ALL THE BETTER TO EAT YOU WITH:
Identifying and characterizing digestive cysteine peptidases in Tribolium castaneum

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T. castaneum as a pest
- Red flour beetle
- Feeds on stored products – wheat, cereals, flour
- Economic loss
- Phosphine fumigation is common
- Many populations are now resistant

Cysteine Peptidases in T. castaneum

- T. castaneum genome has 25 cysteine peptidases
- On chromosomes 3, 7, 8, 10
- Many types, including cathepsin L, B, O, and K

Main objectives
1. Distinguish digestive cysteine peptidase from non-digestive
   - Determine relative expression between feeding (adults and larvae) and non-feeding (egg and pupae) developmental stages
2. Digestive cysteine peptidase function
   - Knock down major gut peptidase
   - Monitor response of all digestive cysteine peptidases

Experimental design
RNA-seq

Results
Heat map

Extract RNA
polyA pull down (method)
cDNA
Ion Torrent PGM chemistry

Twelve cysteine peptidases are up-regulated in feeding stages. Of these, ten have multiple lines of evidence suggesting involvement in digestion. Seven have major role in digestion when on a normal diet. Previous biochemical study found seven enzymes in midgut, hypothesized for digestion (Vinokurov et al. 2009). Seven were up-regulated in responses to inhibitors. Two with low/no expression were further decreased in response to inhibitors. Candidates for pest control are constitutively expressed. Major digestive peptidases are candidates for pest control.

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Cysteine Peptidases in *T. castaneum*

- Microarray - inhibitors

*Tribolium castaneum* compensates for loss of cysteine peptidase activity when fed inhibitors through differential regulation of cysteine peptidase genes.

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Method for knockdown

RNA interference

**Katherine Aronstein, Brenda Oppert and Marce ́D. Lorenzen (2011).** RNAi in Agriculturally Important Arthropods, RNA Processing, Prof. Paula Grabowski (Ed.)

Experimental design

- Target different sections of LOC659441: 3′, middle, 5′, entire gene region
- Controls: non-injected, mock-injected, ADC

**RNA-seq:** Not dependent on gene specific primers & can get global analysis of differentially expressed genes
Results

Off-target knockdown

Results

Over-represented GO terms

Molecular Function:
Serine-type endopeptidase activity
p=4.5e-6

Molecular Function:
Cysteine peptidase activity
p=4.5e-6

Biological Process:
Proteolysis p=1.41e-10

Up-regulation of serine peptidases in the posterior midgut

Summary

Anterior midgut
pH 5.0 - 6.0
85% cysteine activity, 15% serine peptidases

Midgut
pH 6.0 - 7.0
20% prolyl oligopeptidase activity

Posterior midgut
pH 7.0 - 7.5
75% cysteine, 25% serine peptidases

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