Beetle Functional Genomics

Christopher I. Keeling
ckeeling@interchange.ubc.ca

University of British Columbia
Beetle research foci

Dezene Huber, UNBC

Chris Keeling

Cold tolerance

Detoxification

Olfaction

Pheromone biosynthesis
Cytochrome P450s

Usually involved in NADPH-mediated oxidation of substrate

Metabolic functions include:
- Detoxification of plant secondary metabolites
- Hormone biosynthesis
- Pheromone biosynthesis
- Pheromone and hormone degradation
- Odorant degradation
- Omega oxidation of fatty acids
**Apis mellifera**

46 (2) P450s

**Anopheles gambiae**

~105 (7) P450s

**Drosophila melanogaster**

~87 (4) P450s

**Tribolium castaneum**

~134 (10) P450s

(Tribolium Genome Sequencing Consortium. 2008 The genome of the model beetle and pest Tribolium castaneum. Nature 452, 949-955, Table S16)
MPB P450s

From ESTs: ~131 Contigs with annotations to P450s

e.g., CYP4, CYP6, CYP9, CYP28, CYP49, and CYP314

In the process of:
Identifying unique P450s
Identify a full-length cDNA clone for each unique P450, if any
Fully sequence these FL clones
55 Full-length MPB (*) and 134 (10) *Tribolium castaneum* P450s

CYP346
CYP6
CYP345
CYP9
CYP4
CYP3XX
CYP6
P450 Redox Partners

NADPH cytochrome P450 reductase (CPR)

FLcDNA identified in antennae cDNA library
Subcloned and expressed in E. coli and insect cells
Purified from E. coli expression and functionally characterized
Activity confirmed with Ips confusus CYP9T1 expressed in insect cells (clone courtesy of Claus Tittiger)

Cytochrome b5

Can improve stability and activity of some P450s
FLcDNA identified in antennae cDNA library
Subcloned and expressed in insect cells
Will be co-expressed with CPR and P450s in insect cells
MPB Pheromone Biosynthesis

Acetyl CoA → SCoA

SCoA → Mevalonate → Geranyl diphosphate → 6-Methylhept-6-en-2-one → (-)-Frontalin

SCoA → Mevalonate → (Z)-6-Nonen-2-one → (+)-exo-Brevicomin → (-)-trans-Verbenol

SCoA → Mevalonate → Myrcene → (-)-Verbenone → (-)-α-Pinene → (+)-cis-Verbenol

SCoA → Mevalonate → 2-Phenylethanol

SCoA → Mevalonate → 1-Octen-3-ol
Pheromone biosynthesis de novo via the mevalonate pathway

*In situ* hybridization with *HMG-R* probe

**JH-Treated**

**Control**

*Dendroctonus* jeffreyi

Hall, GM et al. (2002) *Naturwissenschaften* 89:79-83


*Ips pini*
**EST sequencing**

**Gene expression**

**Functional characterization of pheromone biosynthetic genes**
Mevalonate pathway in MPB
Prenyltransferases

Cf., *Ips pini* geranyl diphosphate synthase

We have identified two MPB FLcDNA clones, but expression in *E. coli* and preliminary functional characterization suggest they are geranylgeranyl diphosphate synthases.
Six P450s selectively abundant in midgut/fat body cDNA libraries are currently being expressed and functionally characterized.
Discovery

Proteomics

**LC-MS/MS**: midgut/fat body from 16 h JH/acetone treated females identified 125+ MPB proteins

**iTRAQ** (isobaric tag for relative and absolute quantitation): midgut/fat body from 16 h JH and acetone treated males and females, in progress

Transcriptomics

Custom Roche Nimblegen 12x135k microarrays:

midgut/fat body from 8 h JH and acetone treated males and females
Pheromone Production

Using headspace-sampling GCMS to study timing of pheromone production of JH-treated adults
## Olfaction

<table>
<thead>
<tr>
<th>Category</th>
<th>ESTs/TUGs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odorant/Pheromone Binding Proteins &amp;</td>
<td>665 ESTs/39 TUGs</td>
</tr>
<tr>
<td>Chemosensory Proteins</td>
<td></td>
</tr>
<tr>
<td>Odorant/Pheromone receptors:</td>
<td>73 ESTs/15 TUGs</td>
</tr>
<tr>
<td>Sensory Neuron Membrane Proteins:</td>
<td>22 ESTs/2 TUGs</td>
</tr>
<tr>
<td>Odorant degrading enzymes:</td>
<td>1,000+ ESTs/many TUGs</td>
</tr>
</tbody>
</table>
Functional characterization of an antennal P450

The most abundant EST in antenna cDNA library

A CYP6, some similarity to other insect antenna-specific P450s

Has been expressed in insect cells

Functional characterization with pheromone components and host volatiles has not yet shown activity
Chemosensory Protein (CSP)
Odorant Binding Protein (OBP)
Pheromone Binding Protein (PBP)
Odorant Receptor (OR)
Sensory Neuron
Membrane Protein (SNMP)
Discovery

Custom Roche Nimblegen 12x135k microarrays

Male versus female antennae (WTA)
Pheromone Biosynthesis

Proteomics: iTRAQ of midgut and fat body tissues from 16 h JH and acetone treated males and females

Transcriptomics: microarrays of midgut and fat body tissues from time course of JH and acetone treated males and females

Targeted qRT-PCR based upon proteomic and transcriptomic results

Continue functional characterization of P450s

Functional characterization of other proteins in the pheromone biosynthetic pathways
Olfaction

Elaboration and annotation of olfactory gene families
Continue functional characterization of antennal P450
Functional characterization of odorant/pheromone-binding and chemosensory proteins
Targeted qRT-PCR based upon transcriptomic results
Hannah Henderson
P450 Funct. char.
CPR Funct. char.
P450 gene family

Maria Li
P450 Funct. char.
Prenyltransferases

Harpreet Dullat
P450 Funct. char.
Proteomics
Transcriptomics

Mack Yuen
Bioinformatics