SECOND ANNUAL
INTERNATIONAL SCIENTIFIC WORKSHOP

Mountain pine beetle: secondary metabolite detoxification and cold tolerance research

Dezene P. W. Huber and Christopher I. Keeling
Adult emergence
Dispersal & host selection
Aggregation
Host colonization & reproduction
Early larval development

Late larval, pupal, & early adult development

End of overwintering
Spring

Winter

Autumn

Beginning of overwintering

Dendroctonus ponderosae
The main infestation has begun to spread over the Rocky Mountains in northeastern British Columbia and into northwestern Alberta.

Kurz, W.A. et al. (2008)  
*Nature* 452:987
Anopheles gambiae
~105 (7) P450s

Dendroctonus ponderosae
~60 P450s

Drosophila melanogaster
~87 (4) P450s

Apis mellifera
46 (2) 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)
Upregulation of potential homologs upon feeding + presence in certain cDNA libraries

Ips paraconfusus

- male 8h
- male 24h
- female 8h
- female 24h
Heterologous expression of P450s in cultured insect cells

1. PCR amplify gene of interest (GOI) using primers with restriction sites
2. Restriction digest GOI and vector
3. Ligate vector and GOI
4. GOI is inserted into viral DNA via recombination sites
5. Recombinant Baculovirus containing GOI
L: Ladder

Lanes 1,2: N19 (similar to Cyp4BD1)

Lanes 3,4: Cell control

Lanes 5,6: Viral stock

Lanes 7-10: supernatant of microsome prep

Co-expression of P450s and MPB NADPH-cytochrome P450 reductase
Confirmation of the presence of P450 expression

![Graph showing absorption (abs.) at different wavelengths (nm) with peaks at P420 and P450.]
Optimization of multiplicity of infection (MOI)
Co-expression of P450s and MPB NADPH-cytochrome P450 reductase

Each CPR:P450 pair is unique...

...MOI must be optimized in each instance.
Detoxification:

- Insect cell culture system is established and running smoothly.

- We are now able to co-express MPB cytochromes P450 and MPB NADPH-cytochrome P450 reductase.

- Co-expression requires optimization to maximize the amount of P450 in relation to CPR.

- Currently we are expressing three MPB P450s, chosen based upon expression of homologs and upon their presence/absence in certain MPB cDNA libraries.

- Substrate assays are beginning.
**Upcoming detoxification work**

- Large-scale expression analysis of insects following feeding and/or fumigation with synthetic secondary metabolites.

- Proteomics analyses of fed and/or fumigated insects.

- Continuation and expansion of cytochrome P450 functional characterization.

- Targeted qRT-PCR experiments, based upon previous results.

- Other detoxification gene candidates?
The main infestation has begun to spread over the Rocky Mountains in northeastern British Columbia and into northwestern Alberta.

Fall 2008 Daily Maximum, Minimum & Mean Temperatures (10 Sampling Weeks)
Cold tolerance collections:

- >3500 individuals flash frozen at the collection site.
- Represent early-instar to almost-pupal stages.
- 25 total collection dates (10 autumn, 15 spring).
- About 120 individuals/week.
- Broad range of temperature conditions.
- Fine-grained temperature monitoring at two levels on the tree trunks.
EST data were derived from numerous sources

- Midgut/fat body tissue from JHIII-treated adults
- Antennae
- Untreated adults
- JHIII-treated adults
- Overwintering larvae
- Terpenoid fumigation of adults
- Pupal stage
- Early developmental stage (larvae)
- Midgut/fat body tissue from JHIII-treated adults
qRT-PCR primers and probes developed and optimized for 15 genes involved the glycerol biosynthetic pathway.
qRT-PCR primers and probes developed and optimized for two lipolysis genes.
qRT-PCR primers and probes developed and optimized for a putative antifreeze protein gene.
Tenebrio molitor antifreeze protein gene identification and regulation

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Expression of biologically active recombinant antifreeze protein His-MpAFP149 from the desert beetle (Microdera punctipennis dzungarica) in Escherichia coli

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Microdera dzhungarica punctipennis AFP and a putative MPB AFP

Note: TCTxSxxCxxAx or related ACT(X)_9 repeat motifs
Microdera dzhungarica punctipennis AFP and a putative MPB AFP

Note: TCTxSxxCxxAx or related ACT(X)_9 repeat motifs

Tenebrionidae... same infraorder as Curculionidae
Upcoming cold tolerance work

• Large-scale expression analysis and proteomics analysis using collected, stored samples.

• Exploration of the MPB genome for other AFP candidates.

• Potential exploration of promoter region of AFP candidate(s).

• Targeted qRT-PCR experiments, based upon previous results.
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(by Ron Long)
Detoxification

Cold tolerance

MPB
Dendroctonus ponderosae

Environment
Genes
Interacting Genomes

Fungal Associates
e.g. Ophiostoma spp.

Gene by Environment Interactions

Pine Hosts
P. contorta, P. banksiana, and their hybrids
Cytochromes P450s

• Found in organisms from bacteria to plants to mammals to arthropods.

• Usually involved in NADPH-mediated oxidative attack on various substrates.

• Organisms usually possess a large number of P450 genes and pseudogenes.

• Metabolic functions are varied, and include:
  - Biosynthesis of toxins (plants)
  - Detoxification of plant secondary metabolites
  - Hormone biosynthesis
  - Pheromone biosynthesis
  - Pheromone and hormone degradation
  - Omega oxidation of fatty acids
Microdera dzhungarica punctipennis AFP and a putative MPB AFP

MPB

\[\text{MCTFNKNWLVIAIIVTCLCTEYNCOCTGGADCTSCTEACTGCCNPCNAHTCTNSKNCVKA}\]

MPB

\[\text{YTCTGSENVCNLALTCTGSTNCNKAMTCTNSKDCFETATTCTGSTNCYKATACTNSTGCPGS}\]

**Note:** \(\text{TCTxSxxCxxAx}\) or related \(\text{ACT(X)\_x\_x\_x}\) repeat motifs