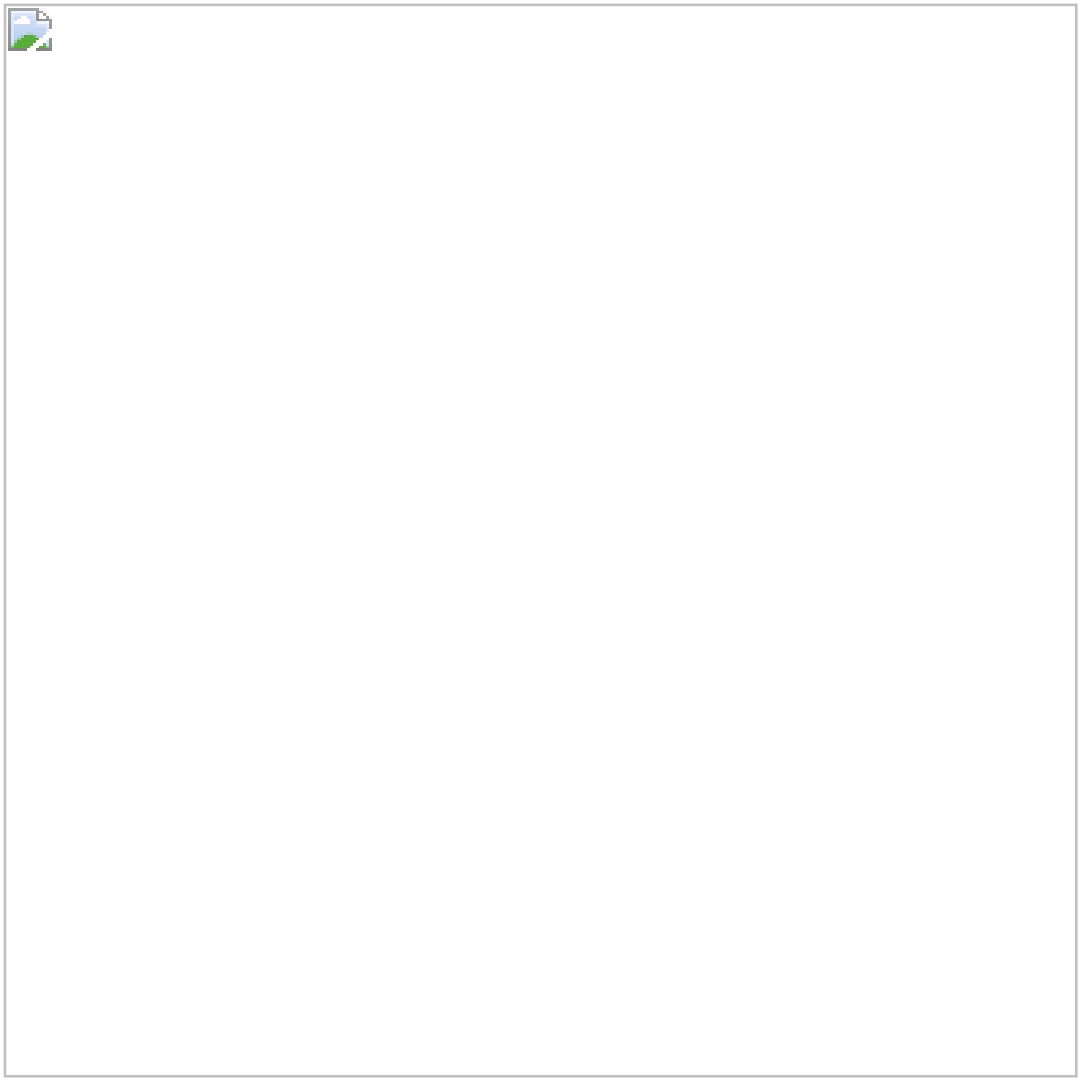

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Abstracts are listed in alphabetical order by the last name of the senior author

Characterization of a novel peptide with allatotropic activity in the fall armyworm, *Spodoptera frugiperda*

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The biosynthesis of juvenile hormone (JH) by the corpora allata (CA) can be either stimulated or inhibited by neuropeptides termed allatotropin (AT) or allatostatin (AS), respectively. To date, only one AT has been identified. It was first isolated from heads of pharate adults of *Manduca sexta* (Manse-AT; GFKNVEMMTARGF-NH2). The gene encoding Manse-AT has been cloned from various lepidopterans, including *S. frugiperda*. Here we report the identification of a second peptide with allatotropic activity from the brain of *S. frugiperda* by molecular techniques. A cDNA that encodes 53 amino acids included one copy of the RVRG NPISCF-OH peptide. This peptide strongly stimulated the synthesis and release of JH in vitro by the CA of female adult S. frugiperda and was code-named Spofr-AT 2. The stimulation was dose-dependent with an app. EC_{50} of ca. 10^{-7} M. CA that were activated with Spofr-AT 2 could be inhibited by the addition of Manse-AS to the incubation medium. Northern blotting and RT-PCR analyses revealed that the preprohormone is expressed in the brain, midgut, and ovary in a developmental-specific manner. Whole-mount in situ hybridisation confirmed the gene expression in various tissues of adult females. Supported by the DFG (Ho 631/15-4).

Juvenile hormone accelerates ovarian development and does not affect age polyethism in the primitively eusocial wasp, *Ropalidia marginata*

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Juvenile hormone modulates post-imaginal reproductive division of labor in primitively eusocial species and promotes the production of queens (e.g., *Polistes*) while it modulates age polyethism and promotes the production of foragers in highly eusocial species (e.g., the honey bee). *Ropalidia marginata* is a primitively eusocial wasp that shows both post-imaginal regulation of reproductive division of labor as well as age polyethism. Hence, *R. marginata* is a particularly interesting model system to study the effect of juvenile hormone. We demonstrate here that a single, topical application of 100 µg of juvenile hormone-III per female wasp accelerates ovarian development of wasps held in isolation. Similar application to wasps released back on to their natal nests has no effect on their rate of behavioral development as witnessed from the age of first performance of feed larva, build, bring pulp and bring food. We conclude therefore that in *R. marginata*, juvenile hormone has retained its function of modulating reproductive division of labor and has not acquired the function of modulating age polyethism.

Uncovering Juvenile Hormone's Mode of Action in *Ips pini* (Say)

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In the pine engraver beetle, *Ips pini*, pheromone components are produced de novo via the mevalonate pathway. Feeding and JH III treatment induce an increase in pheromone biosynthetic gene expression in male midgut cells, especially *3-hydroxy-3-methylglutaryl-CoA reductase (HMG-R)* and *synthase (HMG-S)*. Microarray analysis has identified several other mevalonate pathway genes, as well as a few unknowns, that appear to be JH-responsive.

Further expression analyses using real-time and semi-quantitative RT-PCR prepared from midguts at various time points (within the first 8 hours) after JH treatment have revealed some that are rapid, primary JHresponders, with transcripts visible after only 1 hour. The promoter regions of JH-responsive genes are needed to search for putative JH response elements. Therefore, an I. pini genomic library was screened using JH-responsive clones in order to isolate their 5' flanking regions. Electrophoretic mobility shift assays using nuclear extracts prepared from Sf21 cells treated with either hormone or carrier alone were performed to identify regions of JH-induced protein binding. Also, important regions for induction are being mapped using transcriptional assays with luciferase reporter constructs. The isolation of JH-inducible genes and their regulatory sequences will provide vital information about the mechanism of JH action in the Coleoptera.

Unique structural features of moth farnesyl diphosphate synthase: implications for the biosynthesis of homologous juvenile hormones

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Although the vast majority of insects produce only one form of juvenile hormone (JH III), the Lepidoptera produce a total of five JHs, including four structural homologs that feature one or more ethyl side chains. In a key step of JH biosynthesis, three isoprene units undergo a head-totail condensation catalysed by farnesyl diphosphate synthase (FPPS), generating the C_{15} farnesyl diphosphate – the backbone of JH – or one of its C_{16-18} structural homologs. We cloned and sequenced FPPS cDNAs from two species of moths. A comparison of these sequences with those of all other known FPPSs – cloned from various organisms ranging from *Drosophila melanogaster* to *Homo sapiens* – revealed several amino acid substitutions within the catalytic cavity. Molecular modeling of moth FPPS suggests that some of these substitutions could provide the extra space needed for binding the bulkier ethyl-substituted substrates/products. In assays aimed at assessing the substrate specificity of the recombinant enzymes, we observed that moth FPPS displays a relative activity twice as high as that of *Drosophila's* in the presence of homogeranyl-PP and [^{3}H]-isopentenyl-PP, suggesting that the moth enzyme shows preference for homologous substrates

Juvenile hormone regulation of bark beetle monoterpenoid pheromone biosynthesis

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Juvenile hormone (JH) III regulates de novo aggregation pheromone production in bark beetles. Feeding on host phloem or application of JH III induces the production of ipsdienol in male *Ips pini* and frontalin in male *Dendroctonus jeffreyi* in a dose and time related manner. The incorporation of [^{14}C]mevalonolactone into ipdienol and frontalin demonstrated that these pheromone components were produced via the mevalonate pathway. JH III induction of 3hydroxy-3-methylglutaryl-CoA reductase, a key regulatory enzyme in the mevalonate pathway, was localized to midgut tissue by in situ hybridization. Studies with [^{14}C]acetate demonstrated that midgut tissue from male *I. pini* produced ipsdienol and from male *D. jeffreyi* produced frontalin. This work led to a paradigm shift in which it is now recognized that most, but not all, monoterpenoid pheromone components from bark beetles are produced *de novo*. It also provided the background to current studies using microarrays to examine JH III regulation of mevalonate pathway enzymes in midgut tissue, the characterization and JH III regulation of geranyl diphosphate synthase, the discovery of putative JH III regulatory elements and a novel regulatory motif in *I. confuses*.

The effect of juvenile hormone on the PBAN-receptor and pheromone production in the moth *Helicoverpa armigera*: An aging hormone in adult females?

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The present study was designed to elucidate the effect of Juvenile Hormone (JH) on PBAN activation of pheromone production *in vitro* and on specific binding to a PBAN-receptor (PBANR) in pheromone glands of *H. armigera* females. PBAN can activate pheromone biosynthesis only in pheromone glands of newly emerged adult females, after which levels increase with age and reach maximum levels in 3 and 4 day old female glands, thereafter the pheromonotropic response decreases. Female pharate pupae do not respond to PBAN but, in the presence of JH II or its analog, fenoxycarb (FX), PBAN can induce a response in pharate females. In addition, a photoaffinity-biotin-labeled PBAN-analog, specifically binds to a 50 kD putative PBAN membrane receptor in adult females. This binding is absent in pharate females but can be induced after JH II or FX treatments to levels of newly emerged females, thereby providing evidence that JH up-regulates the PBAN-R in immature females. In contrast, in 3-4 day-old mature adult females, FX causes down-regulation of both pheromone production and PBAN-R binding to levels present in older females. Taken together these data indicate that juvenile hormone treatment shifts the normal age-dependent response to PBAN.

Effect of Precocene II on Fatty Acid Metabolism in the Pea Aphid, *Acyrthosiphon pisum* Under Cold Stress

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Pea aphid, *Acyrthosiphon pisum*, accumulate high levels of myristic acid as triglycerides at 10°C. To investigate whether juvenile hormone (JH) mediates fatty acid metabolism in the aphids, the aphids from 25°C colony were treated with precocene II (a JH antagonist) at 0.5 and 2 µg/aphid, respectively, then reared at 10°C for 16 days. An increase in fatty acid content was observed in the aphids treated with 0.5 µg, whereas no change was observed in 2 µg treatment. When the aphids were retreated with same amount of precocene II 24 h after 1st treatment, the fatty acid content in the aphids did not change in either treatment. A further experiment was conducted to investigate the reproductive fecundity in the aphids treated with 2 µg precocene II. The nymphs per aphid in the treated aphids decreased significantly at either 25°C or 10°C compared to that in the control aphids. However, the nymphs per day in treated aphids at 10°C is similar to that in the control aphids. These results imply that JH may mediate fatty acid metabolism and affect the reproduction in the pea aphids reared at low temperatures.

Molecular characterization of the lipophorin receptor of *Blattella germanica*

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We have cloned and molecularly characterized a *Blattella germanica* lipophorin receptor (BgLpR) cDNA. It has a length of 2595 bp encoding a 865-residue protein with a predicted molecular mass of 96.8 kDa. BgLpR is a member of the low density lipoprotein receptor family with five domains: ligand domain, epidermal growth factor precursor, putative *O*-linked sugar, transmembrane domains, and cytoplasmic tail with the internalization signal FDNPVY. In adult females, BgLpR is expressed in the fat body and the ovary during the 7 days of the first gonadotrophic cycle. In the ovary, mRNA levels are high during the first 5 days and suddenly decrease on days 6 and 7. In the fat body, mRNA levels are high at adult emergence, decrease on days 1 and 2, increase from day 3 to day 5, and slightly decrease on days 6 and 7. The pattern in the ovary corresponds to the dynamics of lipophorin incorporation into the ooplasm during the vitellogenic cycle. That in the fat body may be related with a lipid scavenger activity. The pattern difference in the fat body and in the ovary suggests that the regulatory mechanisms in these two organs are different. The possible role of JH in BgLpR regulation is discussed.

Expression and localization of a putative farnesoic acid o-methyltransferase (FAMeT) in *Drosophila melanogaster*

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Farnesoic acid o-methyl transferase (FAMeT) is an important enzyme in the pathway leading to juvenile hormone biosynthesis. Juvenile hormone participates in the development and reproduction of most insects. Database searches based on sequence identity with crustacean FAMeT has revealed a putative gene product in *Drosophila melanogaster*. This gene encodes a protein with 41% sequence identity to *Metapenaeus ensis*. It is thought that FAMeT may play a rate limiting role in juvenile hormone biosynthesis in *D. melanogaster*; however, no experimental evidence has been reported. In order to elucidate the putative *Drosophila* FAMeT orthologue's role in juvenile hormone biosynthesis, we have investigated protein distribution, activity and *in vivo* transcription in *D. melanogaster*. This has been examined by 1) protein expression to test FAMeT's activity *in vitro* and 2) by fusion of the putative FAMeT gene promoter to green fluorescent protein (GFP) within a p-element vector. Germ-line transformations of this construct will be carried out to provide localization data.

Up-regulation of JH levels in *Lacanobia oleracea* larvae attacked by the parasitoid wasp *Eulophus pennicornis*, is associated with reduction in host JH esterase activity

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The ectoparasitoid wasp *Eulophus pennicornis* attacks 5 th and 6th instar larvae of its host *Lacanobia oleracea*. We measured the activity of juvenile hormone esterase (JHE) daily in unparasitized and parasitized penultimate (5^{th}) and final (6^{th}) instar host larvae. In unparasitized larvae, JHE activity was low throughout the penultimate larval stadium, but was markedly higher in final instar larvae. In parasitized penultimate instar larvae, JHE levels were similar to those in unparasitized insects. However, in parasitized final instar larvae, JHE activity was markedly reduced when compared to the levels in unparasitized larvae. In the light of these differences, we measured

the titres of juvenile hormones in parasitized and unparasitized larvae, on days 2 and 5 of the final larval stadium. Whereas JH levels were virtually absent in unparasitized larvae, substantial quantities of JH (~100-fold higher) were detected in parasitized larvae of the equivalent ages. We have also investigated the JH present in larval and adult stages of the parasitoid. In both parasitoid larvae and in adult wasps, we found high titres of JH III, but little JH II, and no JH I.

The making of a yolky egg: It takes more than juvenile hormone and reserves

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In the majority of insects the production of a yolky egg necessitates the induction of vitellogenin (Vg) by juvenile hormone (JH). Vg, i.e. vitellin (Vt), is the predominent yolk protein. Also, lipophorin (Lp) and hexamerins, contribute quantitatively to the protein yolk of the egg. In cockroaches, normally only JH drives the *de novo* synthesis of Vg. Adequate titers of JH are maintained through the controls of rates of JH synthesis and JH degradation. Using the example of the cockroach *Leucophaea maderae* the complexity of controls that allow a timely making of a mature egg will be discussed. This includes the determinations of 1) the JH and Vg profiles, 2) the JH controlled augmentation of lipophorin (Lp) synthesis, 3) the JH influenced increase in JH esterase titers during vitellogenesis and 4) the assessement of the involvement of the hexamerins. Lp and Vg are the 2 major JH binding proteins in circulation. Consequently, the rate of degradation of JH is efficiently reduced even during periods when we measure the highest titers of the JH esterases. Conversely, in the absence of Lp the circulating JH is short lived and therefore Vg production is reduced resulting in a curtailed egg production.

The role of JH and its effector, Broad in the evolution of insect metamorphosis

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Metamorphosis in Holometabola is regulated by JH and its effector, *Broad*. To begin to understand how complete metamorphosis has evolved in insects, we examined the roles of JH and *Broad* in hemimetabolous insects. We have found, by simulating the JH profile of holometabolous embryos in cricket embryos, that JH is able to redirect embryonic cuticle formation in a stage and dose-dependent way. To determine the role of *Broad* in hemimetabolous insects, we cloned this gene from crickets and bugs. Unlike its expression in Holometabola, where it is expressed exclusively during pupal development, we found that the Z1 isoform of *Broad* is expressed during embryonic development of crickets, first appearing during segmentation. We found that dsRNA knock down of *Broad* resulted in embryos with posterior truncations. Another difference between hemimetabolous and metamorphosing insects is seen during post-embryonic development. Our preliminary data indicates that *Broad* is expressed during the nymphal stages, coincident with high JH levels. Taken together, our data suggests that metamorphosis in insects could have arisen after embryonic and nymphal expression of *Broad* acquired inhibition by JH.

The regulation of vitellogenesis in the lubber grasshopper by JH and nutrition

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Hemolymph levels of vitellogenin (Vg) and juvenile hormone (JH) in the lubber grasshopper (*Romalea microptera*) change in parallel during the oviposition cycle, suggesting that JH may closely regulate Vg production. Likewise, the duration of the oviposition cycle is regulated by the amount of food available. We investigated the relationships between nutrition, JH, and vitellogenesis by feeding animals high (H; 4.5g lettuce and 0.6g oats/d) and low (L; 1.5g lettuce and 0.2g oats/d) diets. JH levels in H-diet animals increased on ~ d10, reached a peak on d20-25, and declined thereafter. The levels of Vg-mRNA in these animals show a similar rise and fall, and ovarian mass increased 30-fold. JH levels in L-diet animals remained low through d30, and Vg-mRNA levels and ovarian mass rose only 4-fold above initial levels. When animals were switched from the L-diet to the H-diet on d20, JH levels and Vg-mRNA levels rose quickly on d25 and reached a maximum on d30. Levels of Vg-mRNA also rose about 9 and 10-fold above initial levels on d25 and 30, respectively, and ovarian mass increased 11-fold above initial levels. Infusion of L-diet animals with physiological levels of JH for 5 days stimulated Vg-mRNA levels, but the increase was intermediate to that of animals switched to the H-diet over this period. These data suggest that nutrition regulates vitellogenesis is complex, and only partly reflects its effects on JH levels. (Supported by NSF grant DBI - 9978810 to DWB).

Juvenile Hormone Binding Proteins and Neuronal Plasticity

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In the black cutworm moth, *Agrotis ipsilon*, Juvenile Hormone (JH) has been shown to control both pheromone production and perception. In particular, most recent studies have shown the ability of males to adapt their pheromone behavior by modulation of neural responses to the olfactory stimuli. This phenomenon may be directly related to the regulation of pheromone sensitivity of interneurons in the antennal lobe. This suggests that, in males, JH may act by controlling neuronal plasticity in the macroglomerular complex. We aim at the molecular mechanism underlying JH regulation of neuroplasticity associated to pheromone perception. With this respect, we have identified Juvenile Hormone binding Proteins (JHBPs) in the brain of *A. ipsilon*. In photolabeling experiments, the tritiated JH I analogue ([^{3}H]-EBDA) bound to a protein with a molecular weight of 30-35 kDa in protein extracts from *A. ipsilon* brains. Complementary DNA encoding cytosolic JHBP was cloned from a brain cDNA library. This suggests the action of JH at the intracellular level in brain cells. In all, we have identified the first JH-Binding Protein from a moth brain and discuss its role in the control of neuroplasticity and pheromone perception.

Selective Inhibition of Juvenile Hormone Biosynthesis by Ammonium Diphosphate Compounds

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Juvenile hormone is biosynthesized by an unusual metabolic pathway that utilizes enzymes of the MVA pathway. Two enzymes, FPP synthase and IPP isomerase are potential sites for inhibitor development, particularly in light of the fact that they play a pivotal role in the construction of JH homologs in Lepidoptera. A series of ammonium diphosphate analogs of dimethylallyl diphosphate (DMAPP) and geranyl diphosphate (GPP) were prepared and tested for their ability to inhibit JH biosynthesis *in vitro*, using corpora allata homogenates of larval *Manduca sexta*. When potencies were compared to those obtained with the corresponding porcine enzymes, differences were seen, indicating that similar design strategies could be useful for the development of selective anti-juvenile hormone agents.

The Isolation and Characterization of the First Animal Geranyl Diphosphate Synthase from the Pine Engraver, Ips pini (Say)

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Bark beetles release aggregation pheromones that coordinate the colonization of their coniferous hosts. Over the years, debate has ensued over whether these monoterpene pheromone components are derived from dietary precursors or produced de novo. We present evidence in support of their de novo biosynthesis via the mevalonate pathway. We have isolated the first animal geranyl diphosphate synthase (GPPS) from a cDNA library of juvenile hormone (JH) III treated male *Ips pini*. GPPS catalyzes the formation of the C10 precursor of ipsdienol, a major aggregation pheromone component in male *I. pini*. We have expressed the recombinant GPPS and assayed for functional activity. Sequence comparisons and structural modeling show conserved motifs and an all ?-helical fold similar to other short-chain isoprenyl diphosphate synthases. In a number of bark beetles, JHIII regulates pheromone production. Northern analysis of *I. pini* shows GPPS transcript levels are up-regulated in a JHIII dose-and time dependent manner, similar to other mevalonate genes involved in aggregation pheromone biosynthesis in male *I. pini*.

The role of juvenile hormone in the endocrine regulation of pheromone production in the pinyon *Ips*

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Bark beetles are among the most economically important forest pests in the northern hemisphere and rely on monoterpenoid aggregation pheromones to coordinate host colonization and mating. In this study, we investigate the interplay between feeding on host phloem and the induction of de novo pheromone biosynthesis in *Ips confusus*, the pinyon *Ips*. *I. confusus* has become a major pest in the southwestern United States, destroying hundreds of thousands of acres of pinyon pines. Juvenile hormone (JH) III regulates pheromone production in a number of bark beetles. Interestingly, it appears that JH III alone does not stimulate pheromone biosynthesis in male *I. confusus* but rather some other regulatory factor, perhaps a brain hormone, is required for pheromone production. We have found that feeding on host phloem, but not JH III treatment, strongly induces pheromone production in male *I. confusus*. Moreover, feeding alone stimulates the activity of a key mevalonate pathway enzyme, 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-R), only in males. Nevertheless, feeding and JH III both significantly up-regulate mRNA levels of HMG-R and other mevalonate pathway genes.

Presence and possible function of allatotropin in developmental stages of *Heliothis virescens* and *Apis mellifera*

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Juvenile hormone biosynthesis is, in part, regulated by stimulatory neuropeptides called allatotropins. Using immunocytochemical techniques, we localized *Manduca sexta* allatotropincontaining cells in the central nervous system (CNS) of selected developmental stages of two insect species, the tobacco budworm *Heliothis virescens* and the honeybee *Apis mellifera*. *H. virescens* produces allatotropin (AT) consistently throughout larval development. The distribution patterns of AT-containing cells in the CNS persisted from one larval instar to the next, and were similar to the distribution patterns in adults. However, the total number of AT-containing cells in the brain gradually increased during larval development. In the honeybee *A. mellifera*, AT-containing cells were only found in a few brains from late last instar larvae (prepupae). AT was present in a group of 6-8 cells in the pars intercerebralis. However, we did not find any AT in brains of early last instar larvae, whose corpora allata (CA) were shown to be more sensitive to in vitro stimulation by AT than prepupal CA. The significance of our results in the context of insect development in general, and in the context of caste development in honeybees will be discussed.

Juvenile hormone in the control of reproductive function in *Drosophila virilis* under stress

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To unravel the role of juvenile hormone (JH) in the control of reproduction under stress, JH degradation and reproduction were studied under heat and nutritional stresses and JH treatment in wild type (*wt*) *Drosophila virilis* females and in a heat stress (*hs*) mutant. The *hs* mutation prevents alterations in JH metabolism under heat stress. Both heat stress and starvation result in a decrease of JH degradation, a delay in oocyte maturation, degradation of early vitellogenic egg chambers, accumulation of mature oocytes, an oviposition arrest and followed by decrease in fertility in *wt* females. JH treatment leads to a decrease in JH degradation and an arrest of oviposition for 24h in *wt* and *hs* females. JH treatment prior starvation seems to protect some oocytes from degradation: in JH-treated *wt* female's fertility increases rapidly following the end of starvation. The dynamics of JH degradation and fertility are similar following stress and JH treatment in *wt* females. In *hs* females, there is no decrease in JH degradation, accumulation of mature oocytes and oviposition arrest under heat stress. However, they show all these alterations under starvation. This suggests that JH controls later stages of oogenesis and oviposition under stress.

Is methyl palmitate an indicator of reproductive maturation in female Medflies?

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Methyl palmitate (MP) is a biosynthetic in vitro product of the corpus allatum (CA) of adult female (*Ceratitis capitata*; Diptera:Trypetidae). The presence of MP in whole-body extracts of virgin females has now been determined by GC/MS. Methyl esters peaks were identified by Total Ion Current chromatography and MP quantified by Single Ion monitoring. In mated *Drosophila melanogaster*, reproductive maturation is primed via enhanced allatal production of JHB3, induced by the N-terminus of the male-derived Sex Peptide (*Drm*SP). We hypothesize that the CA of young female Medflies are deficient in either epoxidase, or in farnesoic acid, the sesquiterpene precursor of JH-biosynthesis, and that biosynthesis is therefore diverted via acetyl– CoA carboxylase to the alternative production of palmitic acid ($C_{16:0}$; PA). During this transition period, the CA methylates PA to MP, which by inference presupposes the existence of endogenous methyl transferase. Accordingly, mating is assumed to enhance the acquisition of enzymes/substrates for production of JH-III and JHB3, the conventional products of mature dipteran CA.

The significance of allatal reproductive maturation: Multiple functions of insect allatotropins

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Juvenile hormone (JH) levels in the hemolymph are regulated in part by the actions of neuropeptides that act on the corpora allata (CA). An allatotropin (Manse-AT) was isolated from *Manduca sexta* that stimulates JH biosynthesis by the adult female CA *in vitro*, and similar peptides have been isolated or predicted from several insect species that exhibit a variety of additional roles on muscles, heart and midgut. In other invertebrate orders, Manse-AT-related peptides exhibit myotropic functions suggesting that this might be the ancestral role for this family of peptides. Tissue- and stage-specific alternative splicing predicts the synthesis of allatotropin-like (ATL) peptides that possess overlapping biological activities in *M. sexta*. The levels of one of the alternatively spliced Manse-AT mRNAs is elevated in larvae that were starved, parasitized, or fed the ecdysteroid

agonist RH-5992. These elevated mRNA levels were seen exclusively in the terminal abdominal ganglion of starved larvae. Although the CA of mated *M. sexta* females exhibit an elevated rate of JH biosynthesis in vitro, levels of Manse-AT mRNAs were similar to those in virgin females, suggesting that other factors might be involved in the elevated CA activity.

JH titers, biosynthesis and metabolism in honey bee workers infected by a microsporidia parasite, *Nosema apis*

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It is known that *Nosema apis* (a microsporadian parasite) accelerates the behavioural development in honeybee workers, but the underlying mechanisms are unknown. Early studies suggested that *Nosema* may be capable of producing juvenile hormone (JH) directly. We studied the JH production and metabolism of *Nosema* infected workers. Infected workers foraged at an earlier age and showed higher haemolymph JH titers than control bees. This suggests that *Nosema* infection induces workers to forage earlier via higher JH titers. *Nosema* infected workers showed higher rates of JH biosynthesis than control bees when workers were 6-8 days old (preforaging). Allatectomized workers fed *Nosema* had no detectable levels of juvenile hormone in hemolymph. The majority of these workers did not show earlier foraging compared to the control group (allatectomized bees with no *Nosema*). Finally, *Nosema*-infected workers also showed higher JH degradation compared to control bees. These results suggest that Nosemainfected workers forage at an earlier age than control bees due to higher JH titers, which was due to increased JH production, and despite of the increased JH degradation in infected bees. Our results also suggest that *Nosema apis* does not produce JH directly, but can induce the host CA to produce higher titers of JH. Earlier foraging of the hosts might be advantageous for the parasite because precious foragers may drift more easily to other colonies.

Transcriptional Integration of JH and Ecdysone Signaling Through Nuclear Receptor Dimer Containing Ultraspiracle

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Using intrinsic fluorescence and anisotropy techniques, we have demonstrated binding of methyl epoxyfarnesoate (juvenile hormone (JH) III) to USP. In an Sf9 cell transfection, JH IIIactivation pathway, JH III can act through the USP ligand binding pocket and through a heterologous USP binding site (direct repeat motif) to activate the reporter core promoter. Additional, exogenous USP further increases the loading of USP onto the direct repeat binding site (by gel mobility shift assay) and increases transcriptional activation by treatment with JH III alone. Exogenous ecdysone receptor (EcR) increases the loading of the USP/EcR dimer onto the direct repeat binding site and increases activation due to treatment with 20-OH ecdysone. Treatment with both JH III and 20-OH ecdysone activates through the direct repeat binding site to a much greater than the additive effect of either hormone alone, and exogenous EcR also enhances this synergism, indicating synergism of the two hormones is mediated by the USP/EcR dimer. However, exogenous EcR suppresses activation by JH III alone, indicating that JH III activation through USP is subordinated by the USP/EcR heterodimer when the EcR partner is unliganded.

Transcriptional Transduction of Juvenile Hormone Signaling by the Nuclear Hormone Receptor Ultraspiracle

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The published crystal structure of the nuclear receptor Ultraspiracle (USP) contributed to a paradigm of USP as a ligandless receptor, in which the AF2 of USP is maintained in an antagonist position over the site corresponding to a coactivator binding surface. Using intrinsic fluorescence and anisotropy techniques, we have demonstrated binding of methyl epoxyfarnesoate (juvenile hormone (JH) III) to USP. The interaction of JH I and JH III with the USP ligand binding pocket is qualitatively different than that of JH II. The binding by JH III promotes receptor oligomerization and repositioning of AF2. In a cell transfection, JH IIIactivation pathway, JH III can act through a heterologous USP binding site (DR12 motif) to activate the reporter core promoter. Specific point mutations to particular ligand binding pocket residues alter or prevent JH III binding, and render the mutant receptor to be a dominant negative in this system. The mutant USP with reduced JH III binding and dominant negative action in the cell transfection, JH III-activation pathway is reduced in its ability as a transgene to replace missing function of the wild type USP in null USP flies.

Evaluation of the vinyl sulfoxide class of anti-juvenoids as inhibitors of isoprenoid forming enzymes

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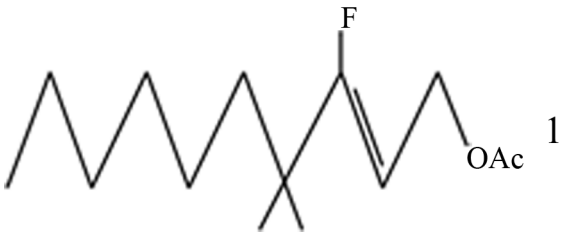
Several vinyl sulfoxides are known to possess topical anti-juvenile

hormone activity. While these compounds inhibit one or more steps prior to farnesoic acid formation, their specific mode of action is unknown. We hypothesized that the most active compound (1) could function as a transition state analog for the isoprenoid forming enzymes, FPP synthase and IPP isomerase, being a mimic of the carbocations that form during catalysis. To test this hypothesis, derivatives of 1 were prepared, including several potential metabolites that could form upon topical application. The compounds were tested for in vitro activity using corpora allata homogenates of *Manduca sexta*. None of the compounds were inhibitory, with the exception of the diphosphate analog of 1, which was a modest inhibitor of FPP synthase. These results suggest that metabolic activation of 1, through hydrolysis and subsequent phosphorylation via endogenous kinases, may be operational.



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Anti-metamorphic effect of an endoparasitoid, *Cotesia plutellae*, on *Plutella xylostella* – Is it caused by alteration of JH titer or ecdysteroidogenesis?

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An endoparasitoid wasp, *Cotesia plutellae*, parasitized the diamondback moth, *Plutella xylostella*, and inhibited larva-pupal metamorphosis. JH esterase (JHE) in the hemolymph of P. xylostella was monitored by use of a surrogate substrate, HEPTAT (methyl 1heptylthioacetothioate). The enzyme kinetic parameters of the fourth instar larvae of the nonparasitized showed that Km was 19.70 μM and Vmax was 228.83 μM/min/μl. In the last larval instar of *P. xylostella*, JHE activity kept high level, but decreased before pupation. Similarly, the parasitized larval showed high level of JHE activity in the initial final instar, which was not significantly different to that of the non-parasitized. Thus, the parasitization by *C. plutellae* did not inhibit host JHE. In comparison, the prothoracic gland showed significant morphological changes between parasitized and non-parasitized larval, even though the application of an ecdystroid agonist, RH5992, did not induce metamorphosis of the parasitized larvae.

Juvenile hormone-mediated gene expression in the midgut of the pine engraver beetle, *Ips pini* (Say)

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The pine engraver beetle is a significant pest of North American coniferous forests. The male is the pioneering sex and attracts mates to its nuptial chamber in the phloem of a host tree with a pheromone. Upon feeding, and regulated by juvenile hormone III (JH), the pheromone component ipsdienol is biosynthesized *de novo* via the mevalonate pathway in the male midgut. Identifying and characterizing genes in the midgut that respond to JH treatment may yield the mode of JH action as well as targets for novel pest management strategies. To identify responsive genes, cDNA microarrays were prepared to represent the unique genes previously identified in a midgut EST project and then hybridized with fluorescently labeled cDNA from midgut tissue from JH-treated and control beetles of both sexes over a time course. Several genes were significantly up or down regulated by JH, including known mevalonate genes, other known genes, and unknown genes. The expression of these genes was further examined by quantitative real-time RT-PCR. Transcript levels in the midgut of both sexes respond to JH although there was a sex-specificity consistent with pheromone biosynthesis.

Juvenile hormone action involves multiple signal transduction mechanisms

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Although, the biological actions of juvenile hormones (JH) in regulating development and reproduction are well studied, the molecular basis of JH action is poorly understood. We discovered that the juvenile hormone esterase gene from *Choristoneura fumiferana* (Cfjhe) is induced by JH I and JH I induction is suppressed by 20-hydroxyecdysone (20E). Cloning and analysis of the promoter region of Cfjhe gene identified a 30 bp region that supports both JH and 20E response observed for the Cfjhe gene. This 30 bp sequence contains a direct repeat element with a 4-nucleotide spacer and is designated as JH response element (JHRE). A reporter gene placed under the control of JHRE is induced by JH in a dose- and time-dependent manner in CF-203, L57, HvE1s and Aa23 cells, and 20E in the presence of EcR was able to suppress JH induction. Nuclear proteins isolated from all four cell lines bound to JHRE. Protein kinase C-mediated phosphorylation decreased the binding of nuclear proteins to JHRE. JH or calf intestinal alkaline phosphatase-mediated dephosphorylation increased the binding of nuclear proteins to JHRE. The results from these studies suggest that JH action and its cross-talk with 20E involve multiple signal transduction mechanisms.

Methyl Farnesoate Controls Adult Male Morphogenesis in a Crayfish

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Insects mature sexually after a terminal molt. Louisiana crayfish, *Procambarus clarkii*, continue to molt as adults, switching between Form Is, primary reproductives, with large chelae and spines on ischiopodites of walking legs 3 and 4, and Form IIs, a non-reproductive type with smaller chelae and lacking ischiopodite spines (Huner, 1994). We investigated the hormonal control of these transitions in two ways, by eyestalk ablation, and by methyl farnesoate (MF) treatments. MF was quantitated by HPLC and ecdysteroids by radioimmunoassay. Of 4 untreated Form I males all molted into Form IIs, while 6 of 7 Form IIs molted into Form Is. After eyestalk ablation 8 of 8 Form Is molted into Form IIs, while 5 of 5 Form IIs molted into Form IIs. MF treatment of intact animals with MF (2 µg/animal/day) resulted in 6 of 7 Form Is becoming Form IIs and 5 of 6 Form IIs becoming Form IIs (significant by chi square analysis $P < 0.01$). MF levels in premolt blood suggested that Form IIs are produced in the presence of 1.3ng/ml MF, while Form Is result from MF levels of 0.5ng/ml. We conclude that the control of morphogenesis of adult primary reproductives (Form Is) depends on low levels of MF prior to the molt, while Form IIs are produced in the presence of elevated levels of MF, since both eyestalk ablation and MF treatments result in the failure of Form IIs becoming Form Is. (Supported by the Sea Grant College Program and CT. DEP)

A comparison of in vivo and in vitro rates of juvenile hormone synthesis and degradation in the lubber grasshopper

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To better understand how juvenile hormone (JH) levels are regulated, we developed a novel method for estimating in vivo rates of both JH synthesis and degradation. This method was used to determine these rates during the oviposition cycle of the lubber grasshopper, *Romalea microptera*. These in vivo rates were compared to in vitro measurements of JH synthesis by corpora allata (using a radiochemical assay) and JH degradation by the hemolymph (using a partition assay). The rates of JH synthesis in vivo paralleled those measured in vitro: JH synthesis was lowest at the beginning, highest during the middle, and intermediate at the end of the oviposition cycle. However, the estimated rates of JH synthesis *in vivo* were 3.4 to 33-fold higher than the rates measured *in vitro*. The rates of JH degradation *in vivo* also paralleled those measured *in vitro*: JH degradation was highest at the beginning, lowest near the middle, and intermediate at the end of the cycle. However, the estimated rates of JH degradation *in vivo* were 10^{-3} to 10^{-5} times lower than the rates measured *in vitro*. These results confirm that changes of JH levels reflect variations in both JH synthesis and degradation. This novel method provides a new approach to study the complex mechanisms involved in regulating JH levels in insects. (Supported by NSF grant DBI - 9978810 to DWB).

Regulation of juvenile hormone synthesis in mosquito: physiological, biochemical and molecular studies

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We aim to understand the regulation of juvenile hormone (JH) synthesis in mosquitoes, and how nutritional signals affect the activity of the neuroendocrine system. JH III synthesis is low immediately after adult emergence, increases in sugar-fed females and decreases after bloodfeeding. *Aedes*-allatotropin makes CA in newly emerged females capable for JH biosynthesis. *Anopheles gambiae* PISCF-amide-allatostatin (allatostatin-C-type) is very effective inhibiting JH synthesis. By raising larvae under different nutritional diets two different adult phenotypes (large and small females) were generated. Teneral reserves (protein, lipids and glycogen) were significantly lower in small females. JH synthesis was significantly reduced in females emerged with low teneral reserves and stimulated by sugar feeding; only when nutrients are appropriate the CA becomes capable of synthesizing enough JH to activate reproductive maturation. EST were sequenced from libraries made from corpora allata-corpora cardiaca complexes from *Aedes aegypti* and *Anopheles albimanus*. A comparison with ESTs from the CA of *Diploptera punctata* confirmed the identity of enzymes involved in JH synthesis and of other important regulatory molecules.

Juvenile hormone activity of novel homo- and polyenehomobenzenes in fruit flies and moths

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Eight mono- and polyenehomobenzene compounds, synthesized via a highly regioselective palladium-catalyzed ?-alkylation of allylic

bromides and chlorides, with substituted and unsubstituted benzylic Grignard reagents, were tested for their juvenile hormone action on larvae and pupae of a dipteran (*Drosophila virilis*) and last instar larvae of a lepidopteran species (*Spodoptera frugiperda*). Three dienes with a para-substituted phenyl ring displayed a strong insecticidal activity against *D. virilis*, whereas mono-, tri- and tetraenes as well as a nonsubstituted diene were not active. Hence, a para-substituted phenyl ring and the length of the aryl chain seem to be important for insecticidal action in *D. virilis*. In contrast, a monoene, a para-substituted diene and a para-substituted triene were active against *S. frugiperda*. The same compounds were also active against another lepidopteran, the map-butterfly *Araschnia levana*. Methoprene, which served as a positive control, was active in both dipterans and lepidopterans. We conclude that some of the tested compounds are specific against certain insect orders and may thus serve as a starting point for the design of more specific juvenile hormone analogs for insect pest control.

Molecular characterization and developmental expression of RXR in *Blattella germanica*

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We have cloned two isoforms of RXR in *Blattella germanica*, a short one (BgRXR-S) and a longer one (BgRXR-L), which are identical except for a deletion/insertion of 23 amino acids in the loop between the helix 1 and 3 of the ligand binding domain. Transcript levels of both isoforms were studied in the fat body of fifth and sixth larval stages, in the ovary and fat body of females during the reproductive cycle and in embryos. BgRXR-S predominates in fat body tissues, whereas BgRXR-L predominates in the ovary. In larval fat body, the expression is quite constant along the stage and only shows a modest peak during molting. In the adult female fat body, mRNA levels are quite constant during vitellogenesis and slightly increase just before oviposition. Conversely, ovarian mRNA levels slightly decrease towards the middle of vitellogenesis. In the embryo, both isoforms show a differential expression, BgRXR-L transcripts being predominant in the first three days and decrease thereafter, whereas those of BgRXR-S are low during these first days, increase from days 3 to 5 and then remain practically constant. Treatment of B. germanica embryo UM-BGE-1 cells with JH did not modify transcript levels of any of the two isoforms.

Cloning of E75 nuclear receptors in *Blattella germanica* and functional characterization in the fat body of during vitellogenesis

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We have cloned the ecdysone-inducible orphan nuclear receptor E75 in *Blattella germanica*. Three isoforms, BgE75A, BgE75B and BgE75C, orthologues of the corresponding isoforms of *Drosophila melanogaster*, were molecularly characterized and their expression was studied in the fat body of adult females during the vitellogenic cycle. mRNA levels of the three isoforms are low in the first days of adult age, increase from days 3 to 5-6 and decrease thereafter. The mRNA increase corresponds to the increase of circulating ecdysteroids that occurs in parallel, and the general expression pattern of E75 looks similar to that of vitellogenin production. However, the patterns of the three isoforms are not completely parallel, the peak of BgE75B being one day delayed with respect to BgE75A and BgE75C. These patterns and the occurrence of a putative E75 response element in the promoter region of the vitellogenin gene of *B. germanica* suggest that E75 might be involved in the regulation of the vitellogenic cycle. Results in line with this hypothesis will be discussed.

Farnesyl dyphosphate synthase of insects targeted to mitochondria

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The NH2-terminal extension of pharnesyl-diphosphate synthase (FPS) sequence of *Drosophila melanogaster* and *Agrotis ipsilon* have the structural features characterising mitochondrial transit peptides. These extensions are enriched in basic, hydroxylated and hydrophobic residues, contain a number of tetrapeptides fitting the consensus sequence RXXS, which is currently reported as a mitochondrial targeting cleavage motif, and may form a positively charged amphiphilic alphahelix. Functional studies using mutant yeast complementation assays have shown that these extensions are able to convey a passenger protein into yeast mitochondria. Moreover, *Drosophila* S2 cells transfected with EGFP showed a green diffuse pattern covering nucleus and cytoplasm, whereas those transfected with the NH2-terminal extension of *D. melanogaster* FPS fused to EGFP exhibited a typical mitochondrial network pattern. The implications of FPS targeting into mitochondria in the context of the regulation of JH biosynthesis is discussed.

Juvenile hormone diol kinase

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The goal of this work was to isolate and characterize the enzyme responsible for producing juvenile hormone diol phosphate, the principal end product of JHI metabolism in *Manduca sexta*, and to investigate its role in juvenile hormone metabolism. This goal was accomplished first by developing a simultaneous preparation of both enantiomers of juvenile hormones labeled at C-10 with tritium at high specific activity. With substrate in hand, juvenile hormone diol kinase (JHDK) was purified and characterized from *Manduca sexta*. Characterization of JHDK indicated a nanomolar Km for JH I diol and a low micromolar value for MgATP. JH II and III diols also serve as phosphate acceptors with low micromolar Km, whereas other diol derivatives of terpenoid esters structurally similar to JH metabolites are not phosphorylated. The gene sequence of JHDK codes for an enzyme that has 59% sequence identity to *Drosophila melanogaster* sarcoplasmic calcium-binding protein-2 (dSCP2). Molecular modeling of JHDK and dSCP2 revealed structural similarity to G-proteins and calcium binding proteins. We conclude that dSCP2 is a homolog of JHDK, and these proteins constitute a novel kinase family that binds nucleotides using the scaffold of a SCP.

Differential responses to allatostatin in migrant and non migrant populations of the true armyworm, *Pseudaletia unipuncta* (Lepidoptera: Noctuidae)

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A long term interdisciplinary project has been examining the reproductive biology of migrant (Quebec) and non-migrant (Azores) populations of the true armyworm, *Pseudaletia unipuncta*. In this species, female sexual maturation is regulated by JH, whose titers vary significantly in response to abiotic cues (temperature and photoperiod) associated with habitat quality. Azorean individuals become sexually mature at a significantly younger age than those from Quebec, and despite having a lower body mass they have a significantly higher lifetime fecundity. However, an examination of JH biosynthesis, as well as JH and JH esterase titers in the hemolymph, clearly shows that the difference between the two populations results from more than just a simple temporal shift for earlier, post-emergence, JH production in the non-migrant population. In addition, new data examining the responses of the corpora allata (CA) from different-aged Quebec and Azorean females to a fixed dose of allatotropin, as well as those from early and late maturing lines selected from the Quebec population, also support the idea that there are complex differences in the reproductive physiology of migrant and non-migrant populations.

Increased juvenile hormone levels after long-duration flight in the grasshopper, Melanoplus sanguinipes

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While migration imposes a cost to reproduction in many insects, in the migratory grasshopper *Melanoplus sanguinipes* long duration flight accelerates the onset of first reproduction and enhances reproductive success over the entire lifetime of the insect. Since juvenile hormone (JH) is involved in the control of reproduction in most insects, we examined JH titers after long flights using a chiral selective radioimmunoassay. JH levels increased in animals flown to exhaustion but not in animals that were flown for short durations (one-hour) or not flown. No differences were observed in the diel pattern of JH titers associated with flight performance. Treatment of grasshoppers with JH III mimicked the effect of long duration flight in the induction of early reproduction. To test the possibility that the post-flight increase in JH titer is caused by adipokinetic hormone (AKH), we injected unflown animals with physiological doses of *Locmi*AKH I to simulate the release of AKH during long flight. This treatment had no effect on JH titers. Grasshopper feeding and digestion was measured in response to flight experience and long-duration fliers displayed increased feeding and lipid digestion. The relationship between feeding and JH levels is being explored. (Supported by NSF grant 0235892 to MAR).

Regulation of juvenile hormone titers in *Coptotermes formosanus* termite by different soldier percentages

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In the Formosan subterranean termite *Coptotermes formosanus*, the soldier caste represents less than 10%, on average, of field collections. In the laboratory, the number of soldiers increased to about 25% or higher, and workers and soldiers appeared to increase JH III titers. In lab experiments with groups containing 25% or higher soldiers, few workers were transformed into pre-soldiers and subsequently to soldiers. The JH titer in these soldiers remained constant. Soldiers and workers from groups containing less than 25%-soldiers showed higher JH titers. Newly formed soldiers had higher JH titers than older soldiers. Groups containing 25% or higher soldiers inhibited further soldier differentiation by possibly suppressing the activity of the corpora allata, eventually showing

lower JH titers in workers.

Pharmacological analysis of ovarial patency in *Heliothis virescens*

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In insects, six homologues of juvenile hormone have been identified, including juvenile hormones (JH) 0, 1, 2, 3, bisepoxide-3, and isomethyl-1. The lepidopterans, particularly moths, are unique in being the only group of insects in which, except for JH-B-3, all of the other homologues have been demonstrated. Using reproduction in *Heliothis virescens* as a model system, we have initiated studies to explain the mechanisms for why moths exhibit multiple JH homologues. As part of these studies we are attempting to determine the JH signal transduction process in the patency of the follicular epithelium. We used pharmacological agents to analyze signaling pathways that JHs use to cause in vitro ovarial patency in *H. virescens*. Our results suggest that, similar to *Rhodnius prolixus*, NaK-ATP-ase is involved in response to JHs in *H. virescens* as well. However, in contrast to *R. prolixus*, at least two kinds of protein kinases appear to mediate the signal transduction process in *H. virescens*. Potential roles of G protein and internal calcium stores are being examined. We will present data on these experiments and discuss the situation in *H. virescens* compared with that in *R. prolixus* and other insects.

Inhibition of vitellogenesis by the *chico[1]* insulin-signaling pathway mutation of *Drosophila melanogaster* does not involve systemic juvenile hormones and ecdysteroids

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Chico[1] is a null mutation in the insulin-receptor substrate protein (CHICO) gene of *Drosophila melanogaster*. Females are sterile and their oocytes do not mature beyond the last previtellogenic stage. We show that the sterility of chico[1] females is not attributable to systemic factors, but instead to an autonomous ovarian phenotype. *Chico[1]* females exhibit approximately wild type rates of juvenile hormone (JH) biosynthesis, and relatively high ovarian and hemolymph ecdysteroid levels. Wild type ovaries transplanted into *chico[1]* females underwent vitellogenesis, indicating that systemic factors present in *chico[1]* mutant females are sufficient to support normal vitellogenesis. *Chico[1]* ovaries transplanted into wild type females did not undergo vitellogenesis indicating that CHICO is necessary in the ovary for vitellogenic maturation. We suggest that a failure of receptor-mediated endocytosis of yolk protein could contribute to the molecular basis of *chico[1]* female sterility. The global organizing hormones in insects, JHs and ecdysteroids, have long been thought to exert overall control on vitellogenesis. However, this work implies that irrespective of systemic regulatory roles of JHs and ecdysteroids, insulin signaling is necessary for vitellogenic oocyte maturation. Supported by NIH GM/OD54905 (DSR) and NIA AG08761, NIH 1R24GM65513-01(LGH)

Parasitism by *Glyptapanteles liparidis* (Hym., Braconidae) alters the juvenile hormone metabolism of its host larva, *Lymantria dispar*

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As shown earlier, parasitization by the larval parasitoid *G. liparidis* induces a dramatic increase in the hemolymph juvenile hormone (JH) titer of its host larva, especially in the JH III homologue, while the activity of the JH degrading enzyme (JHE) is reduced compared to unparasitized controls. Here we investigated the role of the parasitoids and associated factors in JH synthesis and degradation. GC-MS analysis confirmed that the rising hemolymph JH titers coincided with the parasitoids' molt into 2nd instars. Peak values were observed prior to parasitoid emergence from the host larva and titers dropped to negligible levels shortly afterwards. Whole body extracts from 2nd instar parasitoids yielded JH III and trace amounts of JH II. When the host's corpora allata were separated by neck ligation, levels of JH III were elevated in the hemolymph of the posterior section, which contained the parasitoids, but no JH II was found. When parasitoids were kept in *in vitro* culture, they produced and released only JH III. The parasitoids, on the other hand, had little influence on JH degradation. JHE suppression was induced solely by the polydnavirus/venom complex which is injected into the host larva at oviposition. Host JHE gene expression was not affected by parasitization.

Cloning and functional analysis of juvenile hormone acid methyltransferases from the silkworm, *Bombyx mori*, and the fruitfly, *Drosophila melanogaster*

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Juvenile hormone (JH) acid *O-methyltransferase* (JHAMT) is the enzyme that converts JH acids to JH methylesters at the final step of JH biosynthetic pathway. We have recently isolated, by differential display, a cDNA encoding JHAMT from the corpora allata (CA) of the silkworm *Bombyx mori* (*BmJHAMT*). The expression profile of *BmJHAMT* gene in the CA during the penultimate and the last larval stages suggests that this gene is a key regulator of JH biosynthesis at the onset of the metamorphosis in the silkworm. We have further identified a homolog in the genome sequence of *Drosophila melanogaster* (CG17330; *DmJHAMT*). Although the identity of the amino acid sequence between *DmJHAMT* and *BmJHAMT* is quite low (~35%), the recombinant *DmJHAMT* protein produced in *E. coli* indeed converted JH acids to their cognate methylesters. In addition, immunohistochemistry with an antiserum raised against the recombinant protein revealed the localization of *DmJHAMT* protein in the CA cells of the larval ring gland. Thus *DmJHAMT* gene encodes a genuine *JHAMT* working in the CA cells. Functional analysis of *DmJHAMT* gene in vivo is now in progress using the ectopic expression and the inducible RNAi.

Juvenile hormone-binding protein at follicle cells in *Bombyx mori*

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A novel JH binding protein with possibile relationship to hormone signal transduction was identified from silkworm follicle cells. The follicle cells at a stage of pre-choriogenesis were incubated with JHs and related compounds, and showed concentration-dependent patency in contrast with no effect with 20-hydroxyecdysone. To characterize this biological activity, the membrane fraction of the silkworm follicle cell membrane was prepared and examined for binding activity with JH and analogous compounds. Two JH binding assays were employed, dextran-coated charcoal (DCC) assay with tritiated JH III, and a competitive assay with a biotinylated affinity ligand (phenoxyphenoxypropyl biotine) partial structure of JH analogs, fenoxycarb and pyriproxyfen. The highest activity of the binding activity was found at late pupal stage (day 6-8). Ligand binding was inhibited competitively with excess of non-labeled JH. The dissociation constants, KD values were 2.56, 1.77, and 4.32 for JHI, II, and III, respectively with the DCC assay. The binding activity was lost after heating at 100° C for 10 min, or by protease treatment of the follicle cell preparation.

Putative allatostatin peptides and their receptors in *Caenorhabditis elegans*: Analysis of function through RNA inhibition

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Allatostatins are a group of peptides initially identified in *Diploptera punctata*, which function to inhibit juvenile hormone synthesis and gut motility. Since their discovery, allatostatin homologues have been identified in various other arthropods as well as other invertebrates including Hydrozoa, Nematoda, Mollusca, and Platyhelminthes. Allatostatins function in these latter invertebrates varies but can involve inhibition of muscle contraction, inhibition of interneural and endocrine functions, as well as direct action on biosynthetic pathways. There are, however, many organisms for which allatostatin function has not been assigned. This includes the nematode *Caenorhabditis elegans*. The *C. elegans* genome project has identified a gene termed nlp-6 whose precursor polypeptide contains peptide sequences that are similar to allatostatin peptides. Our current research is aimed at determining the function of nlp-6 and its potential receptor ZK455.3 using RNA inhibition and GFP analysis. Preliminary results from RNAi screens suggest increased egg laying rates in RNA inhibited nlp-6 and ZK455.3 worms. Localization of nlp-6 has previously been shown in somatic gonad tissue. The mechanism of action of these peptides is unclear but it is interesting that these peptides have a role in reproduction as has been found in insect model systems.

Enzymology of moth farnesyl diphosphate synthase: Implications for the biosynthesis of homologous juvenile hormones

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Juvenile hormone biosynthesis is derived from the mevalonate pathway and requires the intermediacy of farnesyl diphosphate (FPP). FPP synthase (FPPS), the presumed enzyme responsible for sesquiterpenoid construction in insects, is a metal-dependent homodimeric protein, that catalyzes the sequential 1-4' condensation of two molecules of isopentenyl diphosphate (IPP) with the allylic primer, dimethylallyl diphosphate (DMAPP). Additional complexity for FPPS catalysis is expected to exist in moths, which are known to produce homologous JH structures. We have studied FPPS activity located within the corpus allatum of larval and adult *Manduca sexta*. The two developmental stages show several differences, including enzyme localization, detergent activation, metal preference, substrate specificity, and kinetic behaviour. These properties are distinct from those observed using purified FPPS obtained from whole body *M. sexta* and porcine FPPS, suggesting that the isoprenyltransferase within the corpus allatum has unique enzymological properties.

Regulation of Reproductive Development in the boll weevil, Anthonomus grandis

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Taub-Montemayor, et al. (1997) showed a positive correlation between juvenile hormone (JH) synthesis and ovarian development in the boll weevil, *Anthonomus grandis*. The production of vitellogenin (Vg) is typically restricted to reproductively competent females and no Vg was detected in untreated male boll weevils (Taub-Montemayor & Rankin, 1997). Methoprene stimulates Vg production in the isolated abdomens of both female and male boll weevils (Taub-Montemayor and Rankin, 1997), suggesting that males are competent to produce Vg but are normally not stimulated to do so. Preliminary work indicated that male boll weevil CA produce little or no JH in vitro, suggesting that males may not produce Vg because of very low JH levels. Reports of JH effects in male *A. grandis* induced us to more carefully monitor JH synthesis, degradation, and circulating titres in male *A. grandis* during early adult development. We also examined JH titres in females and re-examined the ability of males to produce Vg with and without hormonal stimulation. Results indicate that male JH titers and JH production are similar to females. The sexually dimorphic effect of JH on Vg production is thus not due to differences in JH production or titers between the sexes.

Regulation of methyl farnesoate production in crayfish: A possible role for allatostatins

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Decapod crustaceans do not appear to produce juvenile hormone (JH III), but rather its immediate precursor, methyl farnesoate (MF). Both MF and its immediate precursor, farnesoic acid (FA) are produced by the mandibular organs (MOs) in crustaceans. The MOs are homologous to the insect corpora allata (CA), the site of juvenile hormone biosynthesis. However, the FGLa ASTs, of which there are about 60 distinct peptides reported from crustaceans, have previously been found to have no effect on MO activity in crustaceans. In light of our recent report on MF production in embryonic CA of the cockroach, *Diploptera punctata* and its modulation by ASTs, we have reexamined the ability of selected FGLa ASTs to modulate MF production in the crayfish, *Procambarus clarkii*. Using MO from adult males of, we have found wide variability between animals in the *in vitro* rates of MF and FA synthesis. Treatment with Dippu-ASTs has a statistically significant stimulatory effect on MF synthesis, but only in MO that are initially producing MF at lower rates. No effect on FA production was observed, suggesting that the FGLa ASTs exert their effect on the o-methyl transferase, the enzyme responsible for the conversion of FA to MF.

Insulin signal regulation of juvenile hormone synthesis in *Drosophila melanogaster*

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Juvenile hormone is a key endocrine regulator of metamorphosis, reproduction, and aging in Drosophila melanogaster. Mutants of the insulin receptor (*InR*) and the insulin receptor substrate homolog (*chico*) are slow to develop, small, infertile, and long-lived. Here we describe the 10-day adult age course of JH synthesis from isolated corpora allata of *InR* and of *chico*. JH synthesis increased in wildtype flies to a maximum of 30 fmol/gland/h at day 10. In contrast, homozygous *InR* mutants produced only 7 fmol/gland/h at 10 days. *InR* mutation disproportionately reduced the synthesis of JH III-bisepoxide, the major JH subtype of the fly. Both homozygous and heterozygous *chico* genotypes reduced JH synthesis, but only to 47% and 67% of wildtype synthesis, respectively, and without influencing the ratio of JH subtypes. Because JH synthetic rate does not correlate with the size of CA among *chico* genotypes, insulin signaling appears not to influence JH by impeding tissue development. Rather, allatotropic positive axons are abundant in the adult brain but less immunoreactive in the *InR* mutant genotype, suggesting that insulin signaling may affect JH synthesis through control of neuropeptides.

Influence of larval haemolymph, JH acids and long term incubation with allatotropin analogue on juvenile hormone biosynthesis in *Manduca sexta* : *In vitro* studies

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0 day Vth instar CA were incubated with the medium for 3hrs containing IIIrd and IVth instar larval haemolymph, no stimulatory effect of JH biosynthesis was observed. However exogenous addition of pharate Vth haemolymph with medium-199 and pharate Vth haemolymph alone increases the JH production by four times than the control. The addition of JH acids with the pharate haemolymph increased the JH production to 14.97 pmol/h/prCA, which is about 15 fold more than the control. The degree of activation of corpora allata with allatotropin analogue was about 4 times for 3-6 hours interval and about 6 times in both 6-9 and 9-12 hrs under the same incubation condition. The preliminary experiments

may suggest the possible existence of a CA stimulatory factor in the pharate Vth instar haemolymph of M. sexta. Dr.B.G.Unni is thankful to Director for permission to present this research work at the conference and also to Professors G. Bhaskaran, K.H. Dahm and Tim Hayes for their collaboration and constant guidance to carry out this piece of research work at the Biology Department, Texas A&M University, USA and United States Educational Foundation in India for Fulbright Fellowship travel Grant.

Regulation of methyl farnesoate synthesis in mandibular organs of the crustacean, *Cancer pagurus* by mandibular organ – inhibiting hormones

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In Crustacea, there is evidence that methyl farnesoate (MF) acts as a juvenile hormone. MF is synthesized in the mandibular organs (Mos) under the negative control of the neuropeptides, mandibular organ-inhibiting hormones (MO-His), which are produced and secreted from the X organ-sinus gland compex of the eyestalk. The latter 78 amino acid residue peptides, which were purified from *C. pagurus*, are members of the crustacean hyperglycemic hormone (CHH) family and their expression is confined to the X-organ. Phylogenetic analysis and gene organization show that MO-IH and MIH (moult-inhibiting hormone) genes are closely related and represent evolutionary divergence of crustacean hormones. We have shown that MO-IH inhibits farnesoic acid (FA) O-methyl transferase (FAMTase), the enzyme that catalyzes the final step of MF biosynthesis. Levels of putative FAMTase transcripts in Mos of female *C. pagurus* fluctuated during vitellogenesis and embryonic development. Evidence strongly supports a role for cAMP in the signal transduction mechanism of MO-IH that leads to inhibition of MF synthesis in Mos.

Type-B [W(X_6)Wamide] allatostatins from the cricket, Gryllus bimaculatus: molecular cloning, expression and tissue-specific localisation

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Five W(X_6)Wamides which inhibited JH biosynthesis *in vitro* of the corpora allata were isolated from cricket brains in a previous work. In addition to their allatostatic function, two of the peptides effectively inhibited ecdysteroid biosynthesis in the ovaries from adult females. Here we report on the partial identification of the cricket allatostatin type-B gene, and experiments were performed to demonstrate spatial and temporal expression patterns of the gene in various tissues of adult crickets. By PCR screening of a random primed adaptor ligated cDNA library and by RACE, a 535 bp 3'cDNA fragment was yielded which encodes a putative translation product of 85 amino acids containing six peptides of the allatostatin B-type. By Southern blot analyses it was proven that the gene is expressed as a single copy per haploid genome. Using RT *in situ* PCR with 10µm tissue sections and RNA dot blot analyses, it was demonstrated that the gene is expressed, for example, in brain, ovary and digestive tract of adult crickets. In the germarium and in primary oocytes, gene expression was detected as condensed signals that changed into separated granules and finally disappeared during oogenesis, whereas in the follicular cells strong signals became apparent. Supported by the DFG (Ho 631/15-4).

Rapid quantification of juvenile hormones (JH) and their metabolites in the haemolymph of insects by liquid chromatography-mass spectrometry (LC-MS)

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Various methods have been developed to determine JH titres in insects by immunological or physicochemical techniques. Physicochemical methods such as gas chromatography-mass spectrometry (GC-MS) have been considered to be most accurate, but extensive sample preparation has been a major disadvantage when using GC-MS for routine analyses, followed by the inability to monitor JH degradation products. Here we present a simple, fast and sensi-tive method for routine determination of JHs, JH-diols, and JH-acids in insect haemolymph by LCMS. Sample clean-up involves the precipitation of proteins by methanol-isooctane (1:1, v/v), centrifugation, and partial evaporation of the solvents. The JH compounds were separated on a ReproSil-Pur ODS-3 C18 column by gradient elution with water and methanol in less than 22 min and analysed by electrospray MS (ESI-MS). The limit of detection and quantification was 6 and 20 pg for JHs, and 8 and 25 pg for JH-diols, respectively. To de-monstrate the applicability of the method, haemolymph samples from crickets, moths, aphids and ants were analysed. Our results reveal that in the haemolymph of cricket adult females, besides JH III significant amounts of JH I are present. Supported by the DFG (GK 678/1).

Crystal structure of juvenile hormone esterase from *Manduca sexta*, with the inhibitor OTFP covalently bound in the JHE active site

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Juvenile hormone esterase (JHE) is a selective and highly efficient enzyme that hydrolyzes the methyl ester of juvenile hormone. JHE belongs to the a/?–hydrolase family of enzymes, which is a large family of enzymes with diverse substrates and a conserved catalytic mechanism. This mechanism involves a nucleophilic attack on the substrate, leading to release of the methyl alcohol and formation of an acyl-enzyme intermediate via a tetrahedral transition state intermediate. This acyl-enzyme intermediate is then hydrolyzed, leading to release of the JH acid and regeneration of enzyme. We present a 2.8 Å crystal structure of JHE from the lepidopteran *Manduca sexta*. Crystals were produced with the covalent inhibitor 3-octylthio-1,1,1-trifluoro-2propanone (OTFP) present, and the inhibitor is clearly present in this crystal structure. The inhibitor mimics the tetrahedral acyl-enzyme intermediate, and positioning of OTFP indicates how the substrate likely binds. This provides insight into the specificity of the enzyme and the potency of various inhibitors. Mutations have been introduced into this protein to help us further understand the catalytic pathway of this highly efficient enzyme.

Allatostatin in the termite *Reticulitermes flavipes*: Content in brain and corpus allatum and effect on juvenile hormone synthesis

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In the subterranean termite *Reticulitermes flavipes*, allatostatins (ASTs) with the C-terminus PheGly-Leu amide, were localized by immunocytochemistry with antibody against cockroach AST. AST-immunoreactivity occurred in the corpus cardiacum and corpus allatum and in the lateral and medial neurosecretory cells of the brain that innervate these organs as well as in many other nerve cells of the brain. This was observed in workers, nymphs, soldiers and secondary reproductives. A radioimmunoassay demonstrated about 40 fmole equivalents of Dippu AST-11 in brains of soldiers and secondary reproductives. The product of the corpora allata in this species was determined to be juvenile hormone III. Its synthesis by corpora allata of secondary reproductives, determined by in vitro radiochemical assay, was inhibited in a dose dependant manner by two cockroach allatostatins, Dippu AST-7 and Dippu AST-11. Thus, as in cockroaches and crickets, AST-containing nerves innervate the corpora allata of this termite species and their production of juvenile hormone is inhibited by these neuropeptides.

The hemolymph JH titer exhibits a large-amplitude, morph-dependent, diurnal cycle in *Gryllus* species in the laboratory and field

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We observed a striking, morph-dependent daily cycle in the hemolymph JH titer in the cricket Gryllus firmus in both the laboratory and the field. In the flight-capable (long-winged, LW) morph, the JH titer rose about 10-100-fold a few hours before lights-off (lab) or sunset (field) and dropped precipitously just after lights-off or sunset. By contrast, the JH titer was temporally invariant in the flightless (short-winged, SW) morph. The JH titer cycle was lost in LW individuals that became flightless due to flight-muscle histolysis. No morph-dependent cycle was observed for the hemolymph ecdysteroid titer. A similar morph-dependent diel cycle for the JH titer was observed in four other cricket species. This represents the most dramatic case of diel variation in the JH titer in an insect, the only case where the titer cycle is morph-dependent, and the only characterization of the JH titer under field conditions. The morph-dependent JH titer cycle has important implications for the endocrine basis of morph specialization, especially the regulation of morph-specific nocturnal flight, and JH endocrinology in general.

A morph-dependent daily cycle of JH biosynthesis underlies a morph-dependent daily cycle of the hemolymph JH titer in a wing-polymorphic cricket

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A dramatic morph-specific daily cycle in rate of JH biosynthesis that covaried tightly with a daily cycle in the hemolymph JH titer was observed in the wing-polymorphic cricket, *Gryllus firmus*. In the flight-capable (LW, long-winged) morph, rate of JH biosynthesis rose 1-3 fold and the JH titer rose 10-100 fold near the end of the photophase, while the rate of JH biosynthesis fell an equivalent amount, as did the JH titer, during the beginning of the scotophase. By contrast, both rate of JH biosynthesis and hemolymph JH titer were relatively invariant during this period in the flightless, SW morph. An equivalent biosynthetic cycle was observed when assays were performed with corpora allata alone, with corpora allata and attached corpora cardiaca, and in males and females. The LW morph also exhibited significantly elevated juvenile hormone esterase activity,

mainly during the period when the JH titer was dropping. To our knowledge, this is the first example, of a diurnal cycle in JH biosynthesis, and one that is strongly associated with a particular morph in a wing-, caste-, or phase-polymorphic insect.

CG14709 putatively mediates the juvenile hormone effect on preventing bristle outgrowth in the developing adult *Drosophila* abdomen

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Juvenile hormones (JH) are not able to prevent the larval-pupal transition in *Drosophila* and other higher flies, unlike that seen in the Coleoptera and Lepidoptera. Yet exogenous JH prevents the outgrowth of adult bristles and causes the formation of second pupal cuticle on the abdomen. We have shown that the formation of the second pupal cuticle is mediated by the transcription factor *broad*. However, loss-of-function analyses suggest that *broad* is involved in bristle specification but not outgrowth. We recently found three P element insertion mutants that are able to form normal bristles even when given a high dose of JH. Interestingly, *broad* is still up-regulated by exogenous JH in these mutants, indicating that JH effects on the developing adult abdomen are likely mediated by two distinct pathways. All these P element insertions have been mapped cytologically to the 86E14 region which hosts two genes: CG14709 and CG31305. Our preliminary data show that the expression pattern of CG31305 is not altered by these P element insertions. We are now determining if the expression of CG14709 is changed in these insertion mutants. The finding of these JH resistant mutants provides new opportunities of revealing the mechanism of JH action.

Isolation and characterization of a novel chymotrypsin-like protease gene that is activated by JH III in mosquito *Aedes aegypti*

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In order to isolate mosquito genes that are specifically up-regulated by JH III in newly-emerged female mosquitoes, we exploit the suppression subtractive hybridization (SSH) technique. Here we report the cloning and characterization of *JA15*, a chymotrypsin-like protease gene. *JA15* mRNA exhibits over a 10-fold increase in response to 10^{-6} M JH III. This hormonal response is blocked by the protein synthesis inhibitor, cycloheximide (CHX), suggesting that novel protein synthesis was obligatory for this hormonal effect. Expression of *JA15 in vivo* is limited almost exclusively to the midgut. In adult females, the *JA15* transcript is scarce at eclosion, and starts to increase approximately 12 h post eclosion (PE). It reaches a plateau by 36 h PE, and remains at a nearly constant level until a blood meal is taken. *JA15*mRNA decreases shortly after feeding, and remains at relatively low levels until 48 h post blood meal (PBM), then begins to increase again. By 72 h PBM, expression of *JA15* is comparable to that before blood meal. The expression profile of *JA15* is closely correlated with the change in JH titers during vitellogenic cycles, suggesting that the transcription of *JA15* in previtellogenic midgut is under the control of JH III.