pathology, University of Milan, Milan, Italy, [4]Inst. for Exploitation of Animal Germoplasm, CNR, Milan, Italy

Random amplified polymorphic DNA (RAPD), intron size polymorphism, and multilocus enzyme electrophoresis (MLEE) analyses were used to detect genetic markers in *C. capitata*.

The genetic segregation of more than 100 RAPD polymorphisms generated with six primers was studied. The presence of these RAPD fragments segregates in agreement with expectation for simple Mendelian inheritance of dominant markers. Their linkage relationships have been assessed, and they have been assigned to the five autosomal linkage groups of *C. capitata*, previously marked with morphological and biochemical markers. In terms of population genetics, the results obtained by RAPD and MLEE, are significantly correlated, and are in agreement with a general trend of decreasing variability from African populations towards peripheral and laboratory ones. However, the RAPD technique reveals more genetic variation than the conventional MLEE, and can improve discrimination within and between populations, where biochemical genetic polymorphisms are often ineffective.

In *C. capitata*, the 1st intron of the *Adh1* gene contains a sequence homologous to the *mariner* transposable element of *Drosophila mauritana*. Using PCR, the intron was isolated from several wild populations. Seventeen size variants were found, each falling into one of three distinct size categories. The number of variants is highest in Kenyan populations. The degree of polymorphism declines rapidly towards the periphery of the global distribution, where one finds only two or three variants. The allelic status of these variants was confirmed by genetic crosses, Hardy-Weinberg equilibrium analysis, and by DNA cross-hybridisation. The small intron category lack the *mariner*-like element. The medium and large intron categories differ in the presence of an insertion/deletion in the second half of the intron. Each of the two widely spread intron size variants were found to be identical in all the peripheral populations analysed. In the ancestral populations, however, restriction site polymorphisms were found in the variants. This reduction in genetic variation further supports the derived nature of the peripheral populations.

C3-109 TWO TANDEMLY REPEATED SATELLITE-LIKE DNA SEQUENCES FROM THE BOLL WEEVIL, *ANTHONOMUS GRANDIS*, Larry J. Heilmann, USDA, ARS, Biosciences Research Laboratory, P.O. Box 5674, Fargo, ND 58105

The boll weevil, *Anthonomus grandis*, is one of the most destructive insect pests in the western hemisphere, attacking cotton crops from the US to Brazil. To develop safer and more effective methods of control I have been studying gene expression and genome structure in the boll weevil. I have cloned two tandemly repeated satellite-like sequences, one of 370 bp defined by Bam HI restriction sites, and one of 515 bp defined by Eco RI restriction sites. The two sequences show considerable homology in sequence and structure. The Eco satellite is composed of the Bam sequence plus another 45 bp of sequence from the 5' end and 100 bp from the 3' end of the Bam satellite. Individual copies of the sequences differ from each other by 2-8%. The two satellites together make up 0.5-0.7% of the genomic DNA which is equivalent to 10,000 to 15,000 copies per haploid genome. No evidence of transcription of the sequence could be detected. The sequence is not detectable in other insect DNAs including two other genera of weevils.

C3-110 **IDENTIFICATION OF QUANTITATIVE TRAIT LOCI THAT AFFECT POLLEN-HOARDING BEHAVIOR IN THE HONEY BEE,** **Greg J. Hunt, Robert E. Page, Jr., and M. Kim Fondrk, Department of Entomology, University of California, Davis CA 95616.**

**A linkage map was constructed for the honey bee that is based on the segregation of 365 random amplified polymorphic DNA (RAPD) markers and three genes in 95 haploid drones. The map was used to study the genetics of pollen-hoarding behavior, a social behavior that was measured as a colony-level phenotype - the area of pollen stored in the combs within the nest. A hybrid queen from a cross between selected high and low pollen-hoarding lines provided the haploid drones for the linkage map. The drones were each individually backcrossed to sister queens from the high-pollen line and then used for DNA extraction. Pollen stores were measured in the resulting colonies containing backcross workers. Three putative quantitative trait loci (QTLs) were identified with LOD scores higher than 2.0 (Lander and Botstein 1989). The effect on pollen foraging behavior was confirmed for two of these loci by correlating the inheritance of specific RAPD markers with individual foraging behavior of bees from a single colony resulting from another cross. The size of the linkage map (3100 cM) indicated an unusually high rate of meiotic recombination in the honey bee.**

C3-111 MOLECULAR EVIDENCE FOR A Wolbachia ENDOCYTOBIONT IN THE PREDATORY MITE, Metaseiulus occidentalis , Denise L. Johanowicz and Marjorie A. Hoy, Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611

Molecular studies to identify a rickettsial-like endocytobiont previously identified by cytological methods were performed on the spider mite predator, *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae). The polymerase chain reaction (PCR) amplified a segment of DNA using *Wolbachia*-specific primers. A portion of this amplified 16S ribosomal DNA was cloned, sequenced, and compared to other eubacterial sequences. The sequence was closely related to other *Wolbachia* detected in several insect species. This microorganism may be associated with previously described mating incompatibilities in crosses between different populations of *M. occidentalis*.

C3-112 **Searching for a phenotypic marker in *Anopheles gambiae*: Identification and cloning of a tyrosine kinase gene**

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As one of the essential steps toward genetic transformation of *A. gambiae* , we are developing a phenotypic marker which will allow for the rapid and easy detection of transformants. Based on the assumption that insect compound eye development follows a similar process to that of *Drosophila melanogaster* , we considered the *Drosophila* rough eye marker encoded by a truncated version of *sevenless* gene to be a good candidate for a phenotypic marker which could be used in all insects with compound eyes.

To ascertain the usefulness of this strategy, we searched and detected a putative *sevenless* homologue in the *A. gambiae* genome. The putative *A. gambiae sevenless* homologue was detected in genomic Southern blots using a *Drosophila sevenless* tyrosine kinase domain probe. The putative gene was isolated by a tyrosine kinase probe derived from PCR amplification. The sequence analysis of a portion of the gene shows high homology with the tyrosine kinase domain of *Drosophila sevenless* and mammalian *c-ros*. A putative transmembrane domain was located at the 5'- end of the tyrosine kinase domain like typical kinase receptors. Using RT-PCR, a transcript of this gene was detected at the early pupal stage in *A. gambiae*. We are presently conducting a functional analysis of the putative *A. gambiae sevenless* in *Drosophila* with the aim to ultimately construct a functional marker for *A. gambiae* when transgenic technology becomes available in this insect.

C3-113 MOLECULAR CHARACTERIZATION OF REPETITIVE DNA FRAGMENTS ISOLATED FROM A NUCLEAR MATRIX FRACTION OF THE *AEDES ALBOPICTUS* CELL LINE C6/36

Margaret J. Kovach, J.O. Carlson, and B.J. Beaty, Department of Microbiology, Colorado State University, Fort Collins, CO 80523

A nuclear scaffold and matrix fraction of protein was isolated from metaphase C6/36 chromosomes by the selective removal of histones and soluble proteins. Digestion of the DNA associated with this fraction of protein with restriction enzyme *Hae*III presented repetitive DNA elements upon probing with total C6/36 DNA. Two repetitive fragments with approximate sizes of 350 bp and 1750 bp were cloned into pBluescriptII (Stratagene) and designated pMRP.25 and pMRP.21, respectively. Fluorescent *in situ* hybridization to metaphase chromosomes with either element displays a uniform pattern of hybridization to each individual mosquito chromosome. Interestingly, *in situ* hybridization with pMRP.21 to metaphase spreads of HSHYG/11, a stably transformed cell line of C6/36 cells that maintains the transfected plasmid DNA in the form of an artificial chromosome, reveals not only uniform hybridization but amplification of the signal in the Mosquito Artificial Chromosome (MAC). The frequency of this element in C6/36 is approximately 1000 copies/cell and is dispersed in a non-tandem arrangement throughout the genome. Sequence analysis of pMRP.21 insert DNA shows homology to repetitive DNA elements isolated from *Anopheles gambiae* and *Caenorhabditis elegans*. A hypothetical open reading frame shows protein homology to the Tx1 transposon from *Xenopus laevis* and to putative reverse transcriptase polymerases from various species including *An. gambiae*. Southern hybridization to DNA from *Aedes aegypti* and *Aedes triseriatus* suggests that this element is not found in these species.

C3-114 *ANOPHELES GAMBIAE*: A STRUCTURAL AND FUNCTIONAL ANALYSIS OF ITS GENOME, Christos Louis, Alessandra della Torre, George Dimopoulos, Guido Favia, Inga Sidén-Kiamos and George Skavdis, Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Crete, Greece

In spite of the fact that *Anopheles gambiae* is the most important vector for the transmission of malaria in Africa the genetic data available for this organism are still relatively scarce. This is a particularly crucial fact, since a detailed knowledge of this mosquitoe's biology could potentially help develop better strategies for the control of malaria. To help overcome this deficiency, we have focused our attention to the genomics of *An. gambiae*, and we have isolated molecular genetic landmarks to be linked to, and integrated in, the microsatellite map under construction by F.C. Kafatos and his collaborators (Heidelberg). Our primary effort consisted in acquiring RAPD (Random Amplified Polymorphic DNA) markers. Several such markers were characterized, mapped cytogenetically by *in situ* hybridization to polytene chromosomes and sequence tagged sites (STS) were obtained. Some RAPDs were also mapped by recombination using microsatellites as reference points. In a parallel series of experiments we attempted the isolation and primary cytogenetic mapping of a second kind of molecular markers, namely "anonymous" cDNAs. For this we improved a technique (Differential Display) that allows for the isolation of genes that are expressed only in specific tissues or times of development. We have, thus far, isolated and partially sequenced several cDNAs expressed in the mosquito midgut after the blood meal, as well as several salivary gland-specific cDNAs.

Furthermore, in collaboration with M. Colluzi's group in Rome we developed a methodology based on arbitrary primers that allows for the rapid differentiation of incipient species of *An. gambiae* s.str.. Given the epidemiological importance of differentiating between strains of different vectorial capacity, which can only be diagnosed by cytological methods, we are still trying, in close collaboration with Rome, to further improve the methodology using PCR and different kinds of primers.

Finally, in a parallel project aiming at studying the mechanism of expression of genes that are normally turned on after a blood meal, we a) isolated and characterized 4 genes encoding serine proteases expressed in the mosquito midgut, and b) we studied a trypsin gene of *An. gambiae* in transgenic *Drosophila melanogaster*. We present data showing that the fruitfly's gene expression machinery still recognizes all sequences that are responsible for the correct local and temporal expression of this gene in the midgut.

C3-115 COEVOLUTION OF GENE-FOR-GENE RESISTANCE IN THE HESSIAN FLY WHEAT INTERACTION, Lubaki Zantoko, Richard H. Shukle and Roger H. Ratcliffe, USDA/Dept. of Entomology, Purdue University, West Lafayette, IN 47907.

The Hessian fly, *Mayetiola destructor* (Say), is a member of the large dipteran family Cecidomyiidae (the gall midges), which includes several economically important species. It is the most important insect pest of wheat, *Triticum aestivum* L., in soft winter wheat areas of the eastern United States. For the past forty years, primary control of the Hessian fly has been effected by planting genetically resistant wheat. This resistance is expressed as larval antibiosis and is generally controlled by single genes that are partially to completely dominant with duplicate epistasis. Hessian fly populations are a collection of biotypes undergoing change in response to selection pressure from resistance genes deployed in wheat cultivars. Previous genetic studies indicated that virulence in the insect is controlled by recessive alleles at different loci and operates on a gene-for-gene basis with resistance in wheat. We have undertaken (1) to further test gene-for-gene resistance in the Hessian fly-wheat system with respect to new undeployed genes for resistance, and (2) to evaluate linkage disequilibrium both among genes controlling virulence and between genes controlling virulence and morphological or molecular markers. Results will be discussed with respect to genetic control of virulence and the emergence of new more virulent biotypes.

C3-116 A DEFECTIVE NON-LTR RETROTRANSPOSON IS DISPERSED THROUGHOUT THE GENOME OF THE SILKWORM, *BOMBYX MORI*, Hideaki Maekawa, Teru Ogura*, Kazuhiro Okano, Kozo Tsuchida, Houko Tsukeda, Kazuo Hashido, Naoko Takada, Divsion of Radiation Control and Biology, National Institute of Health, Tokyo 162, Japan, *Institute for Medical Genetics, Kumamoto University Medical School, Kumamoto 862, Japan

The presence of long repetitive sequences is demonstrated in the genome of the silkworm, *Bombyx mori*. Members of this BMC1 family reveal several features typical of the L1 (long interspersed sequence one) family of mammals and of retroviruses, except for species specific elements. The number of BMC1 elements is estimated to be approximately 3,500 per haploid genome. Elements containing the full length unit of 5.1 kb are dispersed throughout the genome and their restriction sites are conserved, although most members are preferentially truncated to varying extents at their 5'ends. DNA sequencing indicates that this element contains six tandem repeats of 15 base pair CpG rich sequence in the 5'proximal region. It terminates with a 3'oligo(A) stretch, and is flanked at both ends by a 7-10 base-pair target sequence duplication. In addition, there is significant evidence for amino acid sequence homology with reverse transcriptase domains of other L1 families, especially F, Doc and Jockey of *Drosophila melanogaster*. No large open reading frame is present. We discuss the evolutionary events by which the BMC1 element might have been dispersed in the genome by a transposition mechanism involving RNA intermediates. In order to accomplish the gene introduction into the *Bombyx mori* genome, we tried to use the BMC1 element as a transformation vector.

Ogura *et al.*, Chromosoma 103 (5) (1994 in press)

C3-117 SITE-SPECIFIC NON-LTR RETROTRANSPOSONS INTERRUPT THE 28S RIBOSOMAL GENES OF THE APHID PARASITOID *A. ervi* (HYMENOPTERA, BRACONIDAE), C. Malva and P. Varricchio, Istituto *Internazionale di Genetica* e Biofisica, CNR, Via Marconi 10, 80125 Naples, Italy.

We are interested in the genome structure of a set of closely related Aphidiinae species belonging to the genus *Aphidius* Nees, of relevant interest in biological control. We have constructed *A. ervi* genomic libraries, cloned and characterized several rDNA repeating units and sequenced different regions of the rDNA cistrons. We have found that insertion sequences interrupt the *A. ervi* 28S rDNA genes. We have characterized these intervening sequences because, besides the interest in the structure and evolution of ribosomal genes, they represent the first repeated elements in the genus *Aphidius*. The sequence of the two 5' and 3' R1-28S junctions shows that the elements are inserted in the 28S gene at the same position where R1 elements are present in *D. melanogaster*, *D. virilis*, *Bombix mori* and other insect species. In addition, also in the *A. ervi* rDNA, the insertion of the element produces a duplication of the 14 nt target region. The sequence analysis and the site of insertion indicate that the *A. ervi* elements belong to the non-LTR retrotransposon family with a highly conserved reverse transcriptase domain. The data will be presented and discussed in relation to the origin, function and evolution of rDNA insertions within the insect species.

C3-118 ISOLATION OF MATERNALLY-EXPRESSED GENES OF *Manduca sexta* HOMOLOGOUS TO ANTERIOR AND POSTERIOR PATTERNING GENES OF *Drosophila melanogaster*, David J. Marcey, Richard M. Clark, Damon Cooney, and Michael B. Becknell, Department of Biology, Kenyon College, Gambier, OH 43022

*Manduca sexta*, a lepidopteran, uses zygotically-expressed genes homologous to *Drosophila* segmentation genes in the generation of segmental pattern, but the maternal information required to regulate zygotic segmentation is unknown in this insect. We are interested in studying the maternal control of embryogenesis in *Manduca* for several reasons: 1) The nature of maternal positional information is not well characterized in non-dipteran insects; 2) *Manduca* has ovaries containing numerous, large oocytes in various stages of oogenesis which provide a rich source of maternally-expressed gene products for biochemical work; 3) Understanding maternal control of embryogenesis in this model agricultural pest may provide new ideas for mechanisms of biological pest control. We have used PCR amplification and library screening to isolate *Manduca* homologues to two *Drosophila* maternal genes, *exuperantia* (*exu*) and *vasa*. We are characterizing the sequences and expression patterns of these genes. The *Manduca exu* protein is present in nurse cells and early-stage oocytes in a spatial distribution reminiscent of that in *Drosophila* oogenesis. The presence of an *exu* gene in *Manduca* is especially interesting because the only documented role for *exu* in *Drosophila* oogenesis is to localize the *bicoid* mRNA to the anterior pole of the oocyte, and *Manduca* (as well as other non-dipterans) has no *bicoid* homologue. Comparisons of *Manduca exu* and *Drosophila exu* may therefore shed light on the evolution of mRNA localization processes.

C3-119 CLONING OF INVERSION BREAKPOINTS IN THE MALARIA MOSQUITO *ANOPHELES GAMBIAE*

Kostas Mathiopoulos, A. della Torre, R. Saunders*, Guido Favia and M. Coluzzi, Istituto di Parassitologia, Fondazione Pasteur-Cenci Bolognetti, Università "La Sapienza", P.le Aldo Moro 5, 00185 Roma Italy and *Dept. of Anatomy and Physiology, University of Dundee, Dundee, UK.

Paracentric chromosomal inversions distinguish the six sibling species of *Anopheles gambiae*, the principal malaria vector. They also occur as intraspecific inversion polymorphisms which are frequent particularly in *An. gambiae* s.s. and *An. arabiensis*, the two taxa with the widest distribution and the closest association with man. We have undertaken the task of cloning the inversion breakpoints and developing specific molecular probes for identification of the different inversion karyotypes of *An. gambiae*. We wish to develop a diagnosis for specific inversions based on the breakpoint of that inversion for three main reasons. First, because we believe that this would provide unambiguous diagnosis for that inversion. Second, because elucidating the DNA structure of the breakpoint neighbourhood will supply unique information for the first systematic analysis of naturally occurring inversion breakpoints. Third, because generating molecular probes for specific inversions will greatly improve the genetic and physical maps of the *An. gambiae* genome. Two approaches are being taken. First, we are using an *An. gambiae* cosmid library in combination with specific clones that map close to inversion breakpoints. These original clones are used to screen the cosmid library and select cosmids in the vicinity of inversion breakpoints. These cosmids are used for chromosomal walks across the breakpoints. Second, we are performing a double screening of a genomic or cosmid library of a standard karyotype with microdissected DNA probes from the two breakpoints of an inverted karyotype. The positive clones that are common in the two screenings will constitute the clones that span the breakpoints of that particular inversion. Having isolated a clone that contains an inversion breakpoint, we will then sequence across it. Appropriate primers will be constructed for a diagnostic PCR of the breakpoint that will then be tested on field collected material.

C3-120 THE *HERMES* TRANSPOSABLE ELEMENT FROM THE HOUSE FLY, *MUSCA DOMESTICA*, BELONGS TO THE *HOBO, AC* AND *TAM3* (*hAT*) ELEMENT FAMILY. D. A. O'Brochta, P. W. Atkinson (1) and W. D. Warren(2), Center for Agricultural Biotechnology, University of Maryland, College Park, MD 20742-3351, (1) Division of Entomology, CSIRO, Canberra, ACT, Australia, (2) Center for the Application of Molecular Biology in International Agriculture (CAMBIA), Canberra, ACT, Australia.

The house fly, *Musca domestica*, contains an active transposable element system called *Hermes* (PNAS 90: 9693-9697; Genetical Research: in press). Using PCR and inverse PCR we amplified and sequenced overlapping segments of several *Hermes* elements and from these data we have constructed a 2749bp consensus *Hermes* DNA sequence (accession L34807). *Hermes* termini are composed of 17bp imperfect inverted repeats that are almost identical to the inverted terminal repeats of the *hobo* element of *Drosophila melanogaster*. Full length *Hermes* elements contain a single long ORF capable of encoding a protein of 612 amino acids which is 55% identical to the amino acid sequence of the *hobo* transposase. Comparison of the ends of the *Hermes* and *hobo* elements to those of the *Ac* element of *Zea mays*, and the *Tam3* element of *Antirrhinum majus*, as well as several other plant and insect elements, revealed a conserved 'A2G5' terminal sequence motif. Thus *Hermes* is clearly a member of the *hobo, Ac* and *Tam3* (*hAT*) transposable element family. Some strains of *M. domestica* contain exclusively full length *Hermes* elements, whereas others contain both full length and internally deleted elements of various sizes. We have also shown that *Hermes* is capable of acting as a gene vector in both *M. domestica* and *D. melanogaster* (see abstract entitled "*Hermes* is a Functional Non-Drosophilid Gene Vector" by Warren, Atkinson and O'Brochta).

C3-121 **INSECT METAL-RESPONSIVE GENES AS BIOMONITORS OF HEAVY METAL POLLUTION.** Ken Olson, Alfredo Rayms-Keller, Laura Klimowski, Jon Carlson and Barry J. Beaty. Department of Microbiology. Colorado State University. Fort Collins, CO 80523

Metal-responsive genes isolated from aquatic insects, such as mosquito larvae may prove to be useful tools for biomonitoring heavy metal pollution. The toxicological impact of heavy metals on *Ae. aegypti* has been demonstrated by viability and morphological studies. Metal-, dose-, and time-dependant effects on larval mortality rates has been determined. Exposure of *Ae. aegypti* larvae to heavy metals results in the obliteration of the peritrophic matrix (PM). The PM is an extracellular matrix that lines the gut of most arthropods. This is the first demonstration of a heavy metal effect on PM status.

Metal-responsive genes from an *Ae. aegypti* cDNA library have been isolated by a differential screen with radioactively labelled cDNA probes made from unexposed and metal exposed larvae polyA+RNA. Four independent metal responsive cDNA clones have been recovered. Northern blot analysis indicates that these metal responsive cDNAs hybridize to abundant RNA transcripts isolated from larval midguts after heavy metal exposure. Further characterizations of these metal responsive cDNAs, their corresponding genomic DNAs, and their regulatory elements are under way.

C3-122 LINKAGE MAP OF RANDOM AMPLIFIED POLYMORPHIC DNAS (RAPD) IN *BOMBYX MORI*. Amornrat Promboon[1], Toru Shimada[1], Marian R. Goldsmith[2], Akinori Suzuki[1] and Masahiko Kobayashi[1] : [1]Faculty of Agriculture, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, Japan and [2]Department of Zoology, University of Rhode Island, Kingston, RI, USA.

We constructed a linkage map of random amplified polymorphic DNAs (RAPDs) in *Bombyx mori*. We screened 320 10-mer primers, and found 243 clear polymorphic bands between C108 and p50 strains. In the $F_2$ generation, segregation ratios of 168 bands were nearly 3:1 after chi square test, showing Mendelian inheritance. The Mapmaker program sorted 168 bands into 29 linkage groups and 10 unlinked loci at minimum LOD score 3.0, and determined orders of loci in each group, which contained 2 to 11 markers. It also detected typing errors in our data. We calculated map distances between pairs of neighboring loci using recombination values in males and the Kosambi mapping function. Our RAPD map consists of 169 loci including the *p* locus, and the sum of map distances is approximately 900cM. Linkage groups 1 and 2 of our map correspond to chromosomes 1 and 2 on the conventional linkage map because of linkage to sex and *p*, respectively. We are analyzing both RFLPs and RAPDs of the same $F_2$ DNAs to integrate our RAPD map with an independently constructed RFLP map (Goldsmith and Shi, 1993). We integrated 19 linkage groups in our RAPD map, and 21 linkage groups in the RFLP map. Our integrated groups were also correlated with 11 linkage groups in the conventional map.

C3-123 THE HOMOLOGUE OF THE *DROSOPHILA SEX-LETHAL* GENE IN *CERATITIS CAPITATA*: AN APPROACH TO IMPROVE THE STERILE INSECT RELEASE PROGRAMS. Saccone G., Peluso I. and Polito L.C. Department of Genetics, General and Molecular Biology, University "Federico II" of Naples, Via Mezzocannone 8, 80134 Naples, Italy.

As part of our effort to control the agricultural pest *Ceratitis capitata* (medfly), we have focused our attention on improving sterile insect release programs through the development of transgenic medflies that would, potentially, produce only male progeny. Our goal is to isolate sex-specific genes and their regulatory sequences that could be used in in appropriate construct to induce female lethality. In *Drosophila melanogaster* the *Sex-lethal* (*Sxl*) gene is a key regulator of sex determination and sexual differentiation. It has an early female-specific promoter responsible for its transcriptional activation as well as several loss of function alleles resulting in female-specific lethality. In order to examine whether these characteristics of *Sex-lethal* are conserved in the medfly we have isolated its homologue. We have screened cDNA libraries of female adults and embryos with the *Drosophila Sex-lethal* cDNA as a probe and obtained 8 clones. Their sequences were compared with *Drosophila Sex-lethal* and they showed a high percentage of identity both at the nucleotide and the amino acid levels (65% and 80%, respectively) as well as two conserved types of alterna-tive splicing. Two RNA-binding domains found in the predicted amino acid sequence of *Sex-lethal* are very conserved (94% identity) suggesting a functional conservation of the *Ceratitis* homologue as an RNA binding protein. Northern blot and RT-PCR analysis showed that in male and female medflies several classes of transcripts of the same length are present. In contrast, in *Drosophila* there are male and female-specific transcripts of *Sex-lethal* which differ by 200 bp. Studies are currently underway to express medfly cDNAs in *Drosophila* in order to examine whether the medfly protein is able to function in sex determination of *Drosophila*, by complementing appropriate mutations.

C3-124 A COMPARATIVE MOLECULAR ANALYSIS OF SUSCEPTIBLE AND PYRETHROID-RESISTANT SOUTHERN CATTLE TICKS, *BOOPHILUS MICROPLUS*
Julie A. Scott, Carmen Soileau and Ron Davey*, USDA, Agricultural Research Service, Knipling-Bushland U.S. Livestock Insects Research Laboratory, Tick Unit, Kerrville, TX 78028, *USDA, Agricultural Research Service, Cattle Fever Tick Research Laboratory, Mission TX 78573
Southern cattle ticks, vectors of Texas cattle fever, are widespread throughout Mexico and continue to pose a serious threat to the U. S./ Mexican cattle industries. Recently pyrethroid resistance was reported in ticks from Mexico. Currently, toxicity testing for resistance may take as long as 80 days to complete. If pest management programs are to succeed, more rapid detection and characterization of this resistance is needed. As part of a cooperative research program between the U. S. and Mexico, we have begun to characterize the molecular genetics of pyrethroid-resistance in the southern cattle tick. We report here our inital findings regarding the genetic characterization of this resistance using ticks from a resistant colony recently imported from Mexico and susceptible ticks from a reference strain for a qualitative comparison.

C3-125 MOLECULAR AND CYTOLOGICAL ANALYSIS OF A TRANSPOSABLE ELEMENT SYSTEM FROM THE HESSIAN FLY, Richard H. Shukle and Virginia W. Russell, USDA/Dept. of Entomology, Purdue University, West Lafayette, IN 47907
Transposable genetic elements are assumed to be a feature of all eukaryotic genomes. They can serve as vectors in gene-transfer systems and as mutagenic agents for isolation of genes. The Hessian fly, *Mayetiola destructor* (Say), is an agriculturally important pest of wheat, *Triticum aestivum* L., in the United States and other parts of the world. We assessed the presence of *mariner* and *hobo* transposase-like sequences in *M. destructor* by polymerase chain reaction (PCR) assay using primers designed to detect conserved regions of the transposase genes. Nucleotide sequence analysis of PCR and genomic clones, and *in-situ* hybridization to salivary polytene chromosomes have been conducted. Results indicate the presence of an endogenous mobile-element system in *M. destructor*, which might be developed into a gene-transfer system or may serve in gene mapping.

C3-126 THE ARTIFICIAL GENERATION OF MULTIPLE INFECTIONS OF *WOLBACHIA* MAY FACILITATE REPEATED POPULATION REPLACEMENT. Steven P. Sinkins, Henk R. Braig and Scott L. O'Neill. Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520-8034.
The bacterial endosymbiont *Wolbachia* employs unidirectional cytoplasmic incompatibility (CI) as a strategy for spreading itself in insect populations; this process might be exploited to spread useful genes by a process of 'cytoplasmic drive'. *Wolbachia* has been transferred between distantly related insect species (*Aedes* and *Drosophila*) with associated expression of CI, implying that introduction into important vector species should be possible. The *Drosophila simulans* strains Riverside (DSR) and Hawaii (DSH), which are bidirectionally incompatible, harbour *Wolbachia* populations that may be distinguished by restriction analysis of the 16S rRNA PCR product. A line carrying both *Wolbachia* strains was created by the transfer of infected DSH cytoplasm into DSR, using embryonic microinjection. The dual-infected flies displayed unidirectional CI with both DSR and DSH (reduced egg hatch rate when dual-infected males are mated to single-infected females), and should thus be capable of replacing either single-infected stock at the population level. An increase in prevalence of the dual infection was observed with increasing generation number. The results have implications for the artificial generation of insect lines capable of driving genes into populations already infected with *Wolbachia*, and thus also allow more than one chance at population replacement. This could be a critical consideration given the risk of dissociation between the gene of interest and its driving system.

C3-127 MOLECULAR GENETIC ANALYSIS OF COUMAPHOS-RESISTANT SOUTHERN CATTLE TICKS, *BOOPHILUS MICROPLUS* Carmen Soileau, Julie A. Scott and Ron Davey*, USDA, Agricultural Research Service, Knipling-Bushland U.S. Livestock Insects Research Laboratory, Tick Unit, Kerrville, TX 78028, *USDA, Agricultural Research Service, Cattle Fever Tick Research Laboratory, Mission,TX 78573.
Prior to import into the U.S. all cattle from Mexico must be dipped in coumaphos at the U. S.- Mexico border and all ticks must be killed. Southern cattle ticks resistant to coumaphos are now found throughout Mexico and pose a serious threat to the cattle industry as an alternative acaricide is not readily available. As part of a cooperative research program between the U. S. and Mexico to develop improved pest management programs we have begun to characterize the molecular genetics of this resistance. We report here our inital findings regarding the characterization of genes associated with organophosphate-resistance, based on toxicological data, in ticks from a resistant colony recently imported from Mexico.

C3-128 MOLECULAR APPROACHES TO POPULATION GENETICS IN ANOPHELINE MOSQUITOES, Harold Townson, Navid Djadid and Mohammed Oshagi, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK.

An understanding of gene flow in natural populations and the extent of introgression between sibling species is important for both genetic manipulation and insecticidal control strategies for mosquito vectors.

We have used simple sequence repeats (SSRs), mitochondrial DNA (mtDNA) and RAPD PCR to study genetic variation in anopheline vectors of malaria.

Using 3'-anchored inter-SSR PCR, we have obtained molecular fingerprints for species for which there is little prior information on the genome. Changes to the length and sequence of the anchor determine the number of genomic loci that are amplified. These loci can be used to study gene flow or for gene mapping.

RAPD-PCR has provided species diagnostic characters for sibling or cryptic species. It readily permits identification of laboratory stocks of differing geographic origin and, with care, can be used to examine intra-specific variation in natural populations.

Mitochondrial DNA, particularly the cytochrome oxidase I and II genes, is proving useful in the systematics of anopheline mosquitoes and may have special value in the detection of introgression.

C3-129 IDENTIFICATION OF *TC1*-LIKE ELMENTS IN *ANOPHELES GAMBIAE*, Ann Warren and Julian M. Crampton, Wolfson Unit of Molecular Genetics, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK

*Tc1*-like sequences have been identified in the *Anopheles gambiae* genome using PCR and oligonucleotide primers corresponding to conserved amino acid motifs within the transposase region of the *Tc1*-like elements. Sequences of the cloned PCR products were used to search the available protein sequence data banks. One of these cloned PCR products, TRANG, exhibited homology to the transposase region of several *Tc1*-like elements from a variety of organisms, and shares 78% sequence identity with the corresponding region of the active *Tc1* element from the nematode *Caenorhabditis elegans*. Southern blot analysis of genomic DNA from several strains of *An. gambiae* probed with the TRANG clone indicates a very low copy number of its counterparts within genome of this mosquito. Variations in the position of TRANG in the genome further suggests that it is or was capable of transposition. The characterisation and sequence analysis of *An.gambiae* genomic clones containing the TRANG sequence will be presented.

C3-130 POLYGENIC BASIS OF MALATHION RESISTANCE IN A FIELD POPULATION OF *DROSOPHILA SIMULANS*. Michael Windelspecht[1], Heather Stevens[1], Jill Karotam[2], Virginia Walker[2] Rollin C. Richmond[1] and Bruce J. Cochrane[1]. 1. Department of Biology, University of South Florida, Tampa, FL 33611 2. Department of Biology, Queens University, Kingston, Canada.K7L 3N6

We show that organophosphate resistance in a field population of *Drosophila simulans* with a twenty year history of organophosphate exposure is polygenic in nature. Furthermore, resistance levels in the *D. simulans* population are two orders of magnitude higher than those found in the *D.melanogaster* population from the same site. In addition, without a previous exposure history, the *D.simulans* are cross resistant to both pyrethroids and carbamates . Cytochromes P450 and glutathione S-transferases may be involved, but there is no evidence of an esterase contribution to the resistant phenotype. The possibility of a novel resistance mechanism conferring both elevated organophosphate resistance and multiple insecticide cross-resistance will be discussed.

C3-131 Toward Genetic Mapping of the Refractory Mechanism of Encapsulation in *Anopheles gambiae*. L. Zheng[1], M. Benedict[2], A. Cornel[2], H. Voss[1], W. Ansorge[1], F. Kafatos[1], and F. Collins[2]. [1]EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany; and [2]CDC, Malaria Branch F12, Atlanta, Georgia 30333 USA

We have developed a genetic map for *Anopheles gambiae*, a major human malaria vector in Africa, based on polymorphic microsatellite (or simple sequence repeat) markers. The map currently consists of 132 markers, 47 on the X, 57 on the second, and 28 on the third chromosome, respectively. Four morphological markers (*collarless, lunate, red-eye, and white-eye*) and a dieldrin resistance mutation (*Dild*) have been genetically mapped.

A strain of *An. gambiae* had been selected for its ability to encapsulate oocysts of a variety of different *Plasmodium* parasites. Two genetic loci controlling this refractory phenotype were implicated from previous genetic experiments, one of which has been correlated with an esterase phenotype and the *a* inversion on the left arm of the second chromosome (2L*a*). Genetic crosses have been performed between refractory and susceptible strains. Backcrosses between heterozygous F1 to susceptible parent resulted in five families with 167 F2 female offspring. After *P. cynomolgi* B infection, the midgut of each mosquito was dissected and the number of oocysts (normal or encapsulated) oocysts was determined. Genomic DNA was then prepared from rest of the carcass. To identify the location of the gene(s) involved in the encapsulation, we are currently performing microsatellite genotyping on each mosquito.

C3-132 MOLECULAR ANALYSIS OF DpRV, A NOVEL COMMENSAL REOVIRUS OF THE WASP *Diadromus pulchellus* (HYMENOPTERA), Jean-Michel Drezen, Yves Bigot, Alain Rabouille, Marie-Hèléne Hamelin, Georges Périquet,IBEAS, Faculté des Sciences 37200 TOURS, FRANCE

Wild and laboratory populations of the parasitoid wasp *Diadromus pulchellus* are infected by the reoviridae DpRV. The use of rabbit anti-DpRV antibodies showed that the virus is present in the gut, and in smaller quantities in the venom gland. Strikingly, the genome of DpRV differs depending on the ploidy of the wasp. Virus particles purified from haploid insects (functional males) contain 10 segments whereas those of diploid insects (females and sterile diploid males) contain a supernumerary 3.35 kb segment. The supernumerary segment present in the female wasps might be involved in parasitism of the lepidopteran host *Acrolepiopsis assectella*, or might reflect the consequence of diploidy on viral physiology. The DpRV genome was cloned and the complete nucleotide sequence of 7 genomic segments were determined. Each contains one open reading frame, encoding for a putative protein with no significant similarities with data bank sequences. Viral dsRNA was found by RT-PCR in parasitized host and *Diadromus pulchellus* larvae showing that DpRV is actually transmitted to the host during oviposition. The quantification of the amount of DpRV genomic dsRNAs and transcripts ssRNAs in the parasitized host will be helpful in determining the role DpRV plays in parasitism.

*Mechanisms of Immune Responses in Insects; Vector Competence*

C3-200 IDENTIFICATION OF SURFACE MOLECULES OF MOSQUITO SALIVARY GLANDS WHICH MALARIA SPOROZOITES USE AS RECEPTORS FOR INVASION, Catherine Barreau, Musa Touray, Louis Miller and Kenneth Vernick, National Institutes of Health, Bethesda, MD 20892

There is evidence which suggests that malaria sporozoites may recognize mosquito salivary glands by specific receptor-ligand interactions. We are interested in identifying the putative salivary gland receptor(s) for sporozoite invasion. We use an *in vivo* bioassay for sporozoite invasion of salivary glands. In this assay, purified sporozoites from mature oocysts of *Plasmodium gallinaceum* are infectious to salivary glands while sporozoites purified from salivary glands do not reinvade glands. We have raised a rabbit polyclonal antiserum against female *Aedes aegypti* salivary glands which binds to the salivary gland and which blocks sporozoite invasion *in vivo*. One of several lectins that bind to salivary gland also blocks sporozoite invasion. These observations suggest that there are glycoconjugates on the surface of salivary glands which sporozoites must specifically interact with in order to invade. We are now generating monoclonal antibodies directed against the surface molecules of salivary glands by immunizing mice with a salivary gland membrane preparation. Results from these studies will be reported.

C3-201 MIDGUT PENETRATION AS A CONTROLLING FACTOR IN THE SUSCEPTIBILITY OF MOSQUITOES TO FILARIAL WORMS, Brenda T. Beerntsen, David W. Severson, Jennifer A. Klinkhammer, Victoria A. Kassner and Bruce M. Christensen, Department of Animal Health and Biomedical Sciences, 1655 Linden Drive, University of Wisconsin, Madison, WI 53706

Because previous studies have shown that susceptibility to filarial worm infection in mosquitoes is a multigenic trait, strains of *Aedes aegypti* Liverpool (LVP), highly susceptible ($LVP^{sbm}$) and refractory ($LVP^{rbm}$) to *Brugia malayi* infection, were selected from the susceptible LVP strain by pairwise mating. The $LVP^{sbm}$ strain resulting from this selection strategy had a much higher intensity of infection when exposed to the same concentration of microfilariae as compared to the parental LVP susceptible strain. We hypothesized that this increased infection intensity might be related to the ability of microfilariae to migrate out of the bloodmeal and penetrate the midgut epithelium. To test this hypothesis, we compared the ability of *B. malayi* to penetrate $LVP^{sbm}$ and $LVP^{rbm}$ midguts *in vitro* using previously described methods. The number of microfilariae penetrating and the percentage of ingested microfilariae penetrating susceptible midguts (i.e., penetration efficiency) were significantly greater as compared to refractory midguts. To investigate a genetic basis for midgut penetration, we prepared a F2 intercross population consisting of 167 individuals from our $LVP^{sbm}$ and $LVP^{rbm}$ strains, exposed them to *B. malayi*, and evaluated their phenotype relative to midgut penetration. Using RFLP markers, we identified a Quantitative Trait Locus (QTL) for midgut penetration located on chromosome 2. This finding is important because one of the QTL previously identified for susceptibility to *B. malayi*, fsb[2,LF98], also resides in this genome region. This indirectly suggests that the suitability of the midgut for filarial worm penetration could represent the phenotypic expression of one of the genes influencing *B. malayi* susceptibility in *A. aegypti*.

C3-202 CHARACTERIZATION OF HEXAMERIC SERUM PROTEIN EXPRESSION DURING MOSQUITO LARVAL DEVELOPMENT, Helen Beneš, Svetlana E. Korochkina, Alexey Gordadze, and Robb J. Moskow, Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205

During the last stage of preadult development, representatives of all insect orders express one or more hexameric serum proteins rich in aromatic amino acids, termed arylphorins. We report here the first characterization of arylphorin-like protein expression in six species representing the two major subfamilies of mosquitoes. The synthesis of two major, immunologically distinct arylphorin-like serum proteins, LSP-1 and LSP-2, has been demonstrated during the last larval instar of higher Diptera. Homologous proteins from the hemolymph of fourth-instar (L4) mosquito larvae were characterized by western blot analysis. Antisera raised against calliphorin, the LSP-1 homologue of the blowfly, *Calliphora vicina*, or against *Drosophila melanogaster* LSP-2 cross-reacted in each mosquito species with at least one major polypeptide of 70-85 kDa. Antiserum against *Drosophila* LSP-1 crossreacted with a third polypeptide or the same polypeptide exhibiting antigenicity for the calliphorin antiserum. The subunit composition of arylphorin-like hexamers in mosquito hemolymph is being studied by two-dimensional gel electrophoresis. The N-terminal sequences from the two major arylphorin-like polypeptides (SP-1 and SP-2) of *A. aegypti* served to design primers for amplification of two distinct, 2.1 kb cDNA fragments. Northern blot analysis of RNA from all larval stages of both *A. aegypti* and *A. gambiae* indicates that mosquito SP-1 and SP-2 mRNA is abundantly and uniquely expressed during the fourth instar. Cross-hybridization with heterologous *Drosophila* LSP probes suggests that *A. gambiae* genes may be more suitable for future studies of function by gene transfer into *Drosophila* LSP mutant stocks. Further characterization of regulated expression and function of these proteins during metamorphosis should provide valuable tools for biological control of preadult mosquitoes.

C3-203 GLYCOBIOLOGY OF THE MOSQUITO MIDGUT: IDENTIFICATION OF BINDING LIGANDS FOR THE INVADING OOKINETE, Peter F. Billingsley, S. Wilkins and T. Animashaun, Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB, U.K.

Our preliminary studies have suggested that recognition of the mosquito midgut wall is mediated by lectin-sugar interactions. We have therefore begun to examine the molecular, interactive contributions made by both organisms to the establishment of a successful infection in the mosquito. The major glycan residues expressed on the microvilli in the midgut of *Anopheles stephensi* have been characterised. Affinity blotting with a range of lectins, plus various degrees of deglycosylation with specific enzymes, have revealed a restricted number of *N*- and *O*-linked glycans, some of which may be unique among insects. In a series of feeding experiments, a range of monosaccharides, in particular *N*-acetyl glucosamine, *N*-acetyl galactosamine and their derivatives, have demonstrated their ability to inhibit the invasion of *Plasmodium berghei* ookinetes. This does not appear to be due to inhibition of ookinete chitinase. These combined results, which will be presented in detail, suggest that ookinete recognition of the mosquito midgut cells is a complex process. More recent studies are directed at the identification and characterisation of the mosquito glycan-based ligand for ookinete binding.

**C3-204** IDENTIFICATION OF *ANOPHELES STEPHENSI* AND *ANOPHELES GAMBIAE* GENES INVOLVED IN THE PENETRATION OF *PLASMODIUM FALCIPARUM* THROUGH THEIR MIDGUT EPITHELIUM, Catherine Bourgouin[1], Ghislaine Prévot[1], Christine Winter[2], A.M. Feldmann[3] and François Rodhain[1], 1 & 2: Ecologie des Systèmes Vectoriels & Analyse Bi-dimensionnelle, Institut Pasteur, 75015 Paris, France, 3 : IPO, PO Box 9060, 6700 GW, Wageningen, The Netherlands

*Anopheles gambiae* and *Anopheles stephensi* are important vectors of *Plasmodium falciparum*. Our goal is to identify mosquito genes, which could constitute targets for blocking the development of the parasite in this host.

We focus our attention on the penetration of the plasmodium ookinetes through the midgut epithelium of the mosquito, a key stage in malaria transmission .

We are developing two approaches for identifying mosquito genes involved in that process.

The first approache relies on the identification of midgut genes differentially expressed before and at different time points after a blood meal, using the "Differential Display "technique (Liang and Pardee,1992). Results from such analysis in *Anopheles gambiae* will be presented.

A second approach relies on a 2D-gel comparison of the protein composition of midguts isolated from a malaria-susceptible and a malaria-refractory strain of *Anopheles stephensi*. *Plasmodium falciparum* ookinetes are unable to cross the midgut epithelium of the malaria-refractory strain. Such analysis should lead to the identification of mosquito proteins important for the penetration of the parasite across the mosquito midgut. Current data indicate that the two *Anopheles stephensi* strains differ by a limited number of proteins among which some are induced by a blood meal.

Liang, P., & Pardee, A. B. (1992). Science, 257 967-971.

**C3-205** CLONING AND SEQUENCE ANALYSIS OF A PREPROTACHYKININ FROM SALIVARY GLANDS OF THE YELLOW FEVER MOSQUITO, AEDES AEGYPTI, Donald E. Champagne, Center for Insect Science and Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ 85721

Saliva of blood-feeding insects contains a variety of pharmacologically active substances that counteract the normal hemostatic response of their hosts. In addition to their role in blood feeding, some of these substances may affect parasite transmission more directly, for example by acting as immunosupressants. Disruption of these factors may have a direct effect on parasite transmission. Further, identification of regulatory sequences may facilitate specific expression of heterologous genes in salivary glands, and inhibit the ability of parasites to invade these organs en route to the vertebrate host.

Saliva of Ae. aegypti contains vasodilatory tachykinin peptides, named sialokinins, that enhance blood flow to the feeding site, and may also alter the population of activated macrophages. A degenerate primer, designed from the amino-terminal seven residues of Sialokinin I, was used in a 3'-RACE PCR experiment to amplify a 180 base pair fragment of the preprotachykinin cDNA. This product was cloned, sequenced to verify its identity, and used to screen an Ae. aegypti salivary gland cDNA library constructed in lambda ZAP. Six clones were isolated from 12,000 recombinants. The preprotachykinin has a 24 residue signal peptide, followed by a sequence of 38 residues. A single copy of the tachykinin, located at the 3' end of the open reading frame, is preceeded by a cleavage site and followed by a glycine (consistant with the amidation of the mature sialokinin) and a stop codon. The polyadenylation signal is found 60 nt from the stop codon; the entire untranslated 3' end is 83 nt long. This cDNA will be used to screen a genomic library, with a view to identifying salivary gland promotor sequences.

**C3-206** VIRUS-HOST ASSOCIATIONS IN INSECT CELLS LATENTLY INFECTED WITH HZ-1 VIRUS, Yu-Chan Chao[1], Je-Long Lin[1,2], Jin-Ching Lee[1], Jia-Luen Lee[1], Harry Alan Wood[3], and Ming-Liang Lee[2], [1]Institute of Molecular Biology, Academia Sinica; [2]Department of Biology, National Taiwan Normal University, Taipei, Taiwan, The Republic of China; and [3]Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY14853, USA.

Latent/persistent viral infections in insects are prominent though poorly understood. Here, the associations between the Hz-1 virus and insect host cells, previously referred as persistent viral infection, were studied in depth. Less than 0.2% of the cells in different newly established persistently infected cell lines were found to produce infectious viruses sponetaneously. Once re-activation took place, cell deaths were resulted. The persistently infected cell lines may contain extremely high amounts of viral DNA, up to 16% of the total cellular DNA can be of viral origin. Accordingly, there can be 2000-4000 viral genome equivalents/host cell. However, virus particles were not detected under electron microscopy. Using pulsed-field gel electrophoresis all viral DNA was found to be inserted into the host genome and/or circular episome. These data greatly broaden our knowledge of the persistent viral infection in insects and suggests that previously described persistent infection of Hz-1 virus in insect cells should better be described as a latent viral infection.

**C3-207** FAD-GLUCOSE DEHYDROGENASE: ESSENTIAL ROLE IN ENCAPSULATION OF FUNGAL INVADERS IN INSECTS. Diana L. Cox-Foster, Jessica E. Stehr, Wendy Closshey, Michelle Pieffer, and Shaozhi Zheng. Department of Entomology, Penn State University, University Park, PA 16802. USA.

In insects, FAD-glucose dehydrogenase (GLD) activity is tightly regulated and restricted temporally and spatially. This activity is essential for the insect survival in two major physiologies: molting and encapsulation. Previously we have found this enzyme activity induced and localized during encapsulation of abiotic implants in *Manduca sexta*; this activity was in both the non-cellular hemolymph and the plasmatocytes (Cox-Foster and Stehr, 1994). We demonstrate here the induction of GLD activity in *Manduca* in response to implants containing yeast, but not bacteria (*E. coli* and *B. subtilus*), within 30 minutes. Maximal activity occurred at 3 hours and had decreased to low levels by 24 hours, in contrast to the response to latex implants where GLD activity remained elevated. Implants containing chitin, chitotriose, β-glucan, laminarin, zymosan induce GLD activity, but no induction occurred with implants containing n-acetyl-glucosamine, mannose, or cellulose. GLD was also induced in *Drosophila* following an immune challenge with fungi. Injection of yeast into wild-type pharate adults and into rescued-transformed *Gld*-null mutants also induced GLD activity, while no induction occurs in *Gld*-null mutants. The essential requirement for GLD in encapsulation was tested by injecting *Gld*-null mutants, wild-type, and rescued-transformed pharate adults with either live yeast, heat-killed yeast, or saline, and comparing survival rates. Significantly higher mortality resulting from the injection of live yeast occurred in the *Gld*-null mutants in comparison to wild-type adults. Also, significantly higher numbers of yeast survived in *Gld*-null mutants. We hypothesize that GLD is required in both molting and encapsulation through an enzymatic pathway that generates free-radicals. Key to GLD's role is its tightly-bound co-factor FAD. We hypothesize that electron-transfer from $FADH_2$ to quinones results in semi-quinones that subsequently react with oxygen to generate superoxide radicals. These free radicals would be responsible for physiological actions seen during molting and encapsulation. Here we demonstrate that GLD can utilize quinones as electron acceptors. Data on the expression of *Gld* mRNA following the immune challenge will also be presented. These data suggest that GLD is among the key players of the cellular defense system of insects to foreign invaders such as pathogens and parasitoids.

C3-208 *AEDES AEGYPTI* DOPA DECARBOXYLASE: GENE SEQUENCE AND EXPRESSION, Michael T. Ferdig, Jianyong Li, David W. Severson, and Bruce M. Christensen, Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, 1655 Linden Drive, Madison, WI 53706

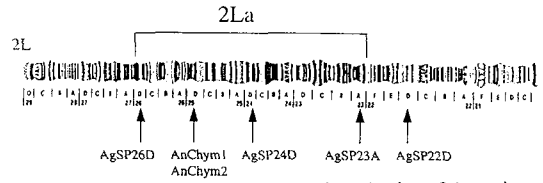
Tyrosine derivatives produced via catecholamine metabolism pathways are essential agents of tanning and other biological activities in mosquitoes. Specifically, the dopa decarboxylase (DDC) catalyzed conversion of L-dopa to dopamine has been shown to be involved in cuticular and egg-chorion tanning, as well as melanotic encapsulation of metazoan parasites. For instance, *Brugia malayi* microfilariae ingested in a bloodmeal by *Armigeres subalbatus* induce increased DDC activity in perfused hemolymph. Because tyrosine utilization pathways produce molecules with diverse biological functions in mosquitoes, we are focusing on the transcriptional-level activity of the enzymes involved in this pathway to better understand the regulatory details of melanization. We present the full DDC-encoding sequence from *Aedes aegypti*, along with its alignment with other reported DDC sequences. We have studied the expression of this gene in order to gain a detailed picture of its regulation in relationship to the biological activities of the organism. In particular, RNA was extracted from whole individuals, fat bodies, and ovaries in order to determine transcriptionally-regulated patterns of this gene in response to development, bloodmeal ingestion, and exposure to filarial worms. We also have obtained genomic clones with the intention of identifying, by way of data-base comparison, upstream regulatory elements. It is anticipated that these data will allow us to recognize molecular events contributing to the tanning/melanization process.

C3-209 CARBOHYDRATES ASSOCIATED WITH *MANDUCA SEXTA* MIDGUT AMINOPEPTIDASE N: ARE THEY *BACILLUS THURINGIENSIS* CRYIAc BINDING DETERMINANTS? Stephen F. Garczynski and Michael J. Adang, Department of Entomology, University of Georgia, Athens, GA 30602

*Bacillus thuringiensis* CryIAc toxin binds to a 120 kDa aminopeptidase in the *Manduca sexta* midgut. We used the detergent, Triton X-114, to solubilize midgut brush border membrane proteins. Enrichment for hydrophobic membrane proteins was achieved by phase separation. The detergent-rich phase contained >80% of the aminopeptidase activity. Ligand blot analysis, using $^{125}$I-CryIAc as a probe, revealed that the 120-kDa aminopeptidase also segregates with the detergent-rich phase. Treatment of the detergent-rich phase proteins with phosphatidylinositol-specific phospholipase C (PIPLC) released a portion of the 120-kDa aminopeptidase to the soluble phase, indicating that this protein is attached to the membrane via a glycosyl-phosphatidylinositol (GPI) anchor. To verify the presence of the GPI-anchor, PIPLC solubilized proteins were subjected to western blot analysis, using antibodies directed against the cross-reacting determinant (CRD) formed by PIPLC cleavage. Lectins, wheat germ agglutinin (WGA) and peanut agglutinin (PNA), were used to detect the presence of N- and O-linked oligosaccharides, respectively. Periodic acid treatment, which disrupts carbohydrate structure, completely abolished $^{125}$I-CryIAc binding to the 120-kDa aminopeptidase. Chemical and enzymatic methods were used to remove the different carbohydrate structures associated with the aminopeptidase. Selective removal of each carbohydrate structure was verified by probing the treated proteins with different lectins and antibodies which recognize the CRD, as well as $^{125}$I-CryIAc to determine toxin binding structures.

C3-210 GENETIC MAPPING OF GENES INVOLVED IN A MELANIZATION RESPONSE IN *ANOPHELES GAMBIAE*, Maureen Gorman[1], Anton Cornel[2], Frank Collins[2], Cristina Salazar[2], Yeon-Soo Han[1] and Susan Paskewitz[1], [1]Department of Entomology, University of Wisconsin, Madison, WI 53706 and [2]Vector Genetics Unit, Centers for Disease Control and Prevention, Atlanta, GA 30333

Colonies of *Anopheles gambiae* have been selected to be either susceptible or refractory to *Plasmodium cynomolgi*. Refractory mosquitoes melanize late ookinetes of this species as well as of many other *Plasmodium* spp. The 2La region of the second chromosome contains at least one gene involved in this response. We have identified six serine protease loci that map by *in situ* hybridization to the 2La region and should be valuable for further genetic mapping studies. Two of the serine proteases, SP24D and SP22D were isolated by cloning following PCR. Two additional clones, Anchym1 and 2, were provided by A. Crisanti and H.M. Mueller of University La Sapienza, Rome, Italy. The final two loci, SP26D and SP23A, were identified by *in situ* hybridization of the polytene chromosomes to SP24D under lowered stringency. RFLPs associated with the 6 loci will be used to genetically map genes in the 2La region that are involved in the immune response.




In addition, serine proteases are important in activation of the major melanization enzyme, phenol oxidase. Thus, any one of the 6 proteases could be important in refractoriness. To begin a functional analysis of the clones, SP24D has been studied by sequencing, Southern blotting and Northern analysis, and this protease is involved in mosquito responses to wounding.

C3-211 MIDGUT LECTINS OF THE MOSQUITO *Aedes aegypti*. Grubhoffer L. and Noriega F. Institute of Parasitology, Branišovská 31, 370 05 České Budějovice, Czech Republic and Department of Biochemistry, University of Arizona, Tucson Az 85721.

Lectins are a structurally diverse class of proteins, their only common features being the ability to bind carbohydrates specifically and reversibly, and to agglutinate cells. They act as mediators of cell recognition in a wide range of biological systems. Lectins from insects may play an important role in host/parasite interaction.

Hemagglutinating activity (HA) was demonstrated and characterized in *Aedes aegypti* midgut extracts. The highest HA was observed using rabbit red blood cells. In a hemagglutination inhibition test the midgut HA showed binding affinity to D-hexosamines (D-mannosamine, D-galactosamine and D-glucosamine), D-glucuronic acid and several complex glycoconjugates, including lipopolysaccharides. The midgut HA strongly binds sialylated glycoproteins (fetuin, bovine submaxillary mucin I). These binding affinities suggest that this HA is a member of the lectin family, probably a LPS-binding protein. Polyclonal antibodies were raised against this HA fraction; they have been used to identify a midgut polypeptide, with a molecular weight of 8 kDa, as a subunit of the lectin complex. The midgut lectin activity is strongly induced by blood or protein meal. Part of the inducible lectin activity seems to be associated with the peritrophic matrix. The patterns of carbohydrate binding specificity of HA in midgut extracts before and after feeding are identical.

C3-212 CHARACTERIZATION AND INDUCTION OF MIDGUT PROTEASES OF *Anopheles albimanus*. Fidel de la Cruz Hernández - Hernández. Ma. Elena Sánchez-Contreras. Ma. Eugenia Pérez-Bonilla. Febe Cázares-Raga. Mario H. Rodríguez*. Dept. of Experimental Pathology CINVESTAV-IPN. Av. I.P.N. 2508. Méx.07000. * CIP. Ministry of Health. P.O. Box 537 Tapachula Chis. Méx. 30700.
Proteolytic activity of enzymes present in midguts of adult male and female *Anopheles albimanus* were studied using zymograms. Midgut extracts were electrophoresed in polyacrylamide gels copolymerized with several proteins (substrates) and incubated under specific conditions to allow digestion. Bands with proteolytic activity were identified by negative staining with Coomassie-blue. A minimum of eleven proteolytic bands were detected in midguts of both sexes. Most of these were similar, but sex-specific bands were also detected. Enzymatic activities in female midguts, were further characterized according to their substrate specificity and susceptibility to protease inhibitors. Eleven gelatinases, 6 caseinases and 5 haemoglobinases most of them serine/cysteine proteases, were identified. Analysis of midgut preparations before and at different times after a bloodmeal indicated the presence of several enzymes before bloodmeal induction, with predominance of a band of 60 kDa. Other specific proteolytic bands began to rise after 5h postfeeding. Of particular interest were two trypsin-like proteases: one of 32 kDa, present before and during the early hours post-feeding ("early trypsin") and others, among them one of 26 kDa ("late trypsin") that appeared after 5h and lasted, at least, until 48h after the bloodmeal. Both enzymes were identified by inhibition with leupeptin and DFP.

C3-213 CHARACTERIZATION OF CALRETICULIN IN CAT FLEAS. Deborah C. Jaworski, James A. Higgins and Abdu F. Azad. Dept. of Microbiology and Immunology, University of Maryland at Baltimore, School of Medicine, 655 W. Baltimore St., Baltimore, MD 21201.
Calreticulin, the major calcium-binding protein of the endoplasmic reticulum, has recently been identified as a component of tick saliva (Jaworski *et al.*, J. Ins. Physiol., in press). In ticks, it is likely that calreticulin has a role in blood feeding through ADP-degrading enzymes (ie. apyrase) or the nitric oxide pathway. We are interested in determining whether cat fleas, *Ctenocephalides felis,* secrete calreticulin. Apyrase has been implicated in flea blood feeding and levels of this enzyme have been determined for flea salivary glands (Ribeiro *et al.*, 1990, Comp. Biochem. Physiol. 95B:215-181). Using the tick calreticulin cDNA, we made a riboprobe to study the expression of calreticulin in fleas. Through Northern blot analysis, we found a transcript for calreticulin in cat fleas. Both male and female, fed and unfed fleas contained this transcript. However, on a per flea basis, the transcript was more abundant in the fed female fleas. This agrees well with studies on tick calreticulin, where calreticulin was increased in feeding female ticks. We are currently localizing calreticulin to flea tissues by both in situ hybridization and immunoflourescent assay. In addition, we are amplifying the gene for calreticulin in fleas. We expect that there may be an enrichment for calreticulin in the flea salivary glands. Little is known about the biology of flea feeding; and what we find out about calreticulin in fleas at the molecular level is likely to impact our knowledge of transmission of flea-borne pathogens. (This study was supported by the National Institute of Health.)

C3-214 SUPPRESSION OF MAJOR PLASMA PROTEIN SYNTHESES BY BACTERIAL INJECTION IN THE SILKWORM *Bombyx mori*. Yusuke Kato, Takashi Okuda, Setsuko Komatsu, Hisashi Hirano, Keiko Kadono-Okuda, Kiyoko Taniai, Seiichi Hara, Masanori Yamamoto, Subrata Chowdhury, Jinhua Xu, Masao Sugiyama, Su-Kyung Choi, Nitish C. Debnath, Akihiro Miyanoshita, Hong-Kyu Choi, Ai Asaoka, Shiro Tomino* and Minoru Yamakawa. Department of Insect Physiology and Behavior, National Institute of Sericultural and Entomological Science, Tsukuba 305, Japan and *Department of Biology, Tokyo Metropolitan University, Tokyo 192, Japan.
There are many examples on the response against critical physiological conditions which results in the suppression of production of some proteins as well as the appearance of some stress induced proteins. This phenomenon is found in heat shock response, cold shock response and hepatic "negative acute phase proteins" in mammalian inflammatory response, etc. During immune responses in insects, selective suppression of production of proteins by a bacterial inoculation was reported (Boman and Steiner, 1981; Trenczek and Faye, 1988; etc), but the regulatory mechanisms and the physiological role of this suppression of proteins remained obscure. In this study, we found that the syntheses of SP2 (an arylphorin type storage protein) and 30K proteins are suppressed by a bacterial injection in the silkworm *Bombyx mori*. The suppression is, at least partially, attributed to the decrease of their mRNAs. A higher decrease of the SP2 mRNA was initiated over 8 h after the bacterial injection. On the contrary, the accumulation of cecropin mRNA was detected within 20 min. Moreover, the suppression needed significantly higher dose of bacteria than that to induce cecropin mRNA. We, therefore, conclude that the suppression of syntheses of some major plasma proteins are regulated in different way from that of the induction of antibacterial protein. The study to reveal the mechanism of this suppression are in progress.

C3-215 GENES INDUCED IN THE *Anopheles* GUT EARLY AFTER A BLOOD MEAL, Francisco J.A. Lemos and Marcelo Jacobs-Lorena, Dept. of Genetics, School of Medicine, Case Western Reserve University, Cleveland OH 44106-4955
Malaria is one of the major causes of disease and death in the tropics. We are searching for mosquito genes expressed early after the blood meal with two main objectives: *1) To find a strong "early promoter" that is induced before peritrophic matrix (PM) formation.* Malaria parasites ingested by a mosquito undergo complex changes and penetrate the midgut epithelium within 24 h, at which time the blood meal is completely surrounded by a thick PM. The PM separates the epithelium, the source of secreted enzymes, from the parasite-containing blood meal. An "early promoter" might be useful in transgenic mosquitoes where genes noxious to parasites could be expressed prior to PM formation. *2) To understand at the molecular level, the early developmental events triggered by blood ingestion.* Secretion of digestive enzymes begins only 6~8 h after the blood meal. The events that occur during this long lag are virtually unknown. By use of the "differential display technique" (comparison of randomly PCR-amplified mRNA 3'UTR sequences) we have identified a gene whose mRNA is relatively rare in the gut of sugar-fed *Anopheles* females but is induced to high levels two hours after a blood meal. Interestingly, sequencing of the cloned cDNA revealed that the early induced mRNA encodes an actin protein. Actin induction correlates with the loss of most of the actin-rich microvilli due to the dramatic abdominal distention and epithelial cell reorganization induced by the blood meal. Current experiments are directed toward understanding the relationship between the reorganization of the gut cell cytoskeleton and actin gene induction. The search for additional "early genes" is also in progress.

C3-216 PCR GENERATED DEFENSIN PROBES FOR THE EVALUATION OF GENE EXPRESSION IN *AEDES AEGYPTI*, Carl Lowenberger, Philippe Bulet[1], Charles Hetru[1], Jules Hoffmann[1] and Bruce Christensen, Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, 1655 Linden Drive, Madison WI 53706 and [1] Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France.

Genetic manipulation of insects, as a means of vector control, requires a detailed understanding of existing mechanisms used by vectors to avoid parasite-induced mortality. In order to investigate the production of immune proteins in vector mosquitoes, we inoculated >1500 newly emerged *Aedes aegypti* with *Escherichia coli* and *Micrococcus luteus*. Twenty-four hours following bacterial challenge, the mosquitoes were frozen and the hemolymph subsequently analyzed for antibacterial compounds. Two peptides found in extremely high levels (>100 µg/g insect) have been sequenced and identified as members of the insect defensin family. We designed primers based on the defensin sequence to amplify *A. aegypti* genomic DNA. The products were cloned and characterized as to sequence, and by Southern and Northern analysis. It is anticipated that the evaluation of expression studies using tissue specific mRNA will allow us to determine the site of production of these immune proteins. The rapid production of these peptides undoubtedly plays a role in protection from prokaryotic pathogens. Studies are currently underway to assess the potential role that these and other immune peptides play in defense response of mosquitoes to eukaryotic organisms such as *Plasmodium* spp. and filarial worms.

C3-217 THE BINDING PROTEIN OF *Bacillus thuringiensis* (Bt) CryIA(c) TOXIN ASSOCIATES WITH A COMPLEX IN MIDGUT BRUSH BORDER MEMBRANE OF *Manduca sexta*, Yang-jiang Lu and Michael J. Adang, Department of Entomology, University of Georgia, Athens, GA 30602

Previous studies identified a 120 kDa protein in the midgut brush border membrane of *Manduca sexta* as a major binding protein for Bt CryIA(c) toxin. Affinity precipitation using toxin-coupled beads, however, revealed a complex of proteins with molecular sizes of 120, 116, 110, 106, 85, 70, 46 and 40 kDa. Among these proteins, only the 120 and 116 kDa proteins bind toxin on blots. When total brush border membrane proteins were separated by gel filtration and ion exchange chromatography, we found that the 116 kDa protein could be purified to near homogeneity while the 120 kDa protein remained co-purified with the other proteins mentioned above. Similar protein composition was also found in a complex obtained by separating total brush border membrane protein by density ultracentrifugation using sucrose gradient. These results indicate that the 120 kDa CryIA(c) binding protein is in a complex form associating with other proteins in midgut brush border membrane of *M. sexta*. Evidence showed that the 116 kDa protein is the hydrophilic form of the 120 kDa protein, which is due to the loss of a hydrophobic GPI-anchor at the C-terminus. Both the 120 kDa and 116 kDa proteins are aminopeptidases, both proteins have the same N-terminus, and the toxin binding inhibited by N-acetyl galactosamine. The organization of toxin binding protein in a complex form may be important to the mechanism of Bt toxin's action.

C3-218 *BACILLUS THURINGIENSIS(BT)* CRYIA(c) AND CRYIC TOXINS POSSESS DIFFERENT BINDING SITES IN MIDGUT BRUSH BORDER MEMBRANE OF *MANDUCA SEXTA*. Ke Luo and Michael J. Adang, Department of Entomology, University of Georgia, Athens, GA 30602

*Bt* CryIA(c) and CryIC toxins are active against *M. sexta*. Both toxins show high-affinity and saturable binding to brush border membrane vesicles (BBMV) prepared from *M. sexta* midguts. Heterologous competition experiments revealed insignificant competition between CryIA(c) and CryIC toxins. The receptor for CryIA(c) toxin has been identified as aminopeptidase N with a molecular size of 120 kDa. CryIC toxin does not bind to this protein on protein blots. These data indicate that CryIC toxin recognizes a different receptor than CryIA(c) toxin . By using toxin affinity chromatography, we identified and purified a putative CryIC binding protein from *M. sexta* BBMV. The size of the protein is about 43-kDa as estimated by SDS-polyacrylamide gel electrophoresis. The binding protein for CryIC toxin in *M. sexta* is similar in molecular size to that (40-kDa) reported in *Spodoptera littorralis* BBMV. Further characterization of the biochemical and molecular properties of this protein should help us better understand the mode-of-action of *Bt* CryIC toxin.

C3-219 CHARACTERISATION OF THE DNA SEQUENCES CONTROLLING GUT-SPECIFIC GENE EXPRESSION IN THE MOSQUITO *ANOPHELES GAMBIAE*, Gareth J. Lycett[1], Hans-Michael Muller[2], Andrea Crisanti[2] and Julian M. Crampton[1], [1]Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK and [2]Institute of Parasitology, University of Rome, Rome, Italy

To direct expression of heterologous genes in specific tissues of insect vectors of disease will require a comprehensive understanding of gene expression in that organism. Towards this goal, we describe the characterisation of the regulatory sequences controlling the expression of several gut-specific genes of the malaria mosquito, *Anopheles gambiae*. Promoter mutagenesis, coupled with reporter gene assays in primary and immortal tissue cultures have been used to analyse putative regulatory regions. The regions defined should control both the blood meal response and gut specificity. Sequence comparisons of regulatory regions from alternative gut-specific genes will also be described.

C3-220 COMPARATIVE STUDIES OF THE PERITROPHIC MATRIX OF *Anopheles gambiae* AND *Aedes aegypti*.

L.A. Moskalyk[1], M.-M. Oo[2], and M. Jacobs-Lorena[2].
[1]Queen's Univ., Dept. of Biology, Kingston ON, Canada K7L 3N6
[2]Case Western Reserve U., Dept. of Genetics, Cleveland OH 44106

*Anopheles gambiae*-transmitted malaria is a major cause of death in the tropical world. Malaria parasites are ingested with the blood and need to pass through the gut to complete their life cycle. The peritrophic matrix (**PM**), a thick acellular layer that lines the midgut, is a physical barrier that the malaria parasite needs to traverse. Our experiments aim at a better understanding of the PM and its biological role. The PM is normally induced by distention of the gut epithelium by the blood meal. The PM can also be induced by feeding latex bead-saline suspension, thus avoiding contamination by exogenous proteins. We have characterized blood-induced and latex-induced PM proteins from *An. gambiae* and *Ae. aegypti* by use of 2D-gel electrophoresis. About 40 proteins are PM-specific in *An. gambiae* and about 20 in *Ae. aegypti*. The proteins of both species tend to be acidic and close to one-half of the latex-induced PM proteins migrate identically to blood-induced PM proteins. *An. gambiae* and *Ae. aegypti* appear to have at least 15 proteins in common, as judged by identical migration on 2D-gels. The glycosylation pattern of PM proteins was investigated by probing protein blots with biotin-tagged lectins. Based on lectin specificity, in both species the majority of the proteins have high-mannose type N-linked glycosylation, and few have O-linked glycosylation. As for the 2D-gel patterns, the lectin staining pattern of latex-induced proteins is less complex than for blood-induced PM proteins. We are currently fractionating latex-induced PM proteins by an initial step-extraction with salts and denaturing agents, followed by HPLC (reverse-phase and gel filtration). Individual proteins isolated in this way will be sequenced.

C3-221 CHARACTERIZATION OF THE TRYPSIN AND CHYMOTRYPSIN-LIKE GENE FAMILIES IN *ANOPHELES GAMBIAE* AND UTILIZATION OF THEIR PROMOTERS FOR TIME AND TISSUE SPECIFIC GENE EXPRESSION.

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We have identified two serine protease gene families in *Anopheles gambiae* : a trypsin gene family and two chymotrypsin-like genes. The characterization of these proteases and their blood meal-dependent regulation will provide new insights in the digestion physiology of *A.gambiae*, as well as the elucidation at the molecular level of parasite vector interactions. The functional characterization of promoter regions derived from blood meal-induced digestive proteases will provide the tools for analyzing time and tissue specific expression of anti-parasitic agents in transgenic mosquitoes.

The *A.gambiae* trypsin gene family consists of seven members clustered within 11kb. Two of them, Antryp1 and Antryp2, are blood meal-induced, whereas the residing 'early' trypsin genes, predominantly Antryp4, are expressed in unfed mosquitoes. The 'early' trypsin mRNA is already translated in unfed females and the products are stored as inactive pro enzymatic form in the midgut epithelium. Soon after blood meal, the 'early' mRNAs become undetectable and the 'early' trypsin Antryp4 is fully released as active form in the gut lumen. Secreted Antryp4 is probably unstable as it is barely detectable at the time when the 'late' blood meal-induced trypsins are secreted in the gut lumen. Feeding on trypsin inhibitor prevented the proteolysis of Antryp4 *in vivo*.

Anchym1 and Anchym2 are induced after blood feeding and their secretory products were located, together with the 'late' trypsins Antryp1 and Antryp2, in the gut lumen. *In vitro* studies using auto-activated Antryp1 or Antryp2 together with the pro enzymatic form of Anchym1 suggest, that the chymotrypsin-like proteases might be activated by trypsin *in vivo*. In contrast to the trypsin genes, the two chymotrypsin-like genes Anchym1 and Anchym2 are interrupted by two short introns which separate the three catalytic domains. The relative positions of these intron sequences are identical to those of corresponding introns occurring in vertebrate trypsin/chymotrypsin genes.

We investigated the promoter activity of the intragenic regions upstream to Antryp1, Antryp2 and Antryp4. Transient expression experiments using dissected guts of unfed and blood fed mosquitoes indicate that the upstream regions of Antryp1 and Antryp2, followed by the sequences coding for the predicted signal peptides, are able to control the expression and secretion of a reporter gene in a blood meal dependent manner.

C3-222 AN *IN VITRO* SYSTEM FOR CULTURING *WOLBACHIA* SYMBIONTS OF ARTHROPODS,

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*Wolbachia* endosymbionts occur commonly in diverse arthropod species where their presence often leads to disruptions in the early fertilization events of the host and subsequent embryonic lethality. This phenomenon (cytoplasmic incompatibility) is most commonly expressed when uninfected hosts mate with other infected hosts, or alternatively, when hosts carrying different *Wolbachia* strains mate with each other. This phenomenon provides a mechanism by which this maternally inherited agent is able to spread rapidly into a host population. It has been proposed that this phenomenon might be utilized as a means to spread transgenes into natural insect populations. Work on this organism has been hampered by its fastidious nature and the lack of an *in vitro* culturing system. In order to overcome this obstacle we have developed a continuous *Aedes albopictus* cell line which supports the growth of this organism *in vitro* and may provide a means to more easily approach questions relating to a molecular understanding of the phenomenon of cytoplasmic incompatibility.

C3-223 STRUCTURE, ORGANISATION AND EXPRESSION OF TRYPSIN GENES IN THE MOSQUITO *AEDES AEGYPTI*.

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Currently, there is much interest in the development of transgenic technology to provide novel strategies for vector control through the manipulation of mosquito genomes. Ultimately, the successful exploitation of this approach may require some degree of control over transgene expression. It may, for example, be useful to have promoters which limit expression to certain tissues (such as the mid-gut) or to certain times (perhaps post blood meal). A group of genes with potential in this respect are the trypsins. These are serine proteases which are expressed at high levels in the mosquito gut after a bloodmeal. We are focusing on the identification, structure, organisation and expression of these genes in the mosquito, *Aedes aegypti*, which is the major urban vector of arboviral disease. We have used oligonucleotides, corresponding to conserved regions of the trypsin coding sequence, as PCR primers to amplify a 453bp product from genomic DNA templates. Both the PCR product and the primers have been used to screen an *Ae.aegypti* total genomic library and several clones have been isolated. These clones are currently being characterised to determine the genomic organisation of the *Aedes* trypsin genes. We are beginning to investigate the expression patterns of these sequences, through Northern blotting at various developmental stages and through *in-situ* hybridisation to sectioned mosquito tissue. The progress of this research will be described. Subsequent analysis of the upstream regions of the trypsin genes should yield promoter and enhancer sequences which are able to constrain transgene expression.

C3-224 URIC ACID AS AN ANTIOXIDANT IN *RHODNIUS PROLIXUS* HEMOLYMPH.
Oliveira, P.L[1], Vicente, A.[1], Petretski, J.H.[2] and Bechara E.J.H.[3] (1) Departamento de Bioquímica Médica, ICB, CCS, Universidade Federal do Rio de Janeiro, 21941-590, Rio de Janeiro, RJ, Brasil. (2) Escola Técnica Federal de Química , Riode Janeiro, RJ, Brasil. (3) Departamento de Bioquímica, Instituto de Química, USP, 20780, São Paulo, SP, Brasil.

Blood-sucking insects usually feed large amounts of blood, which has about 15 g% hemoglobin. Heme, the prosthetic group of hemoglobin is a potent catalyst of the formation of oxygen free radicals. We propose that hematophagous arthropods must oppose an oxidative stress in order to get blood from their vertebrate hosts. This led us to study antioxidant mechanisms in the hemolymph of the blood-sucking bug, *Rhodnius prolixus*.

Several putative antioxidant activities were tested and very high urate concentrations were found in the hemolymph of this insect. Uric acid increased quickly after a blood meal up to about 4 mM, a concentration much higher than that found in extracellular fluids of mammals. An oxidative challenge with an injection of hemin induced increased urate titers in the hemolymph. Fat bodies incubated in *vitro* in the presence of hemin showed 80% higher rates of urate secretion. Therefore, increased urate in the hemolymph following injections of hemin were due to intensification of secretion by the fat body. Exposing the animal to a different oxidative challenge such as 100% oxygen atmosphere also resulted in higher uric acid titer in the hemolymph, suggesting that urate production is a response to the enhanced generation of free radicals and not a consequence of the injection of hemin not related to oxidative stress.

Taken together, these data indicate that urate is an important antioxidant mechanism in *Rhodnius* hemolymph and may have a role as a biochemical adaptation for blood-feeding.

Supported by FINEP and CNPq.

C3-225 BIOCHEMICAL ANALYSIS OF RESPONSES TO SEPHADEX C-25 BEADS IN PLASMODIUM-REFRACTORY AND SUSCEPTIBLE ANOPHELES GAMBIAE,
Susan M. Paskewitz, Michael Riehle, Jaesun Chun, Rick Grendell, and Maureen Gorman, Department of Entomology, University of Wisconsin, Madison, WI 53706

A genetically selected strain of *Anopheles gambiae* (L35) melanotically encapsulates *Plasmodium spp.* while susceptible mosquitoes (4arr) do not. These two strains can also be distinguished by their response to negatively-charged C-25 Sephadex beads because the L35 strain strongly melanizes beads while the 4arr strain does not. This discriminating response is not seen with other beads which are either melanized (A-25, G-25, Dowex 1-X, SP-Sephadex) or not (Dowex 50W-X) in both strains. Male L35s respond to C-25 beads more strongly than females and the response is strongly modulated by blood feeding and age in females. Melanization of beads can be inhibited *in vivo* with the phenol oxidase inhibitor, phenyl thiourea, or the serine protease inhibitor, PMSF, suggesting that phenol oxidase must be proteolytically activated before melanization can occur.

Transfer of C-25 beads from susceptible mosquitoes or susceptible hemolymph to refractory mosquitoes results in a substantial reduction of melanization when compared with beads placed directly into refractory mosquitoes. This suggests that beads are somehow protected from melanization in the susceptible strain. SDS PAGE analysis of beads removed from L35 and 4arr mosquitoes prior to melanization reveals striking differences, with 5-10 strong protein bands associated with beads from refractory mosquitoes but only one weak band associated with beads removed from susceptible mosquitoes. Comparisons between proteins adhering to A-25, C-25 and G-25 beads, all of which are destined to be melanized in the L35 strain, reveal some similarities in the protein profiles. Proteins found on all beads may be involved in the immune response and melanization.

C3-226 **THE EXPRESSION OF EARLY TRYPSIN IN *AEDES AEGYPTI***,
James E. Pennington, Fernando G. Noriega, and Michael A. Wells, The University of Arizona Department of Biochemistry and Center for Insect Science, Tucson, AZ, 85721.

Early trypsin is a female-specific protease present in the *Aedes aegypti* midgut during the first hours after the ingestion of a blood meal. It plays an essential role in the transcriptional activation of the late trypsin form, the major midgut endoprotease involved in the digestion of the blood meal.

Early trypsin is post-transcriptionally regulated by blood feeding. The early trypsin mRNA, absent in larvae, pupae, and newly emerged females, reaches detectable levels 24 h after emergence and attains maximum levels in 4-7 days-old unfed females. Despite the high levels of early trypsin mRNA present in unfed midguts, translation occurs only after a blood meal.

Rabbit polyclonal antibodies raised against recombinant early trypsin strongly recognize a midgut protein present 1-6 h after feeding. This midgut protein is the most abundant polypeptide isolated after benzamidine-sepharose affinity chromatography, binds $^{3}$H-DFP, and the purified protein has tryptic activity towards artificial substrates. The amino terminal sequence of this peptide matches that of the cDNA. At 3 hours after feeding, early trypsin concentration, measured by ELISA, is about 350 ng/midgut.

This work is supported by NIH grant AI31951.

C3-227 A CROSS-SPECIES COMPARISON OF LATE-EXPRESSED PARASITISM-SPECIFIC HEMOLYMPH PROTEINS CHARACTERIZED IN DIFFERENT SPECIES OF INSECT HOSTS,
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Insect endoparasitoids induce a variety of biochemical and physiological alterations in their host organisms. Host insects usually experience immunosuppression, which allows the successful development of the parasitoid. Hosts later undergo developmental arrest, which permits the parasitoids to successfully emerge from the non-metamorphosing host. Many parasitism-specific proteins have been discovered in hosts parasitized by egg-larval, larval-larval, and larval-pupal parasitoids. Interspecies comparisons reveal that these proteins appear at varying times during the course of the host-parasitoid interaction. Proteins expressed soon after the host is parasitized appear to be of polydnavirus origin, and encoded by the virus injected by the female parasitoid into the host; those expressed late originate from varying sources including the virus, teratocytes, and developing parasitoids. Some proteins appear correlated with particular stages of development of the parasitoids, and may be hormonally regulated. Our survey compares proteins expressed in different insect hosts (Lepidoptera, Diptera) using analysis by SDS-PAGE/2-D gels, immuno-blotting, and protein/cDNA sequence comparisons.

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C3-228 GENETICS OF *ANOPHELES GAMBIAE* REFRACTORINESS TO *PLASMODIUM CYNOMOLGI* CEYLON. Patricia Romans, Department of Zoology, University of Toronto, Toronto, ON M5S 1A1, Canada.

Mosquitoes of the malaria refractory line of *A. gambiae* selected by Collins *et al.* (1986, Science 234:607) encapsulate ookinete/oocysts of most *Plasmodium* species. Previous crosses (Vernick *et al.* 1989, Am. J. Trop. Med. Hyg. 40: 585) suggested that one main genetic locus, Pif-C, is responsible for refractoriness to *P. cynomolgi* Ceylon. The refractory line is also fixed for a restriction fragment length polymorphism (RFLP) allele at the diphenol oxidase-A2 locus (Dox-A2), whereas the malaria susceptible line selected at the same time is fixed for an alternative allele. *In situ* hybridization showed that Dox-A2 is located in the middle of chromosome 3R, in region 33C.

In order to determine whether a gene involved in refractoriness to *P. cynomolgi* Ceylon is linked to Dox-A2, backcrosses were performed using the refractory line and G3, its parent laboratory line. F1 females heterozygous for the refractory Dox-A2 allele were crossed with refractory line males. Their female offspring were fed on one of two rhesus monkeys infected with the Ceylon parasite. Six to eight days later, normal and encapsulated oocysts were counted on midguts while DNA was extracted from the remainder of each carcass and Dox-A2 genotype determined. Most mosquitoes fed on one monkey developed low numbers of oocysts ($\leq 100$) and gave no evidence of linkage between a refractoriness gene and Dox-A2, even though about half were clearly refractory (had more encapsulated than normal oocysts). In contrast, mosquitoes fed on the second monkey had much higher numbers of oocysts, sometimes more than 300, and showed clear evidence of linkage between a refractoriness gene and Dox-A2 ($P \leq 0.001$). This gene is located between 10 and 35 cM from Dox-A2, probably on chromosome 3R. The uncertainty of location is due to uncertainty in assigning a refractoriness genotype to each female: its penetrance may be incomplete. Apparently, this refractoriness gene is not expressed unless the infection burden is high. Both the Dox-A2-linked gene and Pif-C were detected in mosquitoes with high oocyst numbers. They may be the same gene.

C3-229 CHARACTERIZATION OF FEMALE MOSQUITO SPECIFIC SALIVARY GLAND GLYCOPROTEINS, Barbara Sina, Lucy Bartels-Andrews and Kathy Ratanavanich, Entomology Department, University of Maryland, College Park, MD 20742

Malaria sporozoites preferentially invade the median and distal lateral lobes of female mosquito salivary glands. Salivary glands of male mosquitoes lack these regions as well as saliva factors associated with blood feeding. Previous work demonstrated that the female specific salivary gland sections are histochemically distinct and covered by a thin basal lamina which binds a unique pattern of lectins. This pattern suggests that glycosylated surface molecules may provide the specificity that limits invasion by sporozoites to these salivary gland sections. Six to seven female salivary gland specific glycoproteins have now been identified in Anopheles gambiae and A. stephensi mosquitoes on blots using biotinylated lectins. Two female specific salivary gland glycoproteins were identified in each species which bound unique lectins. Lectins which identify female salivary gland specific glycoproteins are currently being tested to determine whether they block invasion of sporozoites in vivo. Lectin interference with sporozoite invasion may suggest that the glycoproteins bound represent salivary gland sporozoite receptor molecules.

C3-230 CHARACTERIZATION OF THE *AEDES AEGYTPI* APYRASE, A PLATELET ANTI-AGGREGATION FACTOR. Chelsea T. Smartt, Don Champagne, Jose Ribeiro, Alex P. Kim and Anthony A. James, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717, Department of Entomology, University of Arizona, Tucson, AZ 85721.

The saliva of bloodfeeding arthropods contains several antihemostatic agents that counteract normal vertebrate immune responses. One such protein, apyrase, inhibits platelet aggregation and may thus affect parasite transmission by preventing release of platelet-derived factors involved in the aggregation and activation of neutrophils. Active apyrase was purified from *Aedes aegypti* female salivary glands, subjected to tryptic digestion, and resulting peptides sequenced. The amino acid sequence matched the conceptual translation product of a cDNA clone isolated from an adult female salivary gland library. Sequence comparisons indicate similarities with an ubiquitous family of 5'-nucleotidases. The mosquito protein differs from the other members of this family by lacking the carboxy-terminal hydrophobic region that is switched for a GPI anchor in membrane-bound nucleotidases. Expression of the corresponding gene, designated *Apy*, was shown by Northern analyses and *in situ* hybridization to be restricted to the distal-lateral and medial lobes of the adult female salivary glands. Isolation of the mosquito 5'-nucleotidase gene and comparison with apyrase may reveal how this common cellular gene evolved into one that encodes an activity suited to facilitating bloodfeeding.

C3-231 REL-HOMOLOGOUS PROTEINS IN DROSOPHILA: THE DORSAL-RELATED IMMUNITY FACTOR (DIF) CAN DEFINE THE DORSAL-VENTRAL AXIS OF POLARITY IN THE EMBRYO, Dave S. Stein and Jason Goltz, Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

In Drosophila embryos, dorsal-ventral polarity is defined by an elaborate signal transduction pathway that results ultimately in the regulated nuclear localization of the Dorsal protein. Dorsal is a member of a group of proteins including c-rel and the p50 and p65 subunits of NFκB, which are regulated at the level of nuclear localization. Dorsal protein's ability to act as a transcriptional repressor of some zygotic genes and an activator of others defines structure along the dorsal-ventral axis. Recently Dif, a second Drosophila rel homologue, has been identified and proposed to act as a regulator of the immune response in Drosophila. In response to injury or injection of bacteria, the Dif protein enters the nuclei of cells in the fat body of the larva. In an effort to understand function and regulation of the rel-homologous proteins in Drosophila, we have recently expressed the Dif protein in Drosophila embryos under maternal control. We find that the Dif protein can restore significant dorsal-ventral polarity to embryos lacking Dorsal protein. Furthermore, this polarity is appropriate with respect to the intrinsic polarity of the egg shell. The ability of Dif to restore a dorsal-ventral axis depends on the signal transduction pathway normally regulating Dorsal. Thus, Dif is capable of responding to that signal. The extent of pattern restoration is consistent with the notion that Dif forms a nuclear gradient and is capable of repressing the zygotic genes that Dorsal normally represses but is not capable of activating the genes normally activated by Dorsal. Chimaeric constructs containing various portions of the Dif and Dorsal genes have been transformed into the Drosophila genome to investigate their ability to restore pattern. The results of these experiments will be presented.

C3-232 IDENTIFICATION OF GENE EXPRESSION AFFECTING REFRACTORINESS / SUSCEPTIBILITY OF *AEDES AEGYPTI* TO *BRUGIA MALAYI*, Yongjun Wen, David Severson and Bruce M. Christensen, Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, 1655 Linden Drive, Madison WI 53706

In order to determine the molecular events that account for differences in susceptibility of different strains of the mosquito, *Aedes aegypti*, to the filarial nematode, *Brugia malayi*, mRNA differential display methodologies are being used to identify and isolate differentially expressed genes. Preliminary studies suggest that gene activity from midgut and salivary gland tissues are involved with susceptibility of mosquitoes to *B. malayi*. Total RNA from midgut or salivary glands of susceptible and refractory Liverpool strain *A. aegypti* has been isolated. Following RT-PCR, expressed genes of both groups were compared by alignment on a sequencing gel. Strain-related differences were eluted from the gel, then PCR amplified cDNAs were cloned, sequenced and evaluated in biological databases for similarities with published DNA/RNA or protein sequences. Northern blots are being used to confirm differences associated with susceptibility. Confirmed clones will be mapped to test for linkage to those regions of the genome known to influence susceptibility. Forty different primer sets have been used and more than 20 putative differentially expressed messages have been cloned and are being analyzed.

C3-233 MONOCLONAL ANTIBODIES AGAINST *MANDUCA SEXTA* HEMOCYTES BIND *AEDES AEGYPTI* HEMOCYTES, Elizabeth Willott[1], Carl Lowenberger[2], Bruce Christensen[2], Michael Kanost[1], Biochemistry, Kansas State University[1], Manhattan KS 66506 or Dept of Animal Health and Biomedical Sciences[2], Univ of Wisconsin-Madison, Madison WI 53706

Monoclonal antibodies raised against *M. sexta* hemocytes were tested for binding to *A. aegypti* hemocytes. A subset of six antibodies that bind to both insects' hemocytes has been characterized by immunofluorescence microscopy. Two of the antibodies stain nuclei of both insects; one stains granules or vesicles; two stain in the cytoplasm; and the last stains in a complex pattern.

In order to obtain *A. aegypti* hemocytes suitable for immunofluorescence microscopy, we tested several different conditions for perfusing and collecting the hemocytes.

In addition we have characterized several antibodies that give a different staining pattern on hemocytes from *M. sexta* larvae injected with bacteria compared to those injected with saline.

C3-234 IDENTIFICATION AND CHARACTERIZATION OF THE PEROXIDASE GENE IN THE MOSQUITO, *AEDES AEGYPTI*, Xueling Zhao, Michael T. Ferdig, Jianyong Li and Bruce M. Christensen, Department of Animal Health and Biomedical Sciences, 1655 Linden Drive, University of Wisconsin-Madison, Madison, WI, 53706

Peroxides are multifunctional enzymes that play numerous physiological roles in living organisms. Involvement of peroxidase in hardening of the egg chorion has been demonstrated previously in the sea urchin and in *Drosophila*. Peroxidase activity also has been detected in *Aedes aegypti* ovaries. This enzyme is preferentially synthesized at particular egg developmental stages and is closely related to choriogenesis. To assess the site of synthesis of peroxidase and to evaluate the genetic regulation of this enzyme, an *A. aegypti* cDNA library was screened using a *Drosophila* peroxidase probe. Two hybridizing clones were isolated and cDNA fragments of 1.2 kb and 2.9 kb were subcloned into plasmid pGEM3Z(f-). Southern blotting of digested genomic DNA, from several mosquito species, with the 1.2 kb and 2.9 kb probes suggests a low gene copy number. Presently these cDNA clones are being sequenced. These clones also are being used in Northern blot and *in situ* hybridization studies to determine the timing, tissue-specificity, and the local cell types involved in transcriptional activity of the peroxidase gene during choriogenesis.

C3-235 INACTIVATION OF PHENOL OXIDASE BY MELANOTIC MATERIALS, Jianyong Li, Bradley A. Hodgeman and Bruce M. Christensen, Department of Animal Health and Biomedical Sciences, University of Wisconsin-Madison, 1655 Linden Drive, Madison, Wisconsin 53706

Phenol oxidase in mosquito hemolymph is present as an inactive enzyme, and this enzyme is activated by the invasion of parasites and pathogens. The active phenol oxidase catalyzes formation of melanotic materials, which is part of the components used by mosquitoes to encase and kill the invading pathogens. It was found that phenol oxidase extracted from *Aedes aegypti* mosquitoes catalyzes the hydroxylation of tyrosine to dopa, and the oxidation of dopa and 5,6-dihydroxyindole to dopaquinone and indolequinone, respectively. Indolequinone is a key intermediate in the melanization pathway, which polymerizes to form melanin polymers upon its formation. Data from in vitro studies suggest that mosquito phenol oxidase is inactivated rapidly during its reactions toward either tyrosine or dopa following the darkening of the reaction mixture. In contrast, when oxidation of dopa or tyrosine by the same enzyme is conducted under certain conditions whereby formation of 5,6-dihydroxyindole is suppressed, a several fold increase in the half-life of phenol oxidase is observed. Because blackening of the reaction mixture indicates a formation of indolequinone and polymerization of this indolequinone to form melanin polymers, we believe indolequinone and melanotic materials interact with phenol oxidase, which results in the loss of its activity. Because the products from the phenol oxidase catalyzed oxidation of dopa, as well as some other *o*-diphenolic compounds, are toxic to self materials, the inactivation of phenol oxidase by melanotic materials is suggested to be one of the self protecting mechanisms that prevent the overproduction of toxic materials.

*Hormonal Control of Gene Expression;*
*Target Genes: Reproduction and Development*

C3-300 USE OF SOMATIC CELL MOSAICS TO STUDY THE ROLE OF CALMODULIN IN DROSOPHILA DEVELOPMENT, Richard C. Atkinson and Kathy Beckingham, Dept. of Biochemistry and Cell Biology, P.O. Box 1892, Rice University, Houston, TX. 77251

Calmodulin is an essential protein required for intermediation of calcium dependent responses by a wide variety of target proteins. In addition to general signaling roles, calmodulin is required for specific calcium-mediated responses reserved for unique classes of nerve and muscle cells and in yeast, has been shown to influence the cell division cycle in a calcium independent fashion. While these activities of calmodulin encourage the search for its possible roles in development, such studies are hampered in most multicellular organisms because of genetic redundancy .

We have previously demonstrated that the calmodulin gene is unique in Drosophila and have recently identified a null mutation to the gene that is lethal during the first larval instar when maternal transcripts of calmodulin are depleted. In order to study the roles of calmodulin at stages beyond the point of organismal death for the null mutation, we are using the system of Xu and Rubin to generate somatic clones of the null mutation at various developmental stages. This system uses a heat-shock inducible FLP recombinase and generates clones homozygous for a given mutation that can be identified either by epitope tagged cytological markers or classical genetic markers. Our initial findings are i) calmodulin null clones in the peripheral nervous system result in abnormal differentiation of the sense organs and ii) null clones in all tissues are generally small. In at least some tissues, cells lacking calmodulin are deleted.

We will report on our current screening of flies heat-shocked at various times during development to determine tissue-specific requirements of calmodulin and to further characterize the fate of cellular clones lacking calmodulin.

C3-301 STRUCTURE AND EXPRESSION OF THE HOMEOTIC GENE *ABDOMINAL-A* IN THE MOSQUITO *AEDES AEGYPTI*. Rachel E. Bacon and Paul Eggleston, Wolfson Unit of Molecular Genetics, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.

A homologue of the homeotic *abdominal-A* gene from *Drosophila melanogaste*r has been isolated from the mosquito *Aedes aegypti* by screening a genomic library with fragments of the homeobox gene *Antennapaedia* from *D. melanogaster.* Subsequent analysis of the isolated clone has shown it to have 81% identity at the nucleotide level with *abd-A* from *D.melanogaster.* The majority of nucleotide substitutions can be attributed to variations in codon usage between the two insects. Conceptual translation of the mosquito sequence yields a protein which is identical to the *Drosophila* ABD-A protein throughout the 60 amino acids of the homeodomain. This identity continues for five amino acids upstream and nine amino acids downstream of the homeodomain, ending in each case at intron/exon splice junctions. Recently, homologues of *abd-A* have also been isolated from *Schistocerca, Manduca, Bombyx, Tribolium* and *Apis.* The predicted ABD-A proteins for all but one of these insects are identical throughout the homeodomain. The exception is the *Apis* gene which has only a single amino acid substitution. However, only the *Drosophila* and *Aedes abd-A* genes appear to have an intron immediately downstream of the homeobox. Work is now in progress to further characterise the structure of the *abd-A*gene from *Aedes aegypti* and to investigate its chromosomal location. We are also studying the expression of this gene, both at the levels of transcription and translation, using Northern blotting and in-situ hybridisation throughout the life cycle as well as immuno-histochemical protein localisation in sectioned mosquito tissue.

C3-302 THE BACTERIAL *opd* GENE AS A SELECTABLE MARKER FOR GENETIC TRANSFORMATION: COMPARISON WITH THE *white* GENE IN *D. melanogaster.*

Benedict, M. Q. and Salazar C. E., Emory U. Atlanta, GA

The bacterial parathion hydrolase gene (*opd*) confers resistance to paraoxon and other organophosphates by direct hydrolysis of the insecticide. This gene has potential as a selectable marker in insects and in multitudes of eukaryotic organisms. Due to the ease with which *D. melanogaster* can be transformed, we have tested its potential as a selectable marker. We have developed P element rescue vectors with *hsp70/opd* as the selectable marker and multiple-cloning sites. To determine the rate of false positives and negatives, these were tested using *hsp70/opd* as the sole marker, or in constructs also containing the *D. melanogaster miniwhite* gene as a co-marker. We have recovered 9 transformed lines using *hsp70/opd* as the sole marker. In experiments using the co-marker *miniwhite* to screen 167 $G_0$ families ( > 9000 adult flies ) we recovered 5 transformed lines and encountered no false positives, nor any wild-eye but susceptible flies carrying intact *hsp70/opd* genes. Since the *hsp70* promoter is highly conserved, and the OPD protein functions independently of specific host factors, it should be an excellent selectable marker for use not only in insects, but in almost any eukaryotic organism.

C3-303 A MOLECULAR DISSECTION OF THE COMPONENTS INVOLVED IN THE BIOSYNTHESIS OF ESSENTIAL FATTY ACIDS, Charlotte E. Borgeson and Gary J. Blomquist, Department of Biochemistry, University of Nevada, Reno, NV 89557

The $\Delta^{12}$ desaturase, a crucial enzyme in the lipid biosynthetic pathway, places the second double bond in the essential fatty acid, linoleic acid, [18:2(n-6)]. Linoleic acid is of fundamental importance in animals both as a precursor to arachidonic acid, which in turn serves as a precursor for prostaglandins, thromboxanes and leukotrienes, and as a structural membrane component. Until recently, all animals were thought to lack the $\Delta^{12}$ desaturase and thus required a dietary source of linoleic acid. However, work in our laboratory has shown that several, but not all, insect species, as well several other invertebrate species, possess active $\Delta^{12}$ desaturases.

In those species that can synthesize linoleic acid, one pattern emerges in that no strictly herbivorous species possess the $\Delta^{12}$ desaturase. However, among other species, there is no discernible pattern. Indeed, in two closely related species with identical diets, one, the American cockroach, readily synthesizes linoleic acid, while the other, the German cockroach, does not. Our current work is designed to begin to answer the question as to whether possession of the $\Delta^{12}$ desaturase gene in insects was a primitive state and subsequently lost or silenced in certain species, or whether various species independently evolved this gene. $\Delta^{12}$ desaturase activity requires three protein components, a reductase, a cytochrome $b_5$, and the terminal desaturase. We have constructed a cDNA library from Acheta domesticus and have first screened this library for the cytochrome $b_5$ gene. The reductase gene will then be isolated and the two gene products will be used in reconstitution systems to isolate the desaturase gene. These studies form the groundwork for examinations of the transcriptional and translational regulation of this key component of the eicosanoid biosynthetic pathway.

**C3-304** STRUCTURE AND FUNCTION OF MOSQUITO GONADOTROPINS, Mark Brown and Arden Lea, Dept. Entomology, University of Georgia, Athens, GA 30602; Rolf Graf, University of Zurich, Switzerland; Kristine Swiderek, Beckman Research Institute, City of Hope, Duarte, CA 91010

Three different peptide hormones, ovarian ecdysteroidogenic hormone (OEH) I and II and insulin, stimulate the ovaries to secrete ecdysteroids that, in turn, modulate yolk protein secretion by the fat body and ovarian uptake. Structural analyses of bioactive peptides, isolated from an extract of six million mosquito heads, or their enzymatic fragments resulted in the identification of OEH I and II. The two peptides are structurally unrelated, as indicated by amino acid sequences and molecular weights.

With probes to OEH I and PCR, a genomic DNA fragment was obtained, and it hybridized to two clones from a mosquito head cDNA library. The two clones are structurally similar and encode a peptide that begins with a signal peptide, contains known sequences of the amino-terminal and enzymatic fragments, and is processed to the observed MW. This sequence is related to that of neoparsin A, a locust neurohormone. With an OEH I antiserum and immunocytochemistry, we have confirmed that medial neurosecretory cells in the female brain with axons to the corpora cardiaca are the release site for this hormone; in addition, immunoreactive cells were observed in brains of larvae.

A few insect neuropeptides structurally and functionally related to vertebrate insulins have been characterized. One such group, the bombyxins, stimulate ecdysteroidogenesis in silkworm larvae, along with PTTH, which is unrelated. We have found that vertebrate insulins stimulate ecdysteroidogenesis in mosquito ovaries, and with the characterization of an insulin receptor (tyrosine kinase family) in mosquito ovaries, we believe that a mosquito insulin along with the OEHs regulate ecdysteroidogenesis in mosquitoes in the same way as bombyxins and PTTH in silkworms.

**C3-305** MOLECULAR BIOLOGICAL APPROACHES IN STUDYING THE GENE(S) THAT CONFER PHOSPHINE-RESISTANCE IN INSECTS, Chaudhry M.Q., Central Science Laboratory, Ministry of Agriculture, Fisheries and Food, London Road, Slough, Berkshire SL3 7HJ (UK).

Resistance in stored-product insects to phosphine gas, a major grain fumigant used world-wide, has been reported from more than 45 countries. The mode of action of phosphine and the mechanism of resistance in insects has been a topic of research at CSL. Our previous studies have shown that phosphine interacts with the metabolism of oxygen in insects and causes the generation of oxyradicals. However elevated levels of anti-oxidant enzymes (catalase, peroxidase, superoxide dismutase) were not detected in the resistant insects compared to their susceptible counterparts. Strikingly, the uptake of phosphine in the resistant insects was found to be only a fraction of the amount absorbed by the susceptible ones. A pulse-chase study also revealed that the gas was being excluded by the resistant insects.

We now report results from preliminary investigations into the potential involvement of active transporter genes, such as the mdr genes, in the active exclusion of phosphine in resistant insects. One approach has been to search for DNA sequences showing conserved regions encoding the ATP-binding cassette. We have also approached the problem of identifying the gene(s) that confer phosphine-resistance in insects using subtracted cDNA libraries.

**C3-306** MOSQUITO CATHEPSIN B-LIKE THIOL PROTEASE IS A YOLK PROTEIN PRECURSOR PRODUCED BY THE FAT BODY, ACCUMULATED BY OOCYTES AND ACTIVATED DURING EMBRYOGENESIS, J.-S. Chen[1], A. R. Hays[2], E. S. Snigirevskaya2 and A. S. Raikhel[1,2], [1]Program in Genetics and Program in Cell and Molecular Biology, and [2]Department of Entomology, Michigan State University, East Lansing, MI 48824

In the female mosquito, *Aedes aegypti*, in addition to the major yolk protein precursors, vitellogenin (Vg) and vitellogenic carboxypeptidase (VCP), the vitellogenic fat body also secretes a 44 kDa protein that is deposited in developing oocytes. The cDNA encoding this 44-kDa yolk protein precursor was cloned and sequenced. Its deduced amino acid sequence shares high homology with eukaryotic thiol proteases, especially cathepsin B. Therefore, this novel yolk protein precursor was designated as vitellogenic thiol protease (VTP). *In vitro* transcription/translation expression of the cloned VTP cDNA resulted in a 44-kDa protein which is recognized by anti-VTP antibodies and is similar to the native form secreted by the fat body. The size of VTP decreased to 42 kDa after its internalization by oocytes. Using immunocytochemistry, this protein was localized in organelles of the endocytotic pathway. In mature yolk bodies it is mixed with VCP in the matrix surrounding the crystalline vitellin (a storage form of Vg). At the onset of embryogenesis, VTP is further processed to 33 kDa. We demonstrated that the embryo extract containing the 33-kDa VTP degrades Vg and this Vg degradation can be prevented by a cathepsin B-specific protease inhibitor.

**C3-307** PHENOTYPIC ANALYSIS OF 5 NOVEL EMBRYONIC LETHAL MUTATIONS AFFECTING ANTERIOR CELL FATE IN *TRIBOLIUM CASTANEUM*, Marco DeCamillis, Robin Denell and Richard Beeman. 1USDA-GMRL Biological Research Unit, Manhattan KS 66502, 2Kansas State Univ Dept Biol, Manhattan KS 66506

Using the red flour beetle *Tribolium* castaneum, we are studying the structure and function of homeotic genes found within the beetle homeotic gene complex (HOM-C) that specify anterior developmental fates. In a previous genetic screen animals that carried dominant gain-of-function (GOF) mutations were irradiated and revertant individuals carrying recessive lethality in the region of the HOM-C were selected. The three GOF mutations used affect anterior cell fates. *Lucifer* (*Lu*) and *Antennagalia*$^{Antp^{GOF}}$ (*Ag*$^{Antp^{GOF}}$) are tightly linked to the HOM-C and *maxillopedia*$^{Scr^{GOF}}$; *cephalothorax*$^{Scr^{GOF}}$ (*mxp*$^{Scr^{GOF}}$;*Cx*$^{1}$) is a double mutation carrying a dominant GOF allele of *maxillopedia* (*mxp*-the *proboscipedia* homologue) and a haploinsufficient null allele of *cephalothorax* (*Cx*-the sex combs reduced homologue). Analysis of embryonic lethal phenotypes and interallelic complementation are shown. We have also tested for complementation of each new allele with HOM-C mutations previously isolated including *mxp*. *Cx*. *Prothoraxless* (*Ptl* - the *antennapedia* homologue) and *Abdominal* (*A* - the *Abdominal A* homologue). These data suggest that we have isolated novel homeotic mutations that may include the homologues of *Labial* and/or *Deformed*.

**C3-308** CHITINASES ARE A MULTI - GENE FAMILY IN INSECTS, Humberto de la Vega - Hernandez, Charles A. Specht, Yilun Liu and Phillips W. Robbins. Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139

The N- terminal region of chitinase genes from *Aedes*, *Anopheles* and *Drosophila* were amplified by PCR. The PCR products have been cloned, sequenced and used as probes in Southern hybridizations. In accordance with our results we propose that chitinase genes are a multi - gene family. Chitinases may be regulated differently during development. Expression of each is being studied.

**C3-309** VITELLINE MEMBRANE GENE EXPRESSION IN *Aedes Aegypti* MOSQUITOES, Marten J. Edwards and Henry H. Hagedorn, Department of Entomology and Center for Insect Science, University of Arizona, Tucson, AZ 85721

Attempts to control *Aedes aegypti* through genetic manipulation will benefit from the isolation of stage and tissue specific promoters. Messages from blood-fed *Aedes aegypti* ovarian RNA hybridized with three probes corresponding to three related vitelline membrane genes (15a-1, 2 and 3). RNA messages isolated from ovaries cultured in medium containing 20-hydroxyecdysone hybridized with the probes. Expression was not eliminated by the addition of 0.1 mg/ml cycloheximide to the culture medium. The relative sites and timing of expression of these genes was determined by *in situ* hybridization and northern blot analysis respectively. 1.7 Kb fragments of 15a-1, 2 and 3 genomic clones upstream of the respective coding regions were sequenced. Sequence similarities to the ovarian enhancer, heptamer and ecdysone responsive elements of *Drosophila melanogaster* are reported within the fragments. (This work was supported by grants from the NIH and the MacArthur Foundation).

**C3-310** CHARACTERISATION OF A TRANSMEMBRANE RECEPTOR PTPase EXPRESSED DURING CNS DEVELOPMENT IN THE MOSQUITO *ANOPHELES GAMBIAE*. Paul Eggleston and Susan Spiers, Wolfson Unit of Molecular Genetics, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.

We are interested in the control of neurological development in the mosquito and have recently cloned a type II transmembrane receptor-linked protein tyrosine phosphatase (PTP) from the malaria vector mosquito, *Anopheles gambiae*. Sequence data suggest that this is the homologue of the *Drosophila* Leucocyte common Antigen Related (DLAR) gene and we have named this gene *AnLAR*. The gene was isolated as a PCR product with extensive homology to the protein tyrosine phosphatase catalytic domains of the DLAR gene from *D.melanogaster*. This PCR product was used as a probe to screen an *An.gambiae* total genomic library constructed in the phage vector EMBL4. A positively hybridising 13kb EcoRI genomic fragment was identified, isolated and plaque purified. Following digestion of the EcoRI fragment with XhoI, a series of five subclones corresponding to the genomic *AnLAR* sequence were subjected to sequence analysis. These data show substantial homology to the DLAR gene from *D.melanogaster* throughout many of the major structural motifs. In the fruit fly, this gene has duplicated cytoplasmic PTP catalytic domains and an extra-cellular receptor which comprises three Ig-like repeats and ten FN III-like repeats. Expression is restricted to axons of the central nervous system and the timing and pattern of expression strongly suggest a role in the regulation of axon outgrowth and guidance during embryonic development of the CNS. Initial studies suggest that *AnLAR* has a very similar structure and we are now focusing on a more detailed structural characterisation, as well as on analyses of temporal and spatial expression patterns.

**C3-311** PURIFICATION OF A cDNA ENCODING A TACHYKININ-LIKE NEUROPEPTIDE HORMONE RECEPTOR, Felix D. Guerrero, Frank Clottens and Mark Holman, USDA-ARS Livestock Insects Research Lab, 2700 Fredericksburg Rd., Kerrville, TX 78028

Tachykinin-like peptides have been shown to promote muscle contraction in *Locusta* and function as neuropeptide hormones in physiological processes of mammals. We have initiated a project to clone the tachykinin receptor of the stable fly, *Stomoxys calcitrans*. Utilizing a probe containing a portion of the *Drosophila melanogaster* tachykinin receptor, a stable fly cDNA library was screened and a cDNA clone purified which contains significant homology to the reported sequence of the *Drosophila* tachykinin receptor.

C3-312 CLONING OF DIAPAUSE SPECIFIC GENES OF THE FLESH FLY, *SARCOPHAGA CRASSIPALPIS*. Karl H. Joplin[1], Ronald D. Flannagan[2] and David L. Denlinger[2]. [1]Dept Biological Sciences, East Tennessee State University, Johnson City, TN 37614 and [2]Dept Entomology, Ohio State University, Columbus, OH 43210.

Insects in temperate climates use diapause as an overwintering strategy. This developmental state is signalled by environmental cues, primarily photoperiod. In the flesh fly, *Sarcophaga crassipalpis*, a faculative diapause occurs that is induced by photoperiod, and is centered in the brain. Previous work has shown that brains from diapausing pupae expresse a set of diapause-specific proteins, as determined by 2D PAGE.

We have created a cDNA library from RNA isolated from the brains of diapausing pupae and screened these clones for diapause-specific genes using an elimination hybridization technique. The result of these procedures is 30 clones that are apparently specific for RNA from diapausing pupae or are upregulated in diapause.

Partial sequences (expression tagged sequences) are presented for some of these clones and show homology to proteins of neural origin or protein kinases associated with developmental pathways. We also present preliminary for the use of differential display as a means of isolating additional genes involved in diapause. Further characterization of these clones will allow us to begin analysis on the molecular controls of diapause.

C3-313 CLONING AND CHARACTERIZATION OF MOSQUITO *ULTRASPIRACLE* (USP), THE HETERODIMER PARTNER OF ECDYSTEROID RECEPTOR (EcR), M. Z. Kapitskaya[1], D. E. Cress[2], T. S. Dhadialla[2], and A. S. Raikhel[1], [1]Department of Entomology, Michigan State University, East Lansing, MI 48824 and [2]Rohm and Haas Co. Research Laboratories, 727 Norristown Rd., Spring House, PA 19477.

We cloned a full-length USP cDNA from the mosquito *Aedes aegypti*. The 2,171 bp AaUSP cDNA has an open reading frame of 459 amino acids that reveals high homology to *Drosophila* (DmUSP) and *Bombyx* (BmUSP) Ultraspiracles and to retinoic acid X receptors. The DNA-binding domain of AaUSP shares 97% and 92% identities with the respective domains of BmUSP and DmUSP. In contrast to EcRs, in which the ligand domains are highly conserved, the C-terminal portion of AaUSP corresponding to the ligand binding domain is only 50% identical to that of BmUSP and 52% of DmUSP. Two USP transcripts, 2 and 2.4 kb, were detected by Northern analysis in vitellogenic tissues of the mosquito female.

Full-length cDNAs of AaEcR and AaUSP were expressed using an *in vitro* transcription/translation system resulting in 74-kDa and 52-kDa proteins respectively. Hormone binding of the expressed AaEcR and AaUSP was tested using [$^3$H]-ponasterone A. Neither AaEcR nor USP is capable of hormone binding alone. However, they specifically bind the hormone as a heterodimer. Similarly, only the AaEcR/USP heterodimer is capable of DNA binding as tested by a gel-retardation assay using the *Drosophila* ecdysteroid response element. These experiments demonstrate the functional authenticity of the cloned AaUSP cDNA.

C3-314 MOSQUITO CLATHRIN HEAVY CHAIN GENE: ISOLATION, SEQUENCE ANALYSIS AND DIFFERENTIAL EXPRESSION IN OVARY AND SOMATIC TISSUES, Vladimir A. Kokoza and Alexander S. Raikhel, Department of Entomology, Michigan State University, East Lansing, MI 48824.

The clathrin heavy chain (CHC) is the major structural protein of coated vesicles, the principal organelles of receptor-mediated endocytosis. To understand the role of clathrin in accumulation of yolk protein precursors by mosquito oocytes we isolated and characterized genomic and cDNA clones for CHC of the mosquito, *Aedes aegypti*. Analysis of these sequences demonstrated that AaCHC gene spans more than 15 kb and its coding region is divided into 6 exons with the last exon coding for a 3'-untranslated sequence of mRNA. We identified two variants of CHC mRNA in mosquito tissues. The "ovarian" transcript (6.5 kb) is found only in ovaries of previtellogenic and vitellogenic females, while the "somatic" transcript (7.5 kb) is present in fat bodies, brain and other somatic tissues of females and males. The 3'-untranslated region in the "somatic" transcript is 1 kb longer than in the "ovarian". Both these sequences are encoded by the same exon. We suggest that the mechanism of alternative polyadenylation is involved in generating these CHC transcripts in somatic and ovarian tissues. By whole-mount *in situ* hybridization we localized the "ovarian" CHC mRNA. During the previtellogenic period it first appears in the oocytes of developing primary follicles and only later in their nurse cells. During the vitellogenic period it is present in both oocytes and nurse cells from 3 to 12 hr and disappears from the primary follicles by 24 hr after a blood meal.

C3-315 IDENTIFICATION OF A VERY EARLY GENE WHICH IS CONTROLLED BY A PROMOTER EXPRESSING IN BOTH PRODUCTIVE AND LATENT HZ-1 VIRUS INFECTIONS, Song-Tay Lee[1,2], and Yu-Chan Chao[1], [1]Institute of Molecular Biology, Academia Sinica; [2]Department of Plant Pathology and Entomology, National Taiwan University, Taipei, Taiwan, The Republic of China.

Hz-1 virus is so far the best studied viral latent infection system in insect. A gene encoding a very early transcript has been mapped to the HindIII-I fragment of Hz-1 viral genome. The size of the transcript is 6.2 kb and is transcribed from HindIII-I fragment during both productive and latent viral infections. This very early gene is expressed in host cells in a multi-step-fashion. It is weakly expressed during latent infection, however, is expressed to a greater extent during viral productive infection. During productive infection, accumulation of the transcript was first detected at 0.5 hpi and then significantly increased at 4 hpi. Primer extension experiments revealed that during productive infection, the transcription was initiated from all four nucleotides of the same TACA box. During latent infection, however, these transcription initiation sites switched to the two A nucleotides in the same TACA box. Upon cycloheximide treatment, accumulation of the transcript was greatly induced during productive viral infection but was not induced during latent viral infection. Since this is the first very early transcript being detected of Hz-1 virus during infection, study of this gene and its encoding transcript should be valuable for further analysis of differential regulation of viral gene expression during productive and latent viral infections.

C3-316 **NOVEL GENE SWITCHING IN THE SPRUCE BUDWORM:EXPRESSION OF SAME GENE DURING THE FIRST AND LAST LARVAL INSTARS,** S.R.Palli, R.Kothapalli, A.Pang and A.Retnakaran, Forest Pest Management Institute, Canadian Forest Service, Sault Ste.Marie, Ontario, Canada. P6A 5M7

The Spruce budworm, *Choristoneura fumiferana* exhibits an obligatory overwintering diapause as a second instar larva. The role of select proteins associated with this overwintering diapause was investigated. *Choristoneura* larva produces large quantities of two hexameric proteins (sub unit size 72 and 74 kDa) that are diapause related. These proteins accumulate in the first instar larval hemolymph beginning at four days after emergence and reach maximum levels by seven days after emergence. High levels of these proteins are maintained throughout diapause. These two hexameric proteins were purified to homogeneity and N-terminal sequences were obtained. The amino acid sequences showed similarity to the juvenile hormone suppressible basic hemolymph proteins from *Trichoplusia ni*. Oligonucleotide primers based on the N-terminal sequences were used in RNA-PCR to isolate cDNA fragments coding for these proteins. The mRNAs (2.4 kb) coding for these proteins were present in large quantities during all seven days of the first instar. The mRNAs however decreased to undetectable levels as soon as the larvae molted to the second instar and entered the diapause. No mRNAs could be detected throughout diapause. The mRNAs reappear in the middle of the last larval instar for 2-3 days before they disappear again. Using these proteins as antigens we produced polyclonal antibodies in the rabbit. Western blot analysis showed that these proteins appear in the first instar four days after emergence. They reach maximum levels by 7 days after emergence. These levels remain unchanged throughout diapause. These proteins decrease to minimum levels within seven days of diapause termination and do not appear until the middle of the last larval instar when they appear once again. These observations suggest that the spruce budworm may be using these proteins as an amino acid source during both diapause and pupal life. Supported by Canadian Forest Service and Science and Technology Opportunities Fund.

C3-317 ISOLATION OF ECR AND RXR SEQUENCES FROM THE IXODID TICK, *AMBLYOMMA AMERICANUM* USING RT-PCR, M.J. Palmer, X. Guo, and Q. Xu, Department of Entomology, Oklahoma State University, Stillwater, OK 74078

Ecdysteroids control a number of developmental transitions in ixodid ticks, including those associated with embryogenesis, molting, diapause, and reproduction. However, dissection of ecdysteroid responses in ixodid ticks is complicated by their small size and the lack of defined endocrine, neuroendocrine, and neurohemal tissues. The ecdysone receptor (EcR) and its functional partner Ultraspiracle (USP) are both members of a superfamily of nuclear receptors that encode ligand-activated transcription factors. USP is the invertebrate counterpart of vertebrate RxR receptors, which are cofactors in diverse hormone responses.

We have employed RT-PCR to amplify EcR and RxR/USP sequences from the ixodid tick, *Amblyomma americanum*, to explore the temporal and tissue-specific pattern of their expression. Using degenerate oligonucleotides derived from conserved amino acids in the DNA and/or ligand binding domains, we have amplified a single 738 bp EcR fragment and several distinct 126 bp RxR/USP fragments from the RNA of molting larvae and nymphs. The translated EcR sequence has 48% identity to the corresponding region of the *Drosophila* EcR protein, and is 100% and 58% identical, respectively, to amino acids in DNA and hormone binding domains. The translated RxR/USP sequences show between 60% to 98% identity to the DNA binding domains of USP and RxR proteins. Northern blots detect a single EcR transcript of ~10 kb in RNA from embryos and molting larvae and nymphs. Similar experiments using RxR/USP fragments as probes detect at least two transcripts, suggesting that these genes are alternatively processed in a stage- and/or tissue-specific manner.

C3-318 REGULATION OF THE TRANSFERRIN GENE IN THE TROPICAL COCKROACH *Blaberus discoidalis*, Daphne Q.-D. Pham and John H. Law, Department of Biochemistry and the Center for Insect Science, University or Arizona, Tucson, AZ 85721

Iron is an essential component of many enzymatic processes. Perturbations in iron metabolism play crucial roles in various diseases, including anemia, haemochromatosis and cancer. Although preliminary data on insect iron metabolism show similarities between the insect and vertebrate systems, little is known about iron metabolism in insects. This is unfortunate because past studies in insects have led to findings that eventually resulted in breakthroughs in vertebrate studies. A study of insect iron metabolism will bring new depths to the understanding of iron-binding proteins and other proteins associated with the regulation of cellular iron uptake.

This study is designed to expand our knowledge of iron metabolism. Because not much is known about insect iron metabolism, the current study will focus on establishing the genetic groundwork for transferrin (Tf), an iron-binding protein well known for its involvement in iron metabolism in mammals. Because of its role in iron transport and because iron is a fundamental requirement in numerous basic cellular processes, Tf plays an essential role not only in iron metabolism, but also in cellular differentiation and growth. The specific aim for this proposal is to study the regulation of the Tf gene in the tropical cockroach *B. discoidalis*. Work from our laboratory has indicated that the expression of this gene is under the negative control of juvenile hormone. This study will identify regulatory factors that are involved in the transcriptional regulation of the Tf gene, specifically, *cis*-acting regulatory elements.

C3-319 IMMUNOLOCALIZATION OF A 17 KDA VACUOLAR PROTON ATPASE SUBUNIT FROM *HELIOTHIS VIRESCENS*: A LIGHT AND LASER CONFOCAL MICROSCOPY STUDY, Patricia V. Pietrantonio and Sarjeet S. Gill, Department of Entomology, 5419 Boyce Hall, University of California, Riverside, Riverside, CA 92521

We have previously reported the cloning of a 1.9 kb cDNA from *Heliothis virescens* midgut and Malpighian tubules phage library. This clone contains an open reading frame encoding a protein of 156 amino acids with a predicted molecular weight of 17.2 kDa. Two transcript sizes were observed in Northern blots of both midgut and Malpighian tubules. Expression of this protein in *E. coli* resulted in cell death, therefore, antiserum was developed against a synthetic peptide corresponding to a putative extramembrane region. Specific antibodies were purified on a peptide affinity column and were checked in western blots of Malpighian tubules homogenates where they identified a band of the expected size. In paraffin sections, affinity purified antibodies localized this protein in midgut goblet cell apical membranes and on the apical membranes of Malpighian tubules in both, *Heliothis virescens* and *Manduca sexta* fifth instar 0 day larvae. These antibodies also localized the protein in *H. virescens* pharate males brain.

C3-320 DETOXIFICATION ENZYMES IMPLICATED IN INSECTICIDE RESISTANCE ARE EXPRESSED IN THE ANTENNAE OF DROSOPHILA. C. W. PIKIELNY AND G. HASAN. Dept. of Neuroscience and Cell Biology, UMDNJ, 475 Hoes Lane, Piscataway, NJ 08854 and National Centre for Biological Sciences, Indian Inst. of Sci., Bangalore 560012, India.

We have developed a PCR-based method allowing the construction of subtractive cDNA libraries starting with very small amounts of RNA (nanograms). Using this method, we have generated a cDNA library from the antennae (the main olfactory organ) of *Drosophila melanogaster* from which head cDNAs have been subtracted. Among the clones in this library we have found *Drosophila* homologues of degradation enzymes proposed to be involved in odorant inactivation in vertebrates, cytochrome P450 and UDP-glucuronosyl transferase. Their expression in antennae implies a role in olfaction conserved throughout evolution. Two other degradation enzymes, an esterase and a peroxyde hydrolase may also participate in odorant metabolism. Interestingly, P450, esterase and peroxyde hydrolase have each been implicated in various forms of pesticide resistance, a specialized form of detoxification, in various insects.

C3-321 CLONING AND CHARACTERIZATION OF A *SEC-18* HOMOLOGUE FROM *MANDUCA SEXTA*, Ashok K. Pullikuth and Sarjeet S. Gill, Department of Entomology, University of California, Riverside, CA 92521

Secretion involving vesicle transport between organelles is a conserved process among eucaryotes. Proteins involved in this process show remarkable similarity from yeast to higher eucaryotes. These proteins are implicated in targeting cargo proteins between sub-cellular organelles and in the early steps leading to vesicle docking for neurotransmitter release. A *N*-ethylmaleimide-sensitive fusion protein (NSF) assembles a 20S particle consisting of a number of soluble NSF attachment proteins (SNAPs) which restores intercisternal Golgi transport. This mechanism has also been suggested to operate during tightly regulated forms of exocytosis such as triggered fusion of synaptic vesicles with the presynaptic membrane. NSF, which is encoded by *SEC-18* gene in yeast, is necessary for transport from endoplasmic reticulum to the Golgi. We have isolated a 5.5 kb cDNA, potentially coding for a NSF from *Manduca sexta*. Sequence comparison of this clone with members of the *SEC-18* family show high degree of conservation. Data on molecular characterization of this clone will be presented.

C3-322 CATHEPSIN IN *MUSCA DOMESTICA* EMBRYOGENESIS, Paulo E.M. Ribolla and Antonio G. de Bianchi, Departamento de Bioquímica, Universidade de São Paulo, São Paulo, CP 20780, Brazil.

The degradation of yolk proteins during *Musca domestica* embryogenesis is due to a cathepsin-like proteinase found in unfertilizeted oocytes. This enzyme is composed by a single polypeptide of 41-kDa and its $NH_2$-terminal aminoacid sequence (Ala-Pro-Lys-Tyr-Val-Asp-Tyr-Gly-Glu-Asn-Gly) shows similarities with other cathepsins B and L. This enzyme is stored as a zymogen of 55-kDa during the vitellogenesis. The zymogen activation is dependent of pH lowering and inhibited by cathepsin inhibitors. The autoactivation occurs in two steps by intra and intermolecular cleavages of the zymogen resulting in an intermediary form of 48-kDa that gives origin to the mature form. The zymogen is present into the yolk granules in the mature oocytes. After the oocyte fertilization, the enzyme activity increase to attain a maximum at 3.5 hours after fertilization and, at the end of the embryogenesis, no cathepsin activity was found.

C3-323 MOSQUITO VITELLOGENIN RECEPTOR: PURIFICATION AND DEVELOPMENTAL, BIOCHEMICAL, AND MOLECULAR CHARACTERIZATION, T. W. Sappington, A. R. Hays, and A. S. Raikhel, Dept. of Entomology, Michigan State University, East Lansing, MI 48824.

A modification of the method for extracting the mosquito (Aedes aegypti) vitellogenin receptor (VgR) from ovary membranes resulted in an 11-fold higher yield and 56-fold increase in relative purity of the VgR, in turn permitting purification, antibody production, and microsequencing. Immunoprecipitation and native PAGE experiments suggest that the 205 kDa VgR occurs naturally as a ~390-kDa noncovalent homodimer. A quantitative immunoassay of VgR extracts showed that VgR was present in previtellogenic ovaries on the day of emergence, increasing from 2 ng to more than 10 ng per ovary by day 5. After initiation of vitellogenesis and onset of vitellogenin uptake, VgR quantity increased rapidly between 8 and 24 h after a blood meal, then declined between 24 and 36 h. Immunocytochemistry confirmed the presence of substantial amounts of the VgR in 4-day-old previtellogenic oocytes. In both previtellogenic and vitellogenic ovaries, the VgR was present only in the oocyte, primarily in the cortex. Three tryptic fragments of the VgR were microsequenced (7, 10, and 19 aa) and degenerate primers for PCR were synthesized. One combination of primers produced a ~600-bp cDNA fragment that was partially sequenced and used to screen a cDNA library constructed from ovaries 6-12 h post blood meal. Northern hybridization experiments indicate the mature VgR mRNA transcript is ~7 kb. One of the microsequenced fragments (19 aa) shows 65% similarity to a conserved region of the bovine and rabbit low density lipoprotein receptors.

C3-324 CASEIN KINASE II FROM *RHODNIUS PROLIXUS* OOCYTES - PARTIAL PURIFICATION AND CHARACTERIZATION

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Casein kinase II (CK II) is a cyclic AMP- and calcium-calmodulin independent protein kinase that is expressed in eukaryotes ranging from yeast to mammals. It is involved in several types of cellular processes, ranging from cell proliferation to intermediary metabolism. In the oocytes of *Rhodnius* this enzyme was found to phosphorylate *in vitro* the major yolk protein, vitellin (VT), (Silva-Neto, M.A.C.and Oliveira,P.L.-1993, **Insect Biochem.Mol.Biol,23**:815-823), This enzyme was now purified over 1500 fold, with a recovery of 25 %, by sequential fractionation of the oocyte cytosol through ammonium sulphate precipitation followed by ion exchange and cellulose phosphate chromatography. Enzymatic activity eluted from the cellulose-phosphate step was used for kinetic studies. It accepts both ATP or GTP as phosphate donors (Kms of 36 µM and 86 µM respectively) . Ki for heparin is of 9 nM. H7 and H8 (100 µM), classical inhibitors of protein kinase C and cyclic nucleotide dependent protein kinase,respectively,did not alter CK II activity. The polycationic compounds polylisine (0.1 mg/ml) and spermidine (500 µM) induced a slight activation. These results show that this enzyme exhibits the main properties of CK II studied in other organisms. In order to understand the role of this enzyme after oocyte fertilization CK II activity against endogenous substrates was studied during egg development. Preliminary results show that the activity decreases on day 1 and increases again on day 6.

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C3-325 LIPOPHORIN AND LIPID TRANSFER PROTEIN OF *BOMBYX MORI*

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The complete amino acid sequence of *Bombyx mori* apolipophorin-III (apoLp-III) was determined from its cDNA sequence. The mature protein consists of 160 amino acids with a molecular weight of 17,960. A cDNA sequence identity comparing *Manduca sexta* and *Locusta migratoria* was found to be 73 % and 56 %, respectively.

In a study of lipid metabolism during embryogenesis, we found that apoLp-III was expressed in the fat body of *B. mori* during early embryogenesis. Western blot results showed that apoLp-III is not present until day 2 of the egg stage; subsequently apoLp-III concentration increases from day 3 of the egg stage. After the maximal level on day 5, the concentration of apoLp-III decreased gradually until hatching. ApoLp-III during this period was associated with lipophorin, and low density lipophorin was formed. Nothern hybridization showed that the transcript level for apoLp-III increased rapidly on day 3 of the egg stage and then decreased gradually. The data shows that apoLp-III expression during embryogenesis corresponds to the period of yolk degradation, perhaps involving the utilization of lipid.

We also purified lipid transfer protein (LTP) of *B. mori*. LTP is composed of 350 kDa, 90 kDa and 60 kDa subnits, but it's exact molecular mass is unknown. *B. mori* LTP was able to catalyze the transfer of diacylglycerol among different density lipophorins which then resulted in the production of intermediate density lipophorin. These results were similar to LTPs of *Manduca sexta, Locusta migratoria* and *Periplaneta americana*. LTP was present in hemolymph throughout all stages of *B. mori* development.

C3-326 BIOCHEMICAL, DEVELOPMENTAL, MOLECULAR, AND PHYLOGENETIC STUDIES ON INSECT PYRUVATE CARBOXYLASES

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Pyruvate carboxylase (Pyruvate: carbon dioxide ligase [ADP-forming], EC 6.4.1.1), is a biotin-dependent enzyme catalyzing a two step reaction ligating the carboxyl group to pyruvate. This enzyme was shown to be present in several tissues of ten different species of insects. Pyruvate carboxylase was purified from the thoraces of honeybee, *Apis mellifera*, and was found to be a tetramer consisting of 128 kDa subunits. The activity of this enzyme required acetyl-CoA, ATP, and $Mg^{2+}$. The Kms of the enzyme for bicarbonate and pyruvate were similar to pyruvate carboxylases from other organisms.

Pyruvate carboxylase was also purified from the yellow fever mosquito, *Aedes aegypti* . Two polypeptides of similar molecular weight (133 kDa and 128 kDa) were present and the N-terminal sequences of both were determined. The relative amounts of the 133 kDa and 128 kDa polypeptides were shown to differ in various tissues. The enzyme was found in all tissues examined and was concentrated in the thorax where the amount of the enzyme increased shortly after pupation. Pyruvate carboxylase in thoracic muscles of insects is likely to have an anaplerotic role. Pyruvate carboxylase was also found to be at high levels in the fat body preparations.

Clones covering the complete cDNA of pyruvate carboxylase of *Aedes aegypti* were obtained. The 3952 bp nucleotide sequence including a 3585 bp coding region, was determined from these cDNA clones. The deduced 1195 amino acid sequence has a calculated Mr of 132, 200. Following a putative mitochondrial targeting sequence, three functional domains were identified including biotin carboxylase (BC), carboxyltransferase (CT), and biotin carboxyl carrier protein (BCCP). The mosquito pyruvate carboxylase amino acid sequence showed 55-75% identity to enzymes from other sources. The evolutionary relationship of pyruvate carboxylases among different organisms was analyzed. The evolution of domain structures of the biotin-dependent carboxylases including pyruvate carboxylase was also investigated. The above analysis provided evidence for the coevolution of BC and BCCP domains and early gene duplication events that shaped the family of biotin-dependent carboxylases.

C3-327 ASPECTS OF LIPID METABOLISM IN MOSQUITOES.

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In most insects studied so far, hydrophobic lipids are transported through the aequeous hemolymph by a lipoprotein, called lipophorin. Insect lipophorins distinguish themselves from mammalian lipoproteins by the fact that in general they carry diacylglycerol (DG) as their major neutral lipid, versus triacylglycerol (TG) in the latter. The most characteristic feature of insect lipophorin is that, in contrast to mammalian lipoproteins, it functions as a reusable lipid shuttle due to its ability to load and unload lipid without cellular uptake and disassembly of the lipophorin. A lipophorin was isolated from *Aedes aegypti* total body homogenate and its composition was analyzed. It resembles the typical insect lipophorin in all aspects, with the exception of the lipid composition: its major neutral lipid is not DG but TG (and thus similar to mammalian lipoprbteins). The same result was found for two additional mosquito species, *Culex quiquefasciatus* and *Anopheles albimanus*. The presence of TG may imply a rather different metabolism for mosquito lipophorin as compared to other insects. For instance, *A. aegypti* lipophorin does not readily take up lipid from fat body, in contrast to lipophprins from other insect species. We developed a technique to enzymatically remove TG from the lipophorin and introduce radiolabeled TG. With this radiolabeled lipophorin it will be possible to study metabolic aspects of lipophorin in mosquitoes. Our final goal is to study the role of mosquito lipophorin in the supply of lipid to the developing oocyst of *Plasmodium sp.* in the hemocoel. In collaboration with Dr. A. Warburg (NIH) we demonstrated that phospholipids are taken up from *A. aegypti* lipophorin by *P. gallinaceum* oocysts cultured *in vitro*. We will investigate uptake of TG by developing oocysts, a process that should be receptor-mediated.

This work is supported by NIH grant GM 44876.

**C3-328** BIOCHEMICAL AND MOLECULAR ANALYSIS OF MALATHION RESISTANT CULEX TARSALIS,
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Resistance to organophosphates (OPs) has been associated with the overproduction of esterases. In *C. quinquefasciatus* a 500-fold increase in esterase activity is due to a 250-fold amplification of the general esterase *est B1* gene (Mouches *et al.*, 1986). Similarly, amplifications of the homologous *est B2* and *est B3* genes were implied in OP resistant *C. pipiens* and *C. tarsalis*, respectively (Raymond *et al.*, 1989). Our *C. tarsalis* resistant strain (R) is 150 times more resistant to malathion and has a 20-fold higher malathion carboxylesterase (MCE) activity than the susceptible (S) strain. Although considerable effort was made, R-specific amplified DNAs could not be identified in these mosquitoes. *Est B1* was used to isolate *est B3* from *C. tarsalis* to determine if this esterase was overproduced in R mosquitoes. It has 89% amino acid identity and its gene structure is similar to *est B1*. Genomic Southern blots indicated that the gene is not amplified in the R strain. Malathion resistance cannot likely be attributed to general esterase substrate specificity differences as *est B3* cDNAs from R and S larvae were 98% identical; indeed there were more differences between two cDNAs from R larvae than from the S strain. Northern analysis and cDNA amplification products suggest, however, that the *est B3* transcript may be unusually or inefficiently processed. In order to more directly assess the biochemical mechanism of malathion resistance, MCE was purified from the R and S strains. Kinetic measurements showed that a unique MCE in the R strain hydrolyzed malathion 18 times faster than the enzyme from S insects. It is not an abundant protein but has a high turnover rate. Thus, OP resistance in these mosquitoes is due to a qualitatively altered specific esterase and not to the gene amplification of a general esterase.
*Acknowledgements:* We thank C. Mouches for the B1 probe and A. Downe for support. Funded by NSERC and Insect Biotech Canada.

**C3-329** DELIVERY AND FUNCTIONAL ANALYSIS OF IMMUNOSUPPRESSIVE POLYDNAVIRUS GENE PRODUCTS IN BACULOVIRUS EXPRESSION VECTORS,
Bruce A. Webb, Ana Soldevila, Department of Entomology, University of Kentucky, Lexington, KY 40506.
Although polydnaviruses are known to actively suppress the insect immune system, the isolation and study of individual, immunosuppressive genes has been delayed by the complexity of the polydnavirus and our inability to manipulate the viral genome. We have explored the use of baculoviruses to deliver individual polydnavirus genes for functional analysis. When expressed in baculoviruses under the polyhedrin promoter the VHv1.1 polydnavirus gene inhibited the encapsulation response to parasitoid eggs. Expression of a functionally active polydnavirus gene in baculoviruses has allowed mutagenesis of the VHv1.1 gene to characterize the essential regions the gene. An alternative approach has explored the baculovirus system as a vehicle for the introduction of polydnavirus genomic DNA. Polydnavirus genes in these vectors are expressed under the control of the polydnavirus promoter in the context of an active baculovirus infection. Interestingly, genes expressed in the baculovirus system from polydnavirus promoters are expressed at both early and late times after viral infection.

**C3-330 CLONING AND CHARACTERIZATION OF GENES PREFERENTIALLY EXPRESSED IN THE OVARY OF An. gambiae.**
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The blood meal taken by female mosquito initiates a complex series of events that lead to egg development. Because the sex, tissue and stage specificity of oogenesis its study is interesting as model of gene expression and target for vector control. In order to initiate studies of genes which are preferentially expressed during A. gambiae oogenesis and eventually characterize its control regions we have constructed a cDNA library from ovaries of females that were fed with blood 48 hrs before. We have been following two strategies to search for ovarian cDNA clones. The first is through the use of differential screenings. The second strategy has been using PCR-amplified microdisected DNA from polytene chromosomes presents in the nurse cells. From the differential screening experiments we have been able to identify several genes that are preferentially expressed in the ovary, these results have been confirmed in Northern blot experiments. Until now we have sequenced 3 of these cDNA clones. One of these (Clone 7) encodes for a calcium binding protein that may be involved the import of proteins in the Golgi apparatus. The transcript of the clone 7 is of about 10 times more abundant in ovaries than in other tissues. Another clone (clone 8), encodes for a transcript that is of about 40 times more abundant in the ovaries. Interestingly clone 8 encodes for an a poly-adenilated-isoformo histone H2B-transcript, that is preferentially localized in ovaries. We are in the process of determinating is the accumulation is unique for this isoform or also occurs with other histone genes. The most interesting clone is clone 3, the cDNA encodes for a 268 aa protein that is highly conserved in the evolution, the identity between the mosquito protein and its mammalian homolog is 67%. The protein is very rich in basic amino acids in particular in the NH-terminus. However the function of this protein is still obscure. With all this information we decided to determinate the function of this gene in the ovary and in general in the mosquito. In order to do that we are following different strategies. Initially we are producing antibodies that recognize the C3 product in mosquito and in Drosophila melanogaster. Whole mount immuno-staining experiments in Drosophila ovaries and embryos indicate that the protein in produced in the nurse cells in early oogenesis stages and is preferentially localized in the cytoplasm. During Drosophila development the localization of the C3 product is mostly distributed in all the embryo but some regions in different stages are enriched. Since clone 3 is highly conserved in the evolution and some of the studies to determinate the function of this gene could be easier to perform in Drosophila, we have recently cloned a cDNA clone from the fly. The complete sequence of the Drosophila C3 cDNA indicates that the identity between the mosquito and the Drosophila proteins is almost 100%.

*Gene Transfer: Prospects and Perspectives; Transposable Elements*

**C3-400** PROMOTER SEQUENCES OF *Aedes aegypti* DENSOVIRUS FOR EXPRESSION OF FOREIGN GENES IN MOSQUITO CELLS

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To investigate the infectious clone of *Aedes aegypti* densovirus (AeDNV) virus as an expression vector, the reporter gene encoding β-galactosidase (β-gal) was inserted into four large open reading frames (ORF) downstream of putative promoter sequences (p0.5, p7, and p61) observed in the AeDNV genome (Afanasiev et al. 1990, Virology **185**:323). The resulting recombinant constructs were transfected into *Aedes albopictus* C6/36 cells and the expression of chimeric β-gal was histochemically detected using X-gal as a chromogenic substrate and quantified by ONPG assay. Comparable levels of expression was found from all three ORFs observed on the plus strand of the AeDNV genome: the right ORF (located downstream of the p61 promoter sequence and encoding capsid proteins), the mid ORF (located downstream p7 and encoding putative nonstructural protein 2) and the left ORF (encoding nonstructural protein 1). The expression from the left ORF was shown efficient only when the β-gal gene was fused into this frame downstream of the second putative promoter sequence (p7). Fusion of the reporter gene into the same frame downstream of the first putative promoter sequence (p0.5) but upstream of p7 resulted in a very low level of the β-gal expression. No expression was detected from the ORF located on the minus strand of the AeDNV genome (no canonical promoter sequence had been shown for this ORF). These results indicate that the p7 and p61 promoters sequences of AeDNV genome can serve for efficient expression of foreign genes in mosquito cells.

**C3-401** *hAT* TRANSPOSABLE ELEMENTS AS GENE VECTORS IN THE TEPHRITID, *BACTROCERA TRYONI*, Peter W. Atkinson, Steven Whyard, Hilary A. Mende, Alexandra C. Pinkerton, Craig J. Coates and David A. O'Brochta†, Commonwealth Scientific and Industrial Research Organization, Division of Entomology, GPO Box 1700, Canberra, ACT, 2601, AUSTRALIA and †Center for Agricultural Biotechnology, Maryland Biotechnology Institute, University of Maryland, College Park, MD, 20742.

The *hAT* transposable element family includes the *hobo* element of *Drosophila melanogaster*, the *Ac* element of *Zea mays* and the *Tam3* element of *Antirrhinum majus*. The *Ac* and *Tam3* elements possess the remarkable property of retaining their ability to transpose when introduced into different species. This has led to attempts to use these elements as gene tagging tools in a number of plant species. We demonstrate that the *hobo* element is also capable of transposition when introduced into a species different than its original host. We show that inter-plasmid transposition of *hobo* occurs when it is placed into developing embryos of the tephritid, *Bactrocera tryoni*, indicating that hobo can be used as a gene vector in this insect. To explore this further, we have constructed a *hobo* element containing the neomycin phosphatase gene placed under the control of the *D. melanogster hsp70* promoter. In *D. melanogaster*, expression of this gene enables transgenic individuals to be selected for when larvae are placed on media containing the antibiotic G418. We have determined that *B. tryoni* is very sensitive to this antibiotic and we will present our data arising from experiments using the *hobo-hspneo* plasmid as a gene vector in this insect.

We will also describe the isolation and characterization of an endogenous *hAT* element from *B. tryoni*. This transposable element, called *homer*, is present as middle-repetitive DNA in the genome of *B. tryoni* and has many structural features in common with other insect *hAT* elements such as *hobo* and *Hermes*. The potential of *homer* as a gene vector for use in *B. tryoni* and other tephritid insects will be discussed.

**C3-402** FOREIGN GENE EXPRESSION IN MOSQUITO CELL LINES MEDIATED BY PANTROPIC RETROVIRAL VECTORS, Jane C. Burns[1], Tomoyo Matsubara[1], Stephen Higgs[2], Anthony A. James[3], [1]Dept. of Pediatrics, UCSD School of Medicine, La Jolla, CA 92093, [2]Dept. of Microbiology, Colorado State Univ., Fort Collins, CO, 80523 [3]Dept. of Molecular Biology, UCI, Irvine, CA 92717

Pantropic retroviral vectors, in which the envelope protein of vesicular stomatitis virus completely replaces the retroviral envelope, have a broadened host cell range and can infect insect cell lines. To determine if such integrated retroviruses can mediate foreign gene expression, we constructed the vector LNhsp70ZL, in which the Moloney murine leukemia virus LTR (L) drives expression of neomycin phosphotransferase (N) and the *Drosophila* hsp70 promoter (hsp70) drives expression of *E. coli* β-galactosidase (Z). The cell lines 208F (rat fibroblast) and AG-55 from *An. gambiae* were grown to 50% confluence and infected with $2.7 \times 10^5$ infectious units of LNhsp70ZL in the presence of 4 μg/ml of polybrene. Cells were grown at ambient temperature for 48 h and cell lysates for chemiluminescent detection of β-galactosidase activity were prepared according to the manufacturer's instructions (Galacto-Light, Tropix, Bedford, MA). Results were as follows:

| Cell line | Treatment | Light units/μg protein |
|---|---|---|
| *An. gambiae* | mock infected | 271.8 ± 31.4 |
| *An. gambiae* | infected | 862.0 ± 45.9 |
| 208F | mock infected | 318.5 ± 10.9 |
| 208F | infected | 1,316.2 ± 145.2 |

The hsp70 promoter was a very weak promoter in comparative assays with other promoters in mammalian cell lines. Although enzyme activity was low in the *An. gambiae* cells, similar results were obtained in 4 replicate infections. We conclude that pantropic vectors can mediate gene expression in mosquito cell lines and hold promise as a method for foreign gene expression in transgenic mosquitoes.

**C3-403** RETROTRANSPOSABLE ELEMENTS IN THE MEDICALLY IMPORTANT MOSQUITO, *ANOPHELES GAMBIAE*, Julian M. Crampton, Lynn Paskins and Ann Warren, Wolfson Unit of Molecular Genetics, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK

Malaria remains one of the major health care problems in the World today, causing millions of fatalities annually. The mosquito, *Anopheles gambiae* is the major vector of malaria in Africa. In order to maximise the ability to control vector populations effectively and to assess novel approaches to controlling this vector borne parasitic disease, it is imperative that we understand as much as possible about the biology and molecular biology of this insect. A DNA probe synthesised by PCR using *An.gambiae* genomic DNA as template and primers derived from conserved regions of reverse transcriptases has been used to screen a genomic library of *An.gambiae*. A number of clones selected using this probe have been isolated and mapped using restriction enzymes. Five of the clones were identical and one of these has been selected for further study. A 3kb fragment within the genomic clone hybridised to the original PCR probe and sequence analysis of this fragment will be described. Searches of the DNA and protein data banks indicate that whilst homology exists with previously described retrotransposable and related elements, the element is distinct from the elements already found in *An.gambiae*. Copy number and genomic distribution of this sequence between various strains of *An.gambiae* have been investigated, as has its expression in *An.gambiae* larvae and cultured cells and these will be described in detail. Future studies will be aimed at a full molecular characterisation of the element and a determination of its mobility.

C3-404 STABLE PLASMID TRANSFORMATION OF GLOSSINA MIDGUT SYMBIONTS (RLO),
Colin Dale[1,2], Mark Toleman[1], Susan C. Welburn[1], Julian C. Crampton[2] and Ian Maudlin[1]. [1]Tsetse Research Group, Department of Veterinary Medicine, University of Bristol, BS18 7DU, UK. [2]Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, L3 5QA, UK.
Attention has focused recently on the development of pseudo-transgenic tsetse flies by the introduction of genetically modified symbiotic bacteria (Aldhous, P, 1993. *Science*, 261, 546). With this goal in mind we have cultured midgut symbionts from seven species/sub-species of tsetse. Isolation and characterisation of extra-chromosomal DNA has shown that all strains of symbionts contain a complex array of large plasmid-like molecules. To circumvent possible problems of incompatibility between broad host range cloning vectors and symbiont-borne plasmids, we have developed a unique vector by combining a symbiont plasmid origin of replication with a kanamycin resistance gene from the transposon Tn902. This vector is non-conjugative and is highly stable both in laboratory strains of *Escherichia coli* and *Glossina* symbionts and can thus be used as a shuttle vector for gene transfer between these two bacteria. The presence of *Glossina* symbionts has been shown to have a profound effect upon establishment and maturation of trypanosome infections in tsetse and, with a view to manipulating this relationship to generate refractory flies, we have engineered a unique cloning site into this vector to allow the introduction and expression of foreign genes within these symbionts.

C3-405 I FACTOR ORF-1 ENCODES A NUCLEIC ACID BINDING PROTEIN WITH PROPERTIES SIMILAR TO RETROVIRAL GAG POLYPEPTIDES
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I factors are 5.4kb LINE-like transposable elements present in *Drosophila melanogaster* (1) These elements are responsible for I-R hybrid dysgenesis and transpose at a high frequency in the germ-line of females from a dysgenic cross. Within these elements are two open reading frames (ORFs) encoding proteins with similarity to retroviral Gag polyproteins (ORF-1) and reverse transcriptase (ORF-2)(2).

We have expressed ORF-1 in *E.coli* and purified the protein using Ni-NTA chromatography. The protein encoded by ORF-1 binds to DNA and RNA and contains multiple nucleic acid binding domains. ORF-1 protein forms a high molecular weight complex with DNA in a manner similar to retroviral Gag polyprotein (3).

We have also expressed ORF-1 in baculovirus in order to determine whether the protein has the ability to form virus -like particles (VLPs)

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C3-406 PATHOGEN DERIVED RESISTANCE IN MOSQUITO CELLS BY EXPRESSION OF THE PRM CODING REGION OF DENGUE-2 AND YELLOW FEVER 17D VIRUS IN SENSE AND ANTISENSE ORIENTATIONS.
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Complementary DNA derived from the premembrane (prM) coding regions of dengue type 2 (DEN-2; Jamaican strain) and Yellow Fever 17D (YFV-17D) virus genomes have been inserted in either sense or antisense orientations downstream of the second internal initiation site of double subgenomic Sindbis (dsSIN) virus expression vectors. Four dsSIN viruses, designated TE/3'2J/D2prM, TE/3'2J/D2αprM, TE/3'2J/YFprM and TE/3'2J/YFαprM, have been generated from the dsSIN expression vector. Northern blot analysis confirmed the presence of antisense DEN-2 and YFV-17D prM RNA in *Aedes albopictus* (C6/36) cells infected with TE/3'2J/D2αprM and TE/3'2J/YFαprM viruses, respectively. Additionally, prM protein expression was demonstrated in BHK-21 mammalian cells and C6/36 cells infected with either TE/3'2J/D2prM or TE/3'2J/YFprM dsSIN viruses by in vitro radiolabeling of virus proteins and immunofluorescent assays. All four dsSIN viruses can be used to establish pathogen derived resistance (PDR) in mosquito cells to the homologous flavivirus from which the prM cDNA was derived. Attempts to establish PDR in *Aedes aegypti* mosquitoes using recombinant dsSIN viruses will also be discussed. These dsSIN viruses have been used as a model system to evaluate molecular strategies for establishing PDR in mosquitoes.

C3-407 MATERNAL INJECTION OF A BMNPVIEG PROMOTER BASED EXPRESSION CONSTRUCT. A MODEL TO INVESTIGATE THE POSSIBILITY OF VITELLOGENIC UPTAKE OF FOREIGN DNA IN OOCYTES OF *BOMBYX MORI*.
Roger Huybrechts, Veerle Vulsteke, Naiming Zhou and Jozef Vanden Broeck, K.U.Leuven, Zoological Institute, Naamsestraat 59, B-3000 Leuven, Belgium
The impact of transgenesis for sericulture is evident. Insecticide and disease resistant silk worm strains are two important objectives. The use of transgenic silk worm as a protein factory might be an additional option. However in spite of the intensified efforts of recent years, technical progress in the realization of transgenic *Bombyx* strains is still limited. The original approach of using homologous recombination was not successful sofar. Since for random integration the success can only be guaranteed when large numbers of eggs can be micro-injected we decided to test the possibility of introducing the foreign DNA into the oocytes during vitellogenesis by injecting the DNA into the *Bombyx* pupae (cfr. maternal injection of DNA and transgenesis in mites: Presnail & Hay, Proc. Natl. Acad. Sci. USA, 1992). In addition we decided to protect the injected DNA from circulating nucleases by the addition of a lipofecting agent, DOTAP which also favours transmembranic DNA uptake. For ease of screening we used a BmNPVIEG promoter based construct including the luciferase gene as a sensitive marker for expression. Although the original experiments showed expression in fat body of the emerging moths and presence of vector DNA in their ovarioles a more elaborated study of successive generations made us conclude that *Bombyx* eggs are very reluctant to DNA uptake during vitellogenesis. Maternal injection of foreign DNA sofar did not result in transgene *Bombyx*.

C3-408 TARGETED INSERTION OF EXOGENOUS DNA INTO mariner LOCI OF THE PREDATORY MITE Metaseiulus occidentalis, Ayyamperumal Jeyaprakash and Marjorie A. Hoy, Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611

Random insertions of exogenous DNA into essential mite genomic regions can result in mutations and affect fitness. We searched the genome of the predatory mite *Metaseiulus occidentalis* for inactive *mariner* transposable elements and attempted to insert exogenous DNA into these sites. Degenerate primers were used to amplify a 454 bp *mariner* DNA fragment from the *M. occidentalis* genome by the PCR. A hybrid plasmid pAJ11 containing a *mariner* DNA fragment and *lacZ* construct under the control of a *Drosophila hsp70* promoter was constructed. The plasmid pAJ11 was injected into *M. occidentalis* females by maternal microinjection. Several G6 progenies were tested by PCR using *hsp70*- and *lacZ*- specific primers. Two transgenic lines showed the presence of an expected 611 bp PCR fragment. At least 12 strongly-hybridizing *mariner* DNA bands are found in *M. occidentalis* genomic DNA by Southern blot hybridization. Two *mariner* DNA bands in one transgenic line and three bands in the other line disappeared, indicating multiple pAJ11 insertions possibly occurred at these loci by homologous recombination.

C3-409 SURVEY OF TRANSPOSABLE ELEMENTS IN HONEYBEE (Apis mellifera) USING INFORMATION FROM KNOWN TRANSPOSABLE ELEMENTS, Kiyoshi Kimura, Kazuhiro Amano, Department of Animal Genetics and Breeding, National Institute of Animal Industry, Tsukuba 305, Japan, Mikio Yoshiyama, Faculty of Agriculture, Tsukuba University, Tsukuba 305, Japan, and Koichi Suzuki, Faculty of Agriculture, Iwate University, Morioka 020, Japan

As the first step in surveying transposable elements in honeybees, hybridization analyses and PCR analyses were carried out, using information from known families of transposable elements (16 from *Drosophila*, 6 from *C. elegans* and 2 from *B. mori*). In hybridization experiments, *jockey* element of *Drosophila* was the only probe hybridized with genomic DNA of honeybees. The banding patterns in Southern blots were highly polymorphic among the strains of honeybees. DNAs were successfully amplified using primers designed from *jockey* sequences of *Drosophila* by PCR analyses with honeybee genomic DNA. The amplified DNAs show only a few polymorphic patterns among honeybee strains. Several PCR fragments were cloned and sequenced. Homologies between *Drosophila* sequence and these fragments were around 50%. These results suggest that the *jockey*-like element in honeybee could be transposable but is diverged from the *jockey* element of *Drosophila*. Attempts were made to amplify DNAs using primers designed from several other *Drosophila* transposable elements as well. Using *hobo* and *mariner* primers, DNAs were also successfully amplified. These amplified DNAs were cloned. Sequence analyses will be utilized to compare these sequences and *Drosophila* sequences. Features of honeybee transposable elements will be discussed based on the results of this series of experiments.

C3-410 STABLE TRANSFORMATION OF THE MOSQUITOES *ANOPHELES GAMBIAE* AND *AEDES AEGYPTI*. Anthony N. King, Julian M. Crampton and Paul Eggleston, Wolfson Unit of Molecular Genetics, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.

The lack of a reliable method to transfer genes into insects of medical importance is a major bottleneck in the development of transgenic technology. Until such procedures are routine, progress in the molecular genetic analysis of these insect systems and the exploitation of transgenic technology will be slow. In order to assess their potential as the core of a mosquito transformation system, we have tested the integration efficiency of a number of transposable genetic elements in the genomes of both *Aedes aegypti* and *Anopheles gambiae*. These include members of the *mariner* family of transposable genetic elements, such as the highly active *Mos1* from *Drosophila mauritiana* and *maroon* from the hornfly, as well as the *hobo* element from *Drosophila melanogaster*. Initial results with the *Mos*1 element, using specific PCR primers and Southern blotting, indicate that the introduced construct is capable of episomal transmission through to the $G_1$ progeny, but not to subsequent generations. Such transmission must involve the gametes and most likely the oocytes. We have obtained no evidence for chromosomal integration of the *Mos1* element in mosquitoes. However, the detection of potential transformants is difficult in the absence of a selectable marker and we are currently manipulating the *Mos1* construct to introduce the bacterial *hygromycin* resistance gene under the control of the *Drosophila* actin 5C promoter. The results from these experiments will be described along with those involving other transposable elements which might form the core of a mosquito transformation vector.

C3-411 RECENT HORIZONTAL TRANSFER OF *MARINER* TRANSPOSABLE ELEMENTS ACROSS FOUR INSECT ORDERS, David J. Lampe and Hugh M. Robertson, Department of Entomology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

We used a PCR assay to detect *mariner* elements belonging to the mellifera subfamily in the genomes of insects from four orders. These PCR fragments were employed to obtain multiple genomic clones of these transposons from the European honey bee, *Apis mellifera*; the European earwig, *Forficula auricularia*; a blister beetle, *Epicauta pestifera*; and the Mediterranean fruit fly, *Ceratitis capitata*. The elements in these clones proved to be >90% identical to one another at the DNA level. Within each species clones were more closely related to each other, indicating a single origin within each lineage. Clones from the Med fly and the honey bee were the most divergent, containing stop codons, frameshifts, and deletions indicating that these elements had been in these host species a relatively long time. Elements from the earwig and blister beetle were most similar to each other within their respective lineages, and several clones have seemingly intact transposase genes, indicating recent (and possibly current) activity. These results provide additional striking evidence of interordinal transfer of *mariner* elements in insects.We are currently using the earwig/blister beetle genomic clones to provide the starting material for reconstructing an active version of this subfamily of *mariner* elements.

**References:**

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C3-412 CHARACTERIZATION OF THE *ANOPHELES GAMBIAE* TRANSPOSON, *IKIRARA*, Sheldon Leung, Nada Quercia, Daniel Vasiliauskas, Raja Bhattacharyya, Antonio Colavita, and Patricia Romans, Department of Zoology and Programme in Molecular Genetics and Molecular Biology, University of Toronto, Toronto, Ontario, Canada M5S 1A1

Little progress has been made in controlling *Anopheles gambiae*, the world's most important vector of malaria. An efficient genetic transformation system that reliably and stably introduces genes into the *A. gambiae* genome would make it possible to study genes involved in vector competence *in vivo* and, possibly in the future, to introduce novel malaria toxin genes into vector populations.

We have found an *A. gambiae* DNA to DNA transposon family that we call *Ikirara*, Kinyarwanda for vagabond, which may contain active members. Complete sequence from one element and partial sequence from two others indicates similarities between *Ikirara* and a range of animal and plant transposons used as transformation vectors. Genomic Southern blots have identified *Ikirara* elements in all five species of the *A. gambiae* complex tested. The original *Ikirara* element was found in the intergene region between two vitellogenin genes in some clones but not others. It is present at this location in only one of eight *A. gambiae s.s.* strains examined. This suggests that it may have been inserted recently into this locus because a non-deleted, active element is present elsewhere in the genome.

To test directly whether any endogenous *Ikirara* elements are active, we have designed an excision assay similar to those of O'Brochta and Handler (O'Brochta et al. 1991. Mol. Gen. Genet. 225:387). The initial assays involve transfecting an *A. gambiae* cell line with a reporter plasmid containing the luciferase gene, *luc*, driven by a *trp-lac* promoter, inserted between the 216 bp terminal inverted repeats of *Ikirara* and genomic insertion context sequence. Later, low molecular weight DNAs are recovered from the cells and transformed into *E. coli*. A sample of the *luc*$^-$ colonies will be sequenced to distinguish true excisions from other recombination events.

C3-413 TRANSFORMATION OF *DROSOPHILA VIRILIS* BY THE MOBILE ELEMENT *MARINER*, Allan R. Lohe and Daniel L. Hartl, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138

The transposable element *mariner* is widespread among many species of insects and is therefore a candidate for a universal transformation vector. We have shown previously that the active *mariner* element MOS-1 (isolated from *D. mauritiana*) can transform *D. melanogaster* and also can act as a vector for the introduction of foreign DNA into this species.

To investigate the species limits of *mariner* transformation, MOS-1 was injected into *D. virilis*, a species that shared a common ancestor with *D. melanogaster* about 60 Myr ago. Twenty-seven fertile G0 flies were selfed and pools of G1 progeny examined for the MOS-1 element by PCR. The predicted MOS-1 PCR products were detected in several of the pools and lines were established from single flies. We have shown the following:

- MOS-1 transforms *D. virilis* efficiently. The integrated element segregates in a Mendelian fashion.
- MOS-1 is active in *D. virilis*: copy number increases with the number of generations and a deleted element has also been recovered
- rescue of the 5' and 3' ends from MOS-1 copies at three different genomic locations demonstrates that integration results in a 2-bp TA duplication characteristic of *mariner* insertions
- at least some elements have integrated into heterochromatin, as shown by a reduced signal in Southern hybridizations and lack of signal in hybridizations to polytene chromosomes

These results confirm the ability of *mariner* to transform efficiently a Drosophila species only distantly related to *D. melanogaster*, but caution that the characteristics of transformation may differ from species to species. We are currently attempting to transform *D. virilis* with *mariner* vectors containing selectable markers.

C3-414 HYBRID DYSGENESIS IN THE MEDFLY, *CERATITIS CAPITATA* (DIPTERA, TEPHRITIDAE), A.R. Malacrida[1], C. Torti[1], G. Yannopoulos[2], C. Louis[3,4] and G. Gasperi[1], [1]Department of Animal Biology, University of Pavia, Pavia, Italy, [2]Department of Biology, University of Patras, Patras, Greece, [3]Inst. Molecular Biology & Biotechnology, FORTH, Heraklion, and [4]Department of Biology, University of Crete, Heraklion, Crete, Greece

A syndrome of abnormal genetic effects, which resembles *Drosophila* hybrid dysgenesis, is present in *C. capitata*. This syndrome includes high frequency of partial or complete female gonadal sterility, chromosomal rearrangements (bridges and fragments) at male meiosis and instabilities at the *white eye* locus.

This syndrome was observed in hybrids of *C. capitata* when strains of different origins were mated. The morphology of the undeveloped ovaries recovered in the medfly is apparently very similar to the gonadal dystrophy which in *D. melanogaster* is associated with the P-M and *hobo* mediated dysgenic syndromes. The amount of gonadal sterility observed in medfly hybrids depends on the parental strains, which exhibit specific differences in their inducing abilities. In the considered inter-strain combinations there appears to be quantitative variation in the effect of temperature on GD sterility. The highest level of sterility occurs at 25°C. The pattern of abnormal traits observed in medfly hybrids appears to be the phenotypic expression of a rather complex interacting dysgenic system of inducer and suppresser effects; probably more than one system is activated in the considered crosses.

Genetic instability at the *white eye* (*w*) locus has also been observed. Genetic data support the hypothesis that insertional mutations in the *white eye* locus cause the observed genetic instability.

C3-415 TRANSPOSABLE ELEMENTS IN PINK BOLLWORM, (PECTINOPHORA GOSSYPIELLA). John J. Peloquin and Thomas A. Miller. Department of Entomology, University of California, Riverside, CA 92521

We have been attempting to genetically transform PBW (Pink Bollworm) with nucleic acid constructs as a basis for the genetic control of pest populations of PBW. Transposable elements may be usable as a basis for nucleic acid constructs usable in insect transformation. We have discovered a Mariner or Mariner-like element in PBW. Nucleic acid sequence homology of the putative transposase gene of the PBW Mariner or Mariner-like element indicates that it is most closely related to the Mariner transposase gene sequence reported by H. M. Robertson in *Ephestia cautella*.

C3-416 **IDENTIFICATION AND CHARACTERIZATION OF TWO MOBILE ELEMENTS FROM LYMANTRIA DISPAR USING DEGENERATIVE PRIMERS AND PCR**, Tom A. Pfeifer, Barney A. Lee, Mark Ring and Tom A. Grigliatti. Dept. of Zoology, Univ. of British Columbia, Vancouver, BC Canada V6T 1Z4

Degenerative primers and PCR were used to identify and isolate a mariner-like element and a retrotransposon from the gypsy moth, Lymantria dispar. The mariner-like element was identified using PCR products amplified by degenerative primers as described by Robertson (Nature 362:241-245, 1993). DNA sequencing of the transposase coding region of several cloned gypsy moth mariner-like transposable elements showed 85% similarity at the DNA level to the Cecropia subfamily of mariner elements. A single clone (GMM1) contained an uninterrupted reading frame and showed greater than 90% amino acid identity to the Cecropia counterpart. The cloned amplification product was used to screen a gypsy moth DNA library and several putative clones were identified. Sequencing of these clones will demonstrate whether full length mariner-like elements exist in gypsy moth.

The retrotransposon (Lydia) was isolated by designing degenerative primers designed for amplification of the gag/pol regions of LTR retrotransposons. A 1.1 kb amplification product was used to probe a DNA library and several clones were identified. One 11.0 kb clone was restriction mapped and the entire 6,808 bp element sequenced using TN1000 nested insertions. Lydia contained 300 bp long terminal repeats and produced a 4 bp insertional duplication. DNA sequence demonstrated a high degree of similarity to the Ted (T. ni) element in all three of Ted's open reading frames and lower homology to gypsy and related Drosophila elements. Translation of the Lydia sequence did not produce the expected three defined open reading frames, but stretches of amino acids highly homologous to Ted's putative amino acid sequences were identified.

C3-417 MATERNAL MICROINJECTION OF THE BRACONID WASP Cardiochiles diaphaniae WITH THE PARATHION HYDROLASE GENE OF Pseudomonas diminuta. James K. Presnail and Marjorie A. Hoy, Department of Entomology, University of Florida, Gainesville, FL 32611

Maternal microinjection is the injection of DNA into an adult female in order to deliver DNA to eggs prior to deposition. Here we report the adaptation of this method to the parasitoid wasp *Cardiochiles diaphaniae* (Braconidae). Adult females were injected with the plasmid phsopd containing the parathion hydrolase gene *opd*. Ovaries of injected females were dissected and the DNA extracted and examined by slot blot for the presence of plasmid sequences. The G0 offspring of other injectees were reared to adulthood and examined by the PCR and Southern blot hybridization for the presence of plasmid sequences. Several G0 individuals displayed PCR products indicating uptake of plasmid DNA and one G1 wasp displayed a Southern blot pattern indicating integration of plasmid sequences into the wasp genome. Pesticide bioassays were also performed to examine the expression of the injected sequences. The individuals exhibited significant resistance to ethyl parathion, which suggests *opd* could act as a screen for potential transformants. The results indicate maternal microinjection is a useful method for introducing DNA into the eggs of this internal parasitoid wasp and can result in stable transformation. Furthermore, the *opd* gene appears useful as a preliminary screen for putative transformants.

C3-418 **Research for Genetic Transformation of Diptera Vectors**

E.M.B.Saraiva, E.L.Mialhe, M.Shahabuddin, K.Vernick and L.H.Miller
Laboratory of. Malaria Research, NIAID, National Institutes of Health, Bethesda,MD 20892

We have been working to develop a suitable transfection system towards the goal of obtaining transgenic non-drosophilid insects which will block transmission of human protozoan parasites. The following aspects are being investigated: (1) the development of transfection methods for the introduction of exogenous DNA, (2) analyses of appropriate promoters to control expression of introduced genes, and (3) development of a vector to deliver genes in insects. We have investigated these three topics using either embryos of human malaria vector mosquitoes *(Anopheles gambiae* ) or cell lines from mosquito ( *A. gambiae*) and leishmaniases sand fly vectors ( *Phlebotomus papatasi , Lutzomyia longipalpis* ). We have developed new transfection procedures , essentially based on aqueous microbiolistic and to a lesser extent, nanobiolistic for introduction of DNA. Several parameters that affect reporter gene expression ( luciferase activity under control of the *Drosophila* HSP70 promoter) were tested. Heterologous transposons ( Minos from *D. hydei and* Mariner from *Drosophila mauritiana* ) have been studied either by transfection with selectable marker ( neomycin ) or according to excision-integration assays. Different virus systems (Cytomegalovirus, SV40, Densovirus of *Lepidoptera* and Semliki Forest Virus ) have been tested for their potential transfection ability The results of these studies will be presented.

C3-419 MECHANISTIC ASPECTS OF *HOBO* EXCISION AND TRANSPOSITION. Kenneth J. Saville[1], Peter W. Atkinson[2] and David A. O'Brochta[1], 1) Center for Agricultural Biotechnology, University of Maryland Biotechnology Institute, College Park, MD 20742-3351. 2) Division of Entomology, CSIRO, Canberra, Australia.

We demonstrated previously the ability of the *hobo* transposable element of *D. melanogaster* to excise and transpose in diverse non-drosophilid insects [Atkinson et al (1993) PNAS 90: 9693-9697, O'Brochta et al (1994) MGG 244:9-14]. Recently, we used *hobo* to generate germline transformants of the Queensland fruitfly, *Bactrocera tryoni*, thereby directly demonstrating the utility of *hobo* as a vector for the genetic manipulation of non-drosophilid insects (Atkinson *et al*, this volume). In order to maximize the potential of *hobo* as a gene vector, we are currently investigating the mechanisms of *hobo* excision and transposition to identify the parameters essential for *hobo* movement. Once identified, these parameters may be controllable, permitting the use of *hobo* based vectors in a large array of insects, and allowing for the precise regulation of element movement. The full utility of *hobo* as a gene vector and gene tagging agent will depend on a number of factors including the rate of movement, the stability of inserts, and the degree of target site specificity. Under some conditions, *hobo* shows a significant preference for a particular insertion site. The information conferring this insertion preference is contained within the 39 nucleotides flanking the site of insertion. Results which further characterize the mechanism by which *hobo* insertion sites are selected will be presented.

The characterization of *hobo* excision products has also yielded insight into the mechanism of *hobo* movement. *Hobo* excision is imprecise, resulting in inverted repeats of the sequences originally flanking the element to be created at the excision site. These structures are reminiscent of those generated upon excision of several plant transposons, as well as by V(D)J recombination in developing vertebrate B and T cells. The mechanism by which these excision products are generated will be discussed.

**C3-420** ANALYSIS OF THE *Rdl* GABA RECEPTOR GENE PROMOTER IN THE *AEDES AEGYPTI* MOSQUITO, Frank A. Shotkoski*, Alison Morris#, Anthony A. James#, and Richard H. ffrench-Constant*, *Dept. of Entomology, University of Wisconsin, Madison, WI 53706 and #Dept. Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.

We are interested in defining the promoter and associated regulatory elements governing the expression of *Rdl* GABA receptor subunits in the mosquito nervous system. Two approaches have been taken. 1) The study of Northern anaylsis, RNA protection and primer extension to identify the site of *Rdl* transcription initiation and 2) the transient expression of a luciferase reporter gene from a series of 5' deletion constructs containing the putative *Rdl* promoter following injection into *Aedes aegypti* embryos. Progress towards the identification of *Rdl* regulatory elements and their use in construction of a minigene as a selectable marker in the genetic transformation of mosquitoes will be discussed.

**C3-421** MARINER TRANSPOSASE, Jill Smith, Ivan Clarke and David Finnegan, Institute of Cell and Molecular Biology, Darwin Building, Kings' Buildings, Mayfield Road, Edinburgh, EH9 3JR, Scotland.

The transposable element *mariner* was originally found in *Drosophila mauritiana*, where it was responsible for a somatically unstable mutation of the *white* gene. This element belongs to the class II family of transposable elements, is 1286bp long and contains a single open reading frame.

We have succesfully expressed this open reading frame as a translational fusion protein with Glutathione-S-transferase in *E. coli*. Preliminary experiments with this fusion protein have demonstrated sequence specific binding to a labelled DNA fragment comprising the 190bp from the left hand end of an autonomous *mariner* element. This indicates that the transposase expressed in *E. coli* is functional, allowing further studies of its properties to be made *in vitro*.

Currently we are also studying the *in vivo* activity of *mariner* transposase. To determine if *mariner* transposase, once purified, has retained its ability to stimulate *mariner* transposition we are microinjecting embryos with a genetically marked non autonomous *mariner* element together with purified transposase.

**C3-422** THE *GYPSY* RETROVIRUS OF *DROSOPHILA MELANOGASTER*: PROSPECTS ON INSECTS TRANSGENESIS. Christophe Terzian, Fabienne Chalvet, Alexander Kim, Alain Pélisson, Nicole Prud'homme, Laure Teysset & Alain Bucheton. Centre de Génétique Moléculaire - CNRS, 91198 Gif-sur-Yvette cedex FRANCE.
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Retroviruses are commonly considered to be restricted to vertebrates. However, the genome of many eukaryotes contains mobile sequences known as retrotransposons with long terminal repeats (LTR retrotransposons) showing similarities with integrated proviruses of retroviruses.

The *gypsy* element of *Drosophila melanogaster* has LTRs and contains three open reading frames, one of which encodes potential products similar to gag-specific protease, reverse transcriptase and endonuclease. *Gypsy* belongs to a particular class of LTR retrotransposons because the third ORF encodes a retroviral *env*- like protein (Pélisson *et al.*, 1994; Song *et al.*, 1994). The product of this ORF is translated from an RNA with a structure corresponding to that of the spliced *env* mRNAs of retroviruses (Pélisson *et al.*, 1994). We have characterized a *Drosophila* gene, *flamenco*, that controls *gypsy* activity (Prud'homme *et al.* 1994). We have shown that infectious particles produced in *flamenco* strains can be transferred to stocks devoid of active *gypsy* elements (Kim et al., 1994). These particles have been characterized (Song *et al.*,1994).

We are now working on the host range of the *gypsy* retrovirus: can we infect sibling species of *Drosophila melanogaster* ? Can we infect species outside the *Drosophila* genus ? We have constructed marked *gypsy* elements which will permit to answer these questions.

The potentiality of *gypsy* as a retroviral vector will also be considered.

Kim *et al.*, 1994, PNAS 91:1285-1289. Pélisson et al., 1994, EMBO 13:4401-4411. Prud'homme *et al.*, 1994, Genetics, in press. Song *et al.*, 1994, Genes & Dev. 8:2046-2057.

**C3-423** HETEROLOGOUS GENE EXPRESSION USING *Aedes* DENSONUCLEOSIS VIRUS IN *Aedes albopictus* CLONE C6/36 CELLS, Kelly J. Thibault, Invitrogen Corporation, San Diego, CA 92121

The *Aedes aegypti* densonucleosis virus (AeDNV) is a member of the heterogeneous group of densoviruses from the viral family Parvoviridae. The virus is infectious to mosquito larvae of the genera *Aedes, Culex,* and *Culiseta*. *Anopheles* species may also be infected upon injection of the virus. The virus is infectious at all stages of the mosquito life cycle and causes cytopathological effects in almost all tissues including muscles, hindgut, and ovaries. The genome of AeDNV is approximately 4kb and encodes 2 non-structural proteins (NS) as well as virion capsid protein (VP). The small size of the genome makes it easy to manipulate for cloning and transfection procedures. It has been demonstrated that cloned parvovirus genomes, transfected into eukaryotic cells, can replicate from the plasmid vector as wild-type virus. Additionally, the promoter for the parvovirus capsid gene is very efficient for expression of foreign genes, especially when *trans*-activated by the non-structural proteins. Parvovirus vectors have been used to introduce and express heterologous proteins in tissue culture. Recombinant parvovirus genomes carrying a foreign gene can be encapsidated into infectious particles if complemented in *trans* with the non-strucural proteins. Described here is the use of AeDNV transducing particles as a protein expression system.

(research funded in part by Research Corporation Technologies, Tucson, AZ)

C3-424 ATTEMPS IN GENE TRANSFER IN B MORI, Jean-Luc Thomas, Emmanuel Planel, Bernard Mauchamp, Valérie Tournadre, Pierre Couble and Gérard Chavancy, Unité Nationale Séricicole, INRA 69350 La Mulatière, France. The silkworm *B.mori* can be viewed as a potential system for setting up technics of gene transfer in insects, yet only available in *Drosophila* . Our first trial consisted in inoculating two hours post oviposition, the construct pA3LacZ which contains the cytoplasmic Actin3 promoter. This construct was injected in either linear or circular form, both leading to beta-galactosidase accumulation in vitellophages, but no expression in hatched larvae. In a second step, we obtained from Devauchelle et al. (Pathologie comparée, INRA, Saint Christol-lez-Alès, F.) the pIE1Neo plasmid which has autoreplicating and expression properties in insect cultured cells. From pIE1Neo we have built the pIE1NéoFRT plasmid by inserting FRT sequence from the 2micro-m plasmid of *S cerevisiae* . Genome integration of this plasmid must lead to a FRT strain which would be later the target for insertion of foreign gene colinear with a FRT sequence. After inoculation of this plasmid in eggs, kinetic study performed during embryonic development showed an amplification of the dot blot hybridization signal. The plasmid had been observed in the fourth instar larvae. By Southern blot we found rearranged forms of the plasmid at all analysed stages (i.e.: ten day old eggs and fourth instar larvae), but initial size of the plasmid was retrieved in ten day old embryonic larvae. An alternative to the inoculation consists in the *ex vivo* manipulation of gametic precursors in larvae or pupae. In this way, we used biolistic bombardment on gonads of fifth instar larvae which allowed us to obtain pA3LacZ expression in testes and ova. Oocyte microinjection of explanted ovarioles seems also possible and can give results if they are reimplanted in the parthenogenetic clone PK1.

C3-425 *HERMES* IS A FUNCTIONAL NON-DROSOPHILID GENE VECTOR, W. D. Warren, P. W. Atkinson(1) and D. A. O'Brochta(2). Center for the application of Molecular Biology in International Agriculture (CAMBIA), GPO Box 3200, Canberra, ACT, 2601 Australia. (1) CSIRO Division of Entomology, Canberra, Australia. (2) Center for Agricultural Biotechnology, University of Maryland, College Park, MD.
*Hermes* is a short inverted repeat-type transposable element originally isolated from the genome of the house fly, *Musca domestica* (see abstract by O'Brochta, Atkinson and Warren for a full description). We show that *Hermes* elements are able to transpose from one plasmid to another while being transiently maintained in *M. domestica* embryos. Only sequences delimited by the terminal inverted repeats transposed and insertion resulted in an 8bp duplication of the target site. To test the potential of *Hermes* to act as a broad range insect transformation vector we replaced the transposase coding region of *Hermes* with the *D. melanogaster white* ($w^+$) gene and constructed a 'helper' plasmid containing the *Hermes* transposase gene fused to a heat shock promoter. A mixture of these plasmids was then injected into pre-blastoderm *D. melanogaster* embryos carrying a homozygous $w^-$ mutation. In this way germline transformants could be identified by their pigmented eyes. 23 out of 68 fertile $G_0$ adults produced progeny with pigmented eyes. The number of pigmented individuals recovered from those 23 lines ranged from 1% to 86% of the total progeny, with 9 of the 23 lines producing 10% or more transgenic offspring. Several classes of progeny with differing levels of pigmentation were observed from some $G_0$ parents, suggesting that multiple transposition events occurred in these animals. These results indicate that *Hermes* is capable of efficiently transforming species distantly related to its natural host and that transposition occurs premeiotically. PCR analysis of red-eyed $G_1$ animals confirm the presence of *Hermes* sequences. Data describing the number of insertions within these clusters and their insertion sites will be presented.

C3-426 TECHNIQUES FOR MOLECULAR GENETIC MANIPULATION OF THE VECTOR MOSQUITO, *AEDES AEGYPTI*. Kurt Yardley, Alison C. Morris and Anthony A. James, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.

Genetic control of vectors has been proposed as a method for control of disease transmission. Implicit in this strategy is a need for an efficient means of transforming vectors. We have investigated methods to establish transformation in *Aedes aegypti*. We have examined the major factors needed for transformation and have selected strategies and techniques based on those that have been successful in other organisms. We have optimized some of these techniques for their application to *Aedes aegypti*.

Our goal is to develop a transformation system that will allows us to get stable and heritable incorporation of exogenous DNA into the mosquito genome with a reasonable frequency. To achieve this, a reliable method for introducing DNA into the animals, good selectable markers for distinghuishing transformed individuals, and the ability to screen large numbers of animals must be developed.

Microinjection is used as a method of delivery of the DNA into the embryo. This technique assures consistent delivery of DNA into embryos. The survival rate to adulthood varies between 10% and 20% of injected embryos.

We use an insecticide resistance gene as a selectable marker in our transformation protocols. This gene, organophosphate hydrolase (OPD), detoxifies paraoxon and parathion and in our constructs is driven by the *D. melanogaster* heatshock 70 promoter.

This strategy requires the screening of large numbers of mosquitos in order to detect transformants. Currently we are capable of injecting 500-1000 $G_0$ embryos per week. Resulting adults are mated and $G_1$ animals subjected to selection. Surviving adults are bred and a line establised.

The techniques we have developed work efficiently for *Aedes aegypti* and can be adopted to other species.

C3-427 PERSISTENCE AND EXPRESSION OF PLASMID DNA MICROINJECTED INTO TESTES AND EGGS OF SILKWORM *BOMBYX MORI*, Yusuff Shamila and Sinnakaruppan Mathavan, School of Biological Sciences, Madurai Kamaraj University, Madurai-625021, India.

Plasmid DNA containing CAT reporter gene was injected into testes of V instar silkworm larvae and their persistence and expression were monitored subsequent to injection till eclosen. The injected DNA persisted in extrachromosomal form during the entire period of metamorphosis and was reisolated from the testes of the moths that emerged from the injected larvae. Positive CAT signals of samples extracted from the testes of the injected individuals suggest that the injected DNA might have been enclosed into the nucleus of the sperm. Persistence and expression of injected DNA in the testes offers scope for sperm-mediated gene transfer in the silkworm.

Plasmid DNA (pBm1; $SK^+$ containing Bm1 sequence of *B.mori*) was injected into silkworm eggs 1 to 30 hours after oviposition. Maximum hatchability was recorded in the eggs subjected to microinjection 8 hours after oviposition, which represents preblastoderm stage of the developing egg. DNA was injected manually using a glass capillary microinjector designed in our laboratory. Genomic DNA was extracted every 24 hours from the injected eggs during the entire period of embryonic development and also from larvae successfully hatched from the injected eggs. Slot blot analysis confirmed the persistence of the injected DNA in all the tested samples. Restriction analysis, Southern hybridizations, plasmid reisolation and reverse probing studies showed that the injected plasmid DNA persisted extrachromosomally in different conformational forms during the entire period of embryonic development. Critical analysis of the hybridization patterns reveals amplification of the injected plasmid DNA in the eggs during embryonic development.

C3-428 DNA TRANSFORMATION IN THE MOSQUITO: THE POTENTIAL OF HOMOLOGOUS RECOMBINATION.
Zhao Yu-Guang and Paul Eggleston, Wolfson Unit of Molecular Genetics, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.
The exploitation of transgenic mosquitoes requires methods for the introduction of DNA both into living insects and into cultured cells. Ideally, this will involve a transformation vector which is capable of directing efficient and stable integration into the chromosomes of the recipient. Previous approaches have relied on transposition mediated mechanisms, principally those involving the P element from *D.melanogaster*. However, the few reported cases of mosquito transformation show no evidence for the involvement of transposition, and there are currently no other transposable sequences (either from the mosquito or elsewhere) which have been developed into efficient transformation systems. Homologous recombination between DNA sequences residing in the chromosome and newly introduced cloned DNA sequences (gene targeting) may provide an alternative mosquito transformation strategy. We have developed a transient assay for homologous recombination in mosquito cells which is based on the restoration of luciferase activity from independent substrates which carry non-overlapping deletions. Restored activity following co-transfection of the substrates was demonstrated in a range of cultured mosquito cells. This research is now directed at the construction of gene targeting vectors using both dispersed repetitive sequences and unique sequences, together with optimised selectable marker/promoter combinations. We are also investigating procedures for the enrichment of targeted events through selection against random recombination and the use of conditionally expressed selectable markers.

C3-429 LIPOFECTION MEDIATED TRANSFECTION AS AN APPROACH TO GERMLINE TRANSFORMATION IN THE MEDFLY, L. J. Zwiebel, and F.C. Kafatos, EMBL, D-69117, Heidelberg, GERMANY, IMBB, Heraklion, Crete, GREECE and Department of Cell and Developmental Biology, Harvard University, Cambridge, Mass. 02138 USA
In an effort to establish an effective method for germline transformation in insects other than *Drosophila* we have examined the feasibility of a lipofection based method for DNA transfection. Initial experiments focused on the integration of plasmids designed to confer G418 resistance as well as β-galactosidase expression into the genome of germ cell precursor (pole) cells prepared from early embryos of the medfly. In these studies, preparations of pole cells were generated by differential centrifugation of homogenates prepared from 1-2 hour embryos and used to establish primary cultures of embryonic cells likely to retain characteristics of pluripotent pole cells. Treatment of these primary cultures with a non-toxic lipofection reagent plus marker DNA preparations followed immediately thereafter. Pools of transfected cells were then reintroduced into host medfly embryos by microinjection. Adult $G_0$ (and subsequent generations up to G3) medflies were back crossed to wild-type adults, and resultant progeny were pooled and selected for resistance to the antibiotic G418. In a parallel series of experiments, *in situ* transfection was attempted using direct injection of the lipofection agent together with marker DNA. into early medfly embryos. In addition to initial selection for G418 resistance each individual medfly was assayed for the presence of marker DNA by Southern blots. In a small percentage of cases persistence of marker DNA to the G3 generation was observed, and the Southern patterns suggest putative integration events. The significance of these observations is being explored with a view to developing an alternative to transposon mediated transformation methods that may be used in other insect systems.

*Late Abstracts*

THE MEDFLY Y CHROMOSOME - MAPPING OF THE *MALENESS* FACTOR,
Ute Willhoeft and Gerald Franz, Entomology Unit, Joint FAO/IAEA Programme, Agency's Laboratory, A-2444 Seibersdorf, Austria
*Ceratitis capitata* is one of the major pest insects in agriculture. The Sterile Insect Technique (SIT) is a powerful tool to eradicate and control medfly populations and can also be used for control in fruit growing areas, provided that only males are released. For production of males-only 'Genetic Sexing' (GS) strains, where selectable markers are linked to the male via Y-automosome translocations, already exist. However, strains based on direct molecular manipulation of the medfly sex determination genes would improve the mass rearing and economic feasibility of GS strains. We have mapped the medfly Y chromosome with respect to factors responsible for sex determination and fertility by the use of fluorescence *in situ* hybridization (FISH) on mitotic chromosomes. This was achieved by analysing individuals that had parts of the Y chromosome deleted. No evidence for the existance of any fertility factor was found. A strategy to isolate Y-chromosomal genes that could be used as targets for molecular manipulation of the medfly is discussed.

CATHEPSIN IN *MUSCA DOMESTICA* EMBRYOGENESIS, Paulo E.M. Ribolla and Antonio G. de Bianchi, Departamento de Bioquímica, Universidade de São Paulo, São Paulo, CP 20780, Brazil.

The degradation of yolk proteins during *Musca domestica* embryogenesis is due to a cathepsin-like proteinase found in unfertilizeted oocytes. This enzyme is composed by a single polypeptide of 41-kDa and its $NH_2$-terminal aminoacid sequence (Ala-Pro-Lys-Tyr-Val-Asp-Tyr-Gly-Glu-Asn-Gly) shows similarities with other cathepsins B and L. This enzyme is stored as a zymogen of 55-kDa during the vitellogenesis. The zymogen activation is dependent of pH lowering and inhibited by cathepsin inhibitors. The autoactivation occurs in two steps by intra and intermolecular cleavages of the zymogen resulting in an intermediary form of 48-kDa that gives origin to the mature form. The zymogen is present into the yolk granules in the mature oocytes. After the oocyte fertilization, the enzyme activity increase to attain a maximum at 3.5 hours after fertilization and, at the end of the embryogenesis, no cathepsin activity was found.

**INTRACELLULAR IMMUNIZATION OF C6/36 MOSQUITO CELLS AND *AEDES TRISERIATUS* MOSQUITOES TO BUNYAVIRUSES USING A SINDBIS VIRUS EXPRESSION SYSTEM.**

Ann M. Powers, Kurt I. Kamrud, Ken E. Olson, Steve Higgs, Jon O. Carlson, and Barry J. Beaty. Arthropod Borne and Infectious Diseases Laboratory (AIDL), Colorado State University, Fort Collins, CO 80523.

A double subgenomic Sindbis (dsSIN) virus expression system was utilized to transcribe sequences of LaCrosse (LAC) virus small (S) or medium (M) segment RNA in either sense or antisense orientation. One virus, designated TE/3'2J/ANTI-S, expresses the full length, negative sense S RNA of LAC virus. This virus can be used to intracellularly immunize C6/36 (*Aedes albopictus*) cells against LAC virus as well as closely related heterologous bunyaviruses. The ability of this recombinant virus to provide a similar protective effect *in vivo* was subsequently examined. *Aedes triseriatus* mosquitoes were intrathoracically inoculated with TE/3'2J/ANTI-S and orally challenged with wild type LAC virus. Tissues productively infected by the dsSIN virus were refractory to LAC infection. These studies demonstrate the usefulness of the dsSIN expression system for evaluation of molecular mechanisms of viral interference in mosquitoes.

THE TICK VACUOLAR ATPASE GENE HOMOLOG: CLONING, SEQUENCE ANALYSIS AND DEVELOPMENTAL CHARACTERIZATION, Janis L. McSwain, Chenghua Luo*, Jim Tucker, John R. Sauer, R.C. Essenberg*, Oklahoma State University, Departments of Entomology and Biochemistry*, Stillwater, OK 74078.

A tick vacuolar ATPase gene homolog has been cloned from a 3-day feeding tick salivary gland cDNA library. Sequence analysis reveals an identity of 67% to mammalian clones at the DNA level and 67% (or 83% allowing conservative substitutions) at the protein level. Using 5'RACE (rapid amplification of cDNA ends) and RT-PCR (reverse transcriptase-polymerase chain reaction), the complete sequence has been determined. Northern blot analysis of the gene shows that the gene is expressed in the tick salivary glands at stages corresponding to times when the tick secretes fluid. RT-PCR using poly $A^+$ RNA from the salivary glands dissected from ticks in various feeding stages shows that this gene is present in all feeding stages and in the embryo. Quantitative changes in the level of mRNA during feeding will be determined using ribonuclease protection assays. It appears that this gene product is likely a key enzyme involved in fluid secretion since specific inhibitors of vacuolar ATPase inhibit fluid secretion in tick salivary glands.

**Use of the Sindbis virus replicon system to express heterologous genes in C6/36 cells and whole mosquitoes.** Kurt I. Kamrud, Ken E. Olson, Steve Higgs, and Barry J. Beaty. Arthropod Borne and Infectious Diseases Laboratory (AIDL), Department of Microbiology, Colorado State University, Fort Collins, CO 80523

The Sindbis (SIN) virus double subgenomic vector (dsSIN) pTE/3'2J has been used extensively to express heterologous genes in mosquitoes and mosquito cells. Recombinant pTE/3'2J virus can be generated with little difficulty and can attain high titers. One draw back with the pTE/3'2J vector though is the limit to the size of the heterologous gene which may be inserted. Genes which are larger than 1.5 Kb in size become unstable in the pTE/3'2J vector and may be deleted over time. The SIN Replicon system allows expression of larger genes than the dsSIN virus, thus alleviating the heterologous gene size restriction associated with dsSIN viruses.

We cloned a full length cDNA copy of the Medium (M) genome segment (4495 bp) of La Crosse (LAC) virus into the SIN Replicon vector pSINRep5. In addition, truncated versions of the M segment, coding for the individual glycoproteins, and the bacteriophage T7 RNA polymerase gene were cloned into pSINRep5, representing genes which vary in size from approximately 1 - 5 Kb in size. Production of recombinant Replicon viruses with titers as high as 1 X $10^8$ Infectious Units (IFU)/ml could be reached even with the full length LAC virus M segment. Analysis of the expression products from these recombinant Replicon viruses showed that the proteins being expressed were indistinguishable from wild type by immunoprecipitation and IFA with specific antibodies. *Aedes triseriatus* mosquitoes were inoculated with Replicon virus containing the CAT gene and CAT protein was detected in a similar distribution throughout the mosquito as has been seen with recombinant pTE/3'2J viruses.

The SIN Replicon system holds great promise for expression of exogenous proteins in mosquitoes and mosquito cells which are too large for the dsSIN system. This system should increase the number of genes analyzed for virus/vector interference potential.

INDUCIBLE ANTIBACTERIAL MOLECULES IN *AEDES AEGYPTI*. Rod Chalk, Cleide M. R. Albuquerque, Harold Townson[1] and Peter J. Ham. Biological Sciences, Keele University, Staffs, ST5 5BG, UK.[1]Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK.

*Aedes aegypti* is important as a vector of yellow fever and other arboviruses and is a laboratory host for filariasis and avian malaria. Together with *Anopheles gambiae*, this species has become a principal vehicle for studies aimed at genetic transformation of mosquitoes. We are interested in the inducible immune response of *Aedes aegypti* to discover the mechanism of refractoriness to parasitic infection and to identify genes capable of conferring this property in a transgenic mosquito line. Previously we reported induction of an insect defensin in the haemolymph of this species as a result of bacterial infection (Chalk *et al.*, 1994). Experiments with synthetic cecropins have shown them to be active against the filarial nematode *Brugia pahangi* (Chalk *et al.*, in press). We now report isolation of further antibacterial molecules from *Ae.aegypti*. There are at least 3 molecules with potent activity against live *Micrococcus luteus*. Two of these molecules, with relative molecular weights of 4.1kDa and 4.3kDa, are both believed to belong to the insect defensin family. A third molecule of 13.4kDa also lyses freeze-dried *M.luteus* cells and is believed to be a lysozyme. Unlike the lysozyme gene cloned from *An.gambiae* (Lee, J-Y, pers.comm.) in *Ae.aegypti* this molecule is induced by bacterial infection.

References

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DISTRIBUTION OF THE NLRCth1 NON-LTR RETROTRANSPOSON IS RESTRICTED TO THE CHIRONOMUS GENUS, Alexander G. Blinov, Yuri V. Sobanov, Svetlana V. Scherbik, Karlygash G. Aimanova, Institute of Cytology and Genetics, 630090 Novosibirsk, Russia

The NLRCth1 element of Chironomus thummi described here reveals a strong correspondence to the general structure of non-LTR retrotransposons: the presence of two overlapping ORFs, 1887 bp and 2649 bp in length; 5' and 3' untranslated regions of 153 bp and 185 bp, respectively; a variable oligo(dA) sequence at the 3' end; and a target site duplication flanking element. The ORFs of the NLRCth1 contain cysteine motifs (ORF1), and the conservative RT domains (ORF2) which have been found in all non-LTR retrotransposons investigated. Twenty three species belonging to four genera of the Chironomidae family were investigated by in situ hybridization with a probe containing the NLRCth1. This transposable element has been observed in 18 species. All of these species belong to the Chironomus genus. It has been shown that variability of copy number of this element in Chironomus species is placed in the range of 10 to 150 copies per genome. Total DNA from eight Chironomus species has been tested by Southern-blot hybridization with a probe containing the NLRCth1. It has been shown that a minimum of three different non-LTR retrotransposons are present in the Chironomus genus. All of them contain similar nucleotide sequences in the region of the ORF2 which encodes reverse transcriptase. No sequence similarity has been found in the other parts of the elements. A comparative analysis of two closely related species, Chironomus thummi and Chironomus piger, showed the presence of two variants of the NLRCth1, A and B.