ANALYSIS OF EST’S ENCODING PEA APHID ACYRTHOSIPHON PISUM C002 & THE EFFECT OF ARMET TRANSCRIPT KNOCKDOWN IN TRIBOLIUM CASTANEUM

by

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Approved by:
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Abstract

Aphids mount a remarkable salivary secretion to overcome plant host defenses. Our group has previously reported a gene unique to aphids enriched in the salivary glands of the pea aphid *A. pisum*, C002, which is required for successful feeding on its host plant *Vicia fava*. Here I present an analysis of genetic variation within the available EST data for C002 in pea aphids. From 596 total ESTs, 332 are full-length, and segregate into 8 validated haplotypes based on the criteria I set in place to access the quality of EST data. Additionally, Armet, is a putative multi-functional gene implicated as a neurotrophic factor during development, and as a part of the unfolded protein response during stress. I employ RNA interference in the model organism *T. castaneum* to determine the effect of transcript knockdown during development from early instar larval stages, through pupation, and its effect on adult emergence. I report that knockdown of Armet transcript significantly hinders the ability for beetles to emerge from the pupae.
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Chapter 1 - An Analysis of Pea Aphid *Acythosiphon pisum* C002 ESTs

**Introduction**

*Aphid Saliva & Effector Molecules*

Aphids, an Aphididae family of phytophagous hemipterans, are piercing-sucking organisms that attain their nutrients by accessing the epidermis and sieve element of host plants while concurrently forming a rigid salivary stylet sheath. Finally, the insect is able to ingest the nutrient rich plant phloem sap (Miles 1998). The defense response of the host plant is characterized by a calcium dependent activation of coagulating factors, and a group of unknown responses. The coagulating factors released, in the case of calcium dependence, lead to the occlusion of wounds sustained by the plant (Vos et al. 2009). Microarray analysis of *Arabidopsis thaliana* plants un-infested and infested with green peach aphids *Myzus persicae* demonstrated that plants fed on by aphids indeed responded to aphid feeding (Vos et al. 2009). Also, *A. thaliana* plants over-expressing coagulation factor AtPP2-A1 are refractory to feeding by green peach aphid (Zhang et al. 2011). Additionally, electrical penetration graphs (EPGs) and interaction of vetch aphid *Megoura viciae* saliva with plant forisomes *in vitro* demonstrated that aphid saliva is required to overcome this biochemical barrier (Tjallingii 2005; Will et al. 2007). Within the literature these plant defense suppressing molecules have been deemed avirulence factors. Since, the individual components of arthropod saliva that overcome host defenses have
collectively termed as effector molecules (Hogenhout et al. 2009). Small secreted salivary gland proteins (SSSGPs) is another term in the literature used to describe small salivary secreted protein molecules (Chen et al. 2010).

In the case of phytophagous insects salivary secreted protein molecules are thought to mimic plant defense signaling molecules, thereby modulating the ability of the host plant to mount a response (Bos et al. 2010). Genomic and proteomic techniques have been employed to find candidate effector molecules for further characterization. EST libraries derived from the salivary glands of pea aphid *A. pisum* and green peach aphids were probed to find a multitude of putatively secreted salivary enriched proteins (Bos et al. 2010; Carolan et al. 2011). An analysis of pea aphid salivary glands using both a transcriptomic and proteomic approach found more than 300 genes encoding secretion signals. Interestingly nearly half of these genes have no sequence similarity to anything outside of aphids, and some have been directly observed in aphid saliva (Carolan et al. 2011). Collecting saliva in an artificial diet, concentrating, and analyzing with mass spectrometry yielded an active glucose oxidase (GOX) protein in green peach aphid (Harmel et al. 2008). The same group conducted in pea aphids observed a M1 and M2 metalloprotease, GMC oxidoreductase, and regucalcin; genes that do not necessarily fit into the category of SSSGPs (Carolan et al. 2009). The study found an additional 5 proteins that showed no sequence similarity to proteins in databases (Carolan et al. 2009).

To date, only one potential effector molecule, C002, has RNAi evidence linking it to being required for an aphid to feed on its host plant. Injections of dsRNA encoding the salivary enriched transcript C002 abrogated pea aphids’ ability to feed on its host plant but not on an artificial diet. Additionally, EPGs demonstrated that injected aphids were unable to establish and maintain feeding host plants (Mutti et al. 2006; Mutti et al. 2008). Recently, transiently
expressed dsRNA molecules designed to interfere with *M. persicae* C002 were transiently expressed within the leaves of *A. thaliana* and *Nicotiana benthamiana*, causing a small decrease in individual aphid nymph production (Pitino et al. 2010).

An emerging theme in the field of effector molecules and SSSGPs is one of high coding region variability that is under intense positive selection (Reviewed in Hogenhout et al 2009). In the gall midge there is a peculiar conservation of non-coding regions in SSSGPs, while coding regions contain high variability with a marked positive selection for non-synonymous changes over synonymous (Chen et al 2010). Within aphids, another group demonstrated a high number of positively selected for non-synonymous changes compared to synonymous in C002 across multiple species of aphids. Their evidence suggests that gene duplication and high GC content may attribute to so called fast evolving genes (Ollivier et al 2010).

Here, with C002, I present a pipe-line method for extracting ESTs from the database at NCBI, restricting ESTs to full length coding region, and analyzing ESTs for genetic variance. I report 8 validated haplotypes of C002, and remarkably, no silent mutations within the 8 EST dataset. The results indicate a high level of positive adaptive selection for C002.

**Materials & Methods**

**C002 EST Blast Search**

Blast searches were conducted using the National Center for Biotechnology Information BLAST (Altschul 1990) program (blast.ncbi.nlm.gov). Blastn was employed using an EST encoding full length pea aphid c002 (Accession: HSO93729) as query. The search was against
the Expressed Sequence Tag (EST) library. Sequence searches were restricted to *A. pisum* (taxid: 7029). Max Target Sequences was set to 20,000 to return the maximum number of sequences. Standard penalties were used to assess nucleotide identities and for gaps in sequences. The resulting ESTs were downloaded in batch FASTA format for further processing. Discontiguous blast was performed with the same EST against all ESTs excluding all aphid species.

**EST Processing**

ESTs were processed with the EMBOSS program GetORF (emboss.sourceforge.net) to remove sequences that were not of full length, and to trim the 5’ UTR and 3’ UTR to produce only full length coding region sequences. GetORF was set to show 0 flanking nucleotides with respect to the coding region. Additionally, GetORF was set to find full length coding regions between 625 and 725 bp, and report sequences in batch FASTA format.

**EST Library Origins**

ESTs obtained from blast searches originated from three libraries. The Kirk Pappan library, designated P, was created from adult parthenogenetic female pea aphid salivary glands. This EST library was constructed at Kansas State University in the laboratory of Dr. Gerald Reeck using a non-clonal population of insects. Library P was constructed using the SMART cDNA library construction kit (Clontech, Mountain View, CA). This kit employs the M-MLV reverse transcriptase which is reported to create an error once in 30,000 nucleotides.
(www.promega.com). The Nancy Moran library, M, comes from the whole bodies of the pea aphids at all developmental stages and both the winged and wingless phenotype. M was constructed at University of Arizona. The Wayne Hunter library, H, comes from whole bodies of pea aphids at all developmental stages but wingless. A fourth library created by Navdeep Mutti, DV, was created at Kansas State University in the laboratory of Gerald Reeck using the P library protocol. However, this library, as deposited at NCBI, did not produce any full-length blast hits for C002; partial hits were observed but did not cover the entire coding region.

**Multiple Sequence Alignment and EST Analysis**

Full length ESTs were divided into groups of 25 in order of BLAST score, and Multiple Sequence ClustalW (Thompson 1994) was used to perform alignments on the groups of 25 ESTs. Alignments were analyzed individually, and finally collectively, using the program BioEdit (Hall 1999). ESTs represented 2 or more times were selected for the final analysis. The fourth highest blast score group of 25 ESTs contained only unique sequences or singletons. These 25 singletons were analyzed separately following the same protocol used for the validated haplotypes.
Results

Goal

To explore the evolutionary pressure being applied to the gene encoding *A. pisum* C002 secreted salivary protein, an analysis of the available EST sequences was performed.

Analysis of C002 ESTs from *A. pisum*

A search for C002 ESTs at [http://www.ncbi.nlm.nih.gov/](http://www.ncbi.nlm.nih.gov/) using Blastn restricted to *A. pisum* sequences returned 596 ESTs. Flanking 5’ & 3’ UTR sequences were removed from the coding region of C002 ESTs using the EMBOSS program GetORF ([http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py?form=getorf](http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py?form=getorf)). Additionally, GetORF was used to remove sequences that were not full length, resulting in 332 full length C002 coding region ESTs. Alignments of the remaining 332 sequences were performed using ClustalW ([http://www.ebi.ac.uk/Tools/msa/clustalw2/](http://www.ebi.ac.uk/Tools/msa/clustalw2/)), and were visualized using BioEdit ([http://www.mbio.ncsu.edu/bioedit/bioedit.html](http://www.mbio.ncsu.edu/bioedit/bioedit.html)). For an EST to be established as error-free I required that each such sequence, represented by at least 2 identical ESTs, is taken to be error-free. I call these genetic variants or, alternatively, validated haplotypes. This resulted in 8 validated haplotypes of the C002 transcript. Alignments of the 8 variant sequences at the nucleotide and protein levels are shown in Figure 1 and Figure 2 respectively. At every position where a change in nucleotide sequence is observed, a corresponding change in protein sequence occurs. Additionally, alignments of *A. pisum, A. gossypii, M. persicae, T. citricida* C002 at the
nucleotide and protein levels are provided in Figure 5 and Figure 6 respectively. The accession number for the most prevalent haplotype was chosen for the alignment (EX644002_1). The position specific changes in sequence are compared to the changes observed within *A. pisum* at both the nucleotide and protein levels.

25 ESTs of unique sequence, taken essentially randomly from the singleton ESTs, are aligned at the nucleotide and protein levels in Figure 3 and Figure 4 respectively. These are all very similar in sequence to the 8 validated haplotypes. At the nucleotide level it was determined that there were 24 mismatches among the 25 unique ESTs, corresponding to 14 non-synonymous changes and 10 synonymous.

Representative EST counts for each of the 8 validated haplotypes, nucleotide changes, residue changes, and EST library sources are summarized in Table 1. The frequency values represent the frequency of each validated haplotype used in this study. The ESTs selected in this study were obtained from three different EST libraries. A fourth library, DV, was included but returned no full-length ESTs, and was therefore excluded from the analysis. All DV sequences were truncated at the N-terminus, possibly due to an error in processing of the library sequences. The salivary gland derived EST library designated as ‘P’ provided the majority of ESTs and was represented in 7 of the 8 genetic variants. The two whole body EST libraries designated H and M provided fewer ESTs and was represented two and one times respectively. The 9 genetic validated haplotypes are comprised collectively, of 94 of the 332 full-length ESTs obtained from GETORF. Of the 8 genetic variants 2 predominate: haplotype 3 (46%) and haplotype 7 (30%). Haplotype 3 was found in both the M and P libraries, while haplotype 7 was found in the H and P libraries. The remaining 7 haplotypes range between 2% to 7% and are found only in the P library, except haplotype 5 which was found only in the H library. The two predominant genetic
variants differ at 4 amino acid residues (39, 71, 82, and 141), and one variant, haplotype 5 contains residues from both predominant variants. Two variants haplotype 4 and 6 are predicted to be 7 amino acid residues longer at the C-terminus compared with the other variants. Haplotype 4 shows greater similarity to haplotype 3, while haplotype 6 shows greater similarity to haplotype 7, and are only found in the P library. Remarkably, there are no silent mutations observed in this analysis. Each predicted nucleic acid change results in an amino acid residue change.
Figure 1.1 Nucleotide Alignment of Pea Aphid C002 Sequences.

Alignment of 8 C002 validated haplotypes at the nucleotide level. All EST used are accounted for by accession number in Table 2. Differences in nucleotide sequence are presented in bold face font. Sequences differences are compared across different aphid species nucleotide changes shown in Figure 5.

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<th>120</th>
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</thead>
<tbody>
<tr>
<td>Haplotype</td>
<td>8 ATGGGAAGTTACAATATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA</td>
<td>120</td>
</tr>
<tr>
<td>Haplotype</td>
<td>7 ATGGGAAGTTACAATATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA</td>
<td>120</td>
</tr>
<tr>
<td>Haplotype</td>
<td>1 ATGGGAAGTTACAATATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA</td>
<td>120</td>
</tr>
<tr>
<td>Haplotype</td>
<td>2 ATGGGAAGTTACAATATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA</td>
<td>120</td>
</tr>
<tr>
<td>Haplotype</td>
<td>5 ATGGGAAGTTACAATATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA</td>
<td>120</td>
</tr>
</tbody>
</table>

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| Haplotype | 6 GTTAGATGGCGATGGTCTGCCGCTGAACCGTACGATGAGCAGGAAGAAGCGTCTGGAA | 120 |
| Haplotype | 8 GTTAGATGGCGATGGTCTGCCGCTGAACCGTACGATGAGCAGGAAGAAGCGTCTGGAA | 120 |
| Haplotype | 7 GTTAGATGGCGATGGTCTGCCGCTGAACCGTACGATGAGCAGGAAGAAGCGTCTGGAA | 120 |
| Haplotype | 1 GTTAGATGGCGATGGTCTGCCGCTGAACCGTACGATGAGCAGGAAGAAGCGTCTGGAA | 120 |
| Haplotype | 2 GTTAGATGGCGATGGTCTGCCGCTGAACCGTACGATGAGCAGGAAGAAGCGTCTGGAA | 120 |
| Haplotype | 5 GTTAGATGGCGATGGTCTGCCGCTGAACCGTACGATGAGCAGGAAGAAGCGTCTGGAA | 120 |

*******************************************************************************

| Haplotype | 6 TTACCGATGGAGCCCCTCAGTGCTGCAATCAGCAGTCGGTGGAAGAAGAGAGCCTGGAA | 180 |
| Haplotype | 8 TTACCGATGGAGCCCCTCAGTGCTGCAATCAGCAGTCGGTGGAAGAAGAGAGCCTGGAA | 180 |
| Haplotype | 7 TTACCGATGGAGCCCCTCAGTGCTGCAATCAGCAGTCGGTGGAAGAAGAGAGCCTGGAA | 180 |
| Haplotype | 1 TTACCGATGGAGCCCCTCAGTGCTGCAATCAGCAGTCGGTGGAAGAAGAGAGCCTGGAA | 180 |
| Haplotype | 2 TTACCGATGGAGCCCCTCAGTGCTGCAATCAGCAGTCGGTGGAAGAAGAGAGCCTGGAA | 180 |
| Haplotype | 5 TTACCGATGGAGCCCCTCAGTGCTGCAATCAGCAGTCGGTGGAAGAAGAGAGCCTGGAA | 180 |

*******************************************************************************

| Haplotype | 6 AGCAACAGAGGAGGCTATGCAGCTGTAGGAAATAACGTATTTAATACAGGTTAGGAATTAGGCA | 240 |
| Haplotype | 8 AGCAACAGAGGAGGCTATGCAGCTGTAGGAAATAACGTATTTAATACAGGTTAGGAATTAGGCA | 240 |
| Haplotype | 7 AGCAACAGAGGAGGCTATGCAGCTGTAGGAAATAACGTATTTAATACAGGTTAGGAATTAGGCA | 240 |
| Haplotype | 1 AGCAACAGAGGAGGCTATGCAGCTGTAGGAAATAACGTATTTAATACAGGTTAGGAATTAGGCA | 240 |
| Haplotype | 2 AGCAACAGAGGAGGCTATGCAGCTGTAGGAAATAACGTATTTAATACAGGTTAGGAATTAGGCA | 240 |
| Haplotype | 5 AGCAACAGAGGAGGCTATGCAGCTGTAGGAAATAACGTATTTAATACAGGTTAGGAATTAGGCA | 240 |

*******************************************************************************
ATAAAACGTCCGAAAACGAGGAATTGGTCAAGCAGAGGCTTAAGAGGATAACCAATGCT 600
ATAAAACGTCCGAAAACGAGGAATTGGTCAAGCAGAGGCTTAAGAGGATAACCAATGCT 600
ATAAAACGTCCGAAAACGAGGAATTGGTCAAGCAGAGGCTTAAGAGGATAACCAATGCT 600
ATAAAACGTCCGAAAACGAGGAATTGGTCAAGCAGAGGCTTAAGAGGATAACCAATGCT 600
ATAAAACGTCCGAAAACGAGGAATTGGTCAAGCAGAGGCTTAAGAGGATAACCAATGCT 600
ATAAAACGTCCGAAAACGAGGAATTGGTCAAGCAGAGGCTTAAGAGGATAACCAATGCT 600
******************************************************************************
CCC GCAGATGGCTATGGGAGCCATAAATAATTTT--GGAAGTTTCTTTCGACGTTTTTA 659
CCC GCAGATGGCTATGGGAGCCATAAATAATTTT--GGAAGTTTCTTTCGACGTTTTTA 657
CCC GCAGATGGCTATGGGAGCCATAAATAATTTT--GGAAGTTTCTTTCGACGTTTTTA 657
CCC GCAGATGGCTATGGGAGCCATAAATAATTTT--GGAAGTTTCTTTCGACGTTTTTA 657
CCC GCAGATGGCTATGGGAGCCATAAATAATTTT--GGAAGTTTCTTTCGACGTTTTTA 660
CCC GCAGATGGCTATGGGAGCCATAAATAATTTT--GGAAGTTTCTTTCGACGTTTTTA 657
******************************************************************************
TAAGCGGTCCATACAGAC 678
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Figure 1.2 Protein Alignment of Pea Aphid C002 Sequences.
Alignment of 8 validated haplotypes variants at the protein level named after single EST representatives (See Table 2). Amino acid residue changes in protein sequences are represented in bold face font. Changes in amino acid sequences were compared to species specific changes provided in Figure 6.

```
6       MGSYKLYVAVMAIAIAVQEVRCDSWSEPEYDEQEEASFELPMHERQCDEYKSKIWDFKAF 60
8       MGSYKLYVAVMAIAIAVQEVRCDSWSEPEYDEQEEASFELPMHERQCDEYKSKLDFKAF 60
7       MGSYKLYVAVMAIAIAVQEVRCDSWSEPEYDEQEEASFELPMHERQCDEYKSKIWDFKAF 60
5       MGSYKLYVAVMAIAIAVQEVRCDSWSEPEYDEQEEASFELPMHERQCDEYKSKIWDFKAF 60
1       MGSYKLYVAVMAIAIAVQEVRCDSWSEPEYDEQEEASFELPMHERQCDEYKSKIWDFKAF 60
2       MGSYKLYVAVMAIAIAVQEVRCDSWSEPEYDEQEEASFELPMHERQCDEYKSKIWDFKAF 60

******************************************************************************

6       SNQEAMQLMIEITFNTGKEGSLNSCVSDTTRAIFNFVSDMATQNHYSLGMMMNLMLAFI1 120
8       SNQEAMQLMIEITFNTGKEGSLNSCVSDTTRAIFNFVSDMATQNHYSLGMMMNLMLAFI1 120
7       SNQEAMQLMIEITFNTGKEGSLNSCVSDTTRAIFNFVSDMATQNHYSLGMMMNLMLAFI1 120
5       SNQEAMQLMIEITFNTGKEGSLNSCVSDTTRAIFNFVSDMATQNHYSLGMMMNLMLAFI1 120
1       SNQEAMQLMIEITFNTGKEGSLNSCVSDTTRAIFNFVSDMATQNHYSLGMMMNLMLAFI1 120
2       SNQEAMQLMIEITFNTGKEGSLNSCVSDTTRAIFNFVSDMATQNHYSLGMMMNLMLAFI1 120

******************************************************************************

6       REVDTTSNKFETKEVFERIKTPEIRDYKIKHTTARTVDLLKEPVIRGLFVKVKAEG1L 180
8       REVDTTSNKFETKEVFERIKTPEIRDYKIKHTTARTVDLLKEPVIRGLFVKVKAEG1L 180
7       REVDTTSNKFETKEVFERIKTPEIRDYKIKHTTARTVDLLKEPVIRGLFVKVKAEG1L 180
5       REVDTTSNKFETKEVFERIKTPEIRDYKIKHTTARTVDLLKEPVIRGLFVKVKAEG1L 180
1       REVDTTSNKFETKEVFERIKTPEIRDYKIKHTTARTVDLLKEPVIRGLFVKVKAEG1L 180
2       REVDTTSNKFETKEVFERIKTPEIRDYKIKHTTARTVDLLKEPVIRGLFVKVKAEG1L 180

******************************************************************************

6       IKPSENEELVQRLKRITNPAMGAMGINKFGSFLRFYKRVHTD 226
8       IKPSENEELVQRLKRITNPAMGAMGINKFGSFLRF-------- 219
7       IKPSENEELVQRLKRITNPAMGAMGINKFGSFLRF-------- 219
5       IKPSENEELVQRLKRITNPAMGAMGINKFGSFLRF-------- 219
1       IKPSENEELVQRLKRITNPAMGAMGINKFGSFLRF-------- 219
2       IKPSENEELVQRLKRITNPAMGAMGINKFGSFLRF-------- 220

******************************************************************************
```

Figure 1.3 Nucleotide alignment of 25 unique ESTs of Pea Aphid C002

25 unique ESTs (by accession number) are aligned at the nucleotide level. Differences are marked in bold face.

FP889223_1     ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS094889_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS093815_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS092939_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS092790_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS093201_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS094575_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS092845_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS096096_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS095744_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS093225_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS094600_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS092884_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS096119_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS093801_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
HS092279_1      ATGGGAAGTTACAAATTATACGTAGCCGTCATGGCAATAGCCATAGCTGTAGTACAGGAA  60
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**Figure 1.4 Protein alignment of 25 unique ESTs of Pea Aphid C002**

25 unique ESTs were translated to protein sequence and aligned. Differences in amino acid residues are indicated in bold face font.

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HS093801_1      IKPSENEELVKQRLKRITNAPAKMAMGAIN----KFGSFLRRF  219
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HS094993_1      IKPSENEELVKQRLKRITNAPAKMAMGAIN----KFGSFLRRFYKRVHTD  226
HS095664_1      IKPSENEELVKQRLKRITNAPAKMAMGAIN----KFRFLRRFYKRVHTD  226
HS093201_1      IKPSENEELVKQRLKRITNAPAKMAMGAIN----KFGSFLRRF  219
HS092884_1      IKPSENEELVKQRLKRITNAPAKMAMGAIN----KFGSFLRRF  219
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HS092939_1      IKPSENEELVKQRLKRITNAPAKMAMGAIN----KFGSFLRRF  219
HS094889_1      IKPSENEELVKQRLKRITNAPAKMAMGAIISLEVSD**V**FNKRVHTD  226

***************:**********:.*    .*    .*    .*    .
Figure 1.5 Nucleotide Alignment of Aphid C002 Sequences.

Alignment of C002 ESTs from *A. pism* against: *A. gossypii*, *M. persicae*, and *T. citricida*.

Changes in protein sequence are highlighted in Figure 6 with bold face font.

A. gossypii

ATGGGACGTACAAATTAAATATGTGGCCGTACATGGCAATAATCTAGCTTAGGCTGTAATACAG

T. citricida

ATGGGACGTACAAATTAAATATGTGGCCGTACATGGCAATAATCTAGCTTAGGCTGTAATACAG

A. pism

ATGGGAAGTTAACAAATTAAATACGTAGCCGTCATGGCAATAAGC

M. persicae

ATGGCAAGTTAACAAATTAAATGAGCAGGATGATGATCAGGAAGGATATTACGAT

**** * ***** **** **  * ****** ******** *   * **** * * * ***

A. gossypii

AAAGCTAGTTGTGCTGGTGGGCCTAAC

T. citricida

AAAGCTAGTTGTGCTGGTGGGTCTAAC

A. pism

GAAGTGAATGCAGA----TGCTGCACGC

M. persicae

GAAATTAGTTGCAAGGTCAGCAGAAGATCAGGGAAGAGATACCAGGATATTACGAT

** ** ** * * **

A. gossypii

-----------ACCGAACAATA-------------------CATTGAGTCAAAGA------ 125

T. citricida

-----------ACTGAAACAATA-------------------CGATGAATCAAAGA------ 125

A. pism

-------------TGAAACCTGAA-------------------CGATGACAGAAGAAGCCT-- 112

M. persicae

GATGAGGGAGGTGAAGGAGAGAAGCAGAAGCAGAAGAGATACAGGAGAGAGAGAAC

****  **                *  * *      ***

A. gossypii

GAAATGGAGCATCATCAGTGTGATGAATACAAATCGAAAATCTGGAATAAGGCATTTAGC

T. citricida

GAAATGGAGCATCATCAGTGTGATGAATACAAATCGAAAATCTGGAATAAGGCATTTAGC

A. pism

CCGATGGAGCATCATCAGTGTGATGAATACAAATCGAAAATCTGGAATAAGGCATTTAGC

M. persicae

GAGATGGAGCATCATCAGTGTGATGAATACAAATCGAAAATCTGGAATAAGGCATTTAGC

GAGATGGAGCATCATCAGTGTGATGAATACAAATCGAAAATCTGGAATAAGGCATTTAGC

******** * ****** ** *** ****** ******** ** * ************

A. gossypii

AACCAGGCCGCTATGCAACTGCTGATGAAATAACGAAAATCTGGAATAAGGACGACG

T. citricida

TACCAGGCCGCTATGCAACTGCTGATGAAATAACGAAAATCTGGAATAAGGACGACG

A. pism

AACCAGGCCGCTATGCAACTGCTGATGAAATAACGAAAATCTGGAATAAGGACGACG

M. persicae

AACCAGGCCGCTATGCAACTGCTGATGAAATAACGAAAATCTGGAATAAGGACGACG

*** ******** * *** ** * * ** ****** ******** ** **
A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae

A. gossypii

T. citricida

A. pisum

M. persicae
Figure 1.6 Alignment of Aphid C002 Amino Acid Sequences.

Alignment of translated validated C002 haplotypes for *A. pisum*, *A. gossypii*, *M. persicae*, and *T. citricida*. Variable positions in amino acid sequence observed in Figure 2 are compared to species-specific changes and are represented by bold face font. Of the 8 changes observed in Figure 2, 7 are represented in the main sequence, and 2 are represented by the immediate C-Terminus.

A. *gossypii*  
MGRQLYVAVMAISSLAVIQKASCAGGP=NAYNTTEQYI------------------------ 38

T. *citricida*  
MGRQLYVAVVAAISSLAVIQKASCAGGS=NAYPSTEQYD------------------------ 38

A. *pisum*  
MGQLYAVMAIA-IAVQEVRCWDWAAPYDEQE----------------------------- 35

M. *persicae*  
MASYKLYAVMAIAVIAVQESCKQ<=SEQDDQEGYYDEEGVNDNQGEENDNQGEEN 60

A. *gossypii*  
------------DSEQGDSECTIONKDCDEYKSKIWNAFSPAMQLVVDVLKTEKMG 84

T. *citricida*  
------------EASVFLPMEHRQCDEYKSKIWDKAFSNQEAAMQLMELTFNGLGS 81

A. *pisum*  
----------------DENQGEENDNQGErikaESEFEIYKQIEEYKSIIWAdaSNPKAMLKLTFTNGLGS 120

M. *persicae*  
---------------* :**:**:**::*:*:* :* : :  . :  

A. *gossypii*  
DNVCDTIRVLNSFIDVATNQNSYHYSVGLAKMMLAIARDTSKFRDTEKVFDRIV 144

T. *citricida*  
NDVCDTIRVLNSFIDVATNQNSYHYSVGLAKMMLAIREVDTSKFRDTEKFDRIAA 144

A. *pisum*  
NEVCDTTRALPFVDMATNPQAHYSLGMFKMLAIREVDTTSKFRDTEKFDRIAA 141

M. *persicae*  
NEVCDTTRALPFVDMATNPQAHYSLGMFKMLAIREVDTTSKFRDTEKFDRIAS 180

A. *gossypii*  
QNADIRDYIRNTASRVDLLKFLPMRNLARVFCAFETLYNSQSMNQIKQRINGLTNP 204

T. *citricida*  
KNADIRDYIR------------------------------------------------------ 155

A. *pisum*  
KTPERIDYKHTTARTVLKLFVIRGRLFKVKAEGLIKFSNIELVQRLKRINTAP 201

M. *persicae*  
KTPERIDYINSAKTVDDLEPKIRALRFKVMKAFESLbbbbbbbbbbbb 240

A. *gossypii*  
SKIAMGMNKVGNMFQNV 222

T. *citricida*  
-----------------------------------------------

A. *pisum*  
AKMAMGAINKPGSFLRRF 219

M. *persicae*  
VKLAKGMKTGVGFRHRF 258
Table 1.1 Pea Aphid EST Analysis

Summary of the amino acid and nucleotide changes for the 8 validated haplotypes. An EST count and frequency of the EST found out of the 94 ESTs from the dataset is provided in columns 2 & 3 of the nucleotide & amino acid sequences respectively. Discreet nucleotide & amino acid sequence changes are provided in columns 4-12 in the table respectively. The C-terminal amino acid sequence & library source are provided in columns 13 & 14 respectively.

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*Kirk Pappan EST Library of Pea Aphid salivary glands from adult parthenogenetic females


**Nancy Moran EST library of Pea Aphid whole body from nymph, adult, winged, and wingless parthenogenetic females


***Wayne Hunter EST library of Pea Aphid whole body from nymph and adult parthenogenetic females


The number parenthesis represents the number of ESTs obtained from each library

Frequency is the percentage of ESTs representing each validated haplotype with respect to the total number of ESTs in the validated haplotypes. Frequency does not to describe the frequency of haplotypes in nature or within the total number of C002 ESTs found at NCBI.
Table 1.2 Table of all ESTs included within each validated haplotype
94 ESTs used to create the 8 validated haplotypes are provided. EST accession numbers presented in Figures 1-4 are shown in bold face font.

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Chapter 2 - Effect of Armet Transcript Knockdown in *Tribolium castaneum*

**Introduction**

*Armet Etymology*

The Armet sequence was first discovered from a human cDNA, and designated arginine rich protein (ARP) due to a 55 tri-nucleotide repeat at the 5’ end encoding arginine (Shridhar et al., 1996). Polymorphisms of Armet were later found in a variety of early stage tumors, and Armet was renamed arginine rich mutated in early state tumors (Armet) (Shridhar et al., 1996, 1997). Later, Armet was cloned from human ventral mesencephalic cell line 1 (VMCL1). This translated product was found to not contain the 55 tri-nucleotide repeat, but was instead a secretory protein containing a signal peptide. Both the arginine rich and tumor based mutations of Armet turned out to be incorrect. The designation of mesencephalic astrocyte-derived neurotrophic factor (MANF) was assigned to Armet (Petrova et al., 2003). Armet is still a prevalent title for the gene in the literature and will be in this thesis.

*Armet in Endoplasmic Reticulum Stress (ER Stress) & Unfolded Protein Response (UPR)*

Armet was first implicated in ER stress response when the addition of chemical ER stress inducers (tunicamycin, thapsigargin, and DDT) to mouse pancreatic β cells induced an increase
in both transcript and protein levels. The increased expression correlated to increases of two other known ER stress genes, Grp78 and Hsp70 (Mizobuchi et al., 2007). Additionally, microarray analysis of U2OS and HEK293 challenged by the same chemical inducers found Armet to be up-regulated. Armet was found to be localized in the ER, Golgi, and secreted. Knockdown of Armet showed a higher propensity towards apoptosis, while over-expression conferred a degree of protection to cells (Apostolou et al., 2008).

The protective effects of Armet have been studied mainly in neural and cardiac tissues. It has been demonstrated that expression of recombinant human Armet and addition to dissected mouse mesencephalic tissue provided increased protection to dopaminergic neurons (Petrova et al., 2003). Immunohistochemical studies showed that Armet is expressed during neural development and in adult mice. Induction of epilepsy caused a transient increase of Armet in the thalamus and hippocampus. Also, forebrain ischemia induced a large increase in Armet expression in the hippocampus and a small, restricted increase in the cerebral cortex (Lindholm et al., 2008). Armet has been shown to decrease apoptosis and necrosis in mice that have undergone cerebral ischemia. Recovery of mouse behavior is also associated with the action of Armet (Airavaara et al., 2009; Yu et al., 2010). Armet is also secreted by ventricular myocytes, and during simulated cardiac ischemia (Tadimalla et al., 2008; Glembotski 2011).

**Structure of Armet**

The structure of human Armet was determined using x-ray crystallography (Parkash et al., 2009). Additionally mouse and human Armet structures were determined using solution state
nuclear magnetic resonance (Hoseki et al., 2010; Hellman et al., 2010; Hellman et al., 2010). All structural studies showed a protein containing two domains separated by a cysteine-bridge linker region. The N-terminal domain has a saposin like structure while the C-terminal end is predicted to be intrinsically unstructured.

**Armet and Arthropod Development**

*D. melanogaster* lines containing P-element insertions to abrogate Armet expression showed a distinct phenotype. Larvae homozygous for defective Armet died as first instar larvae. Lack of Armet led to loss of dopaminergic cells and the degradation of the developing larval axonal bundles. It was also noted that the cuticle of the larvae was malformed by the observations that antibodies could pass through without permeabilization (Palgi et al., 2009), likely due to a lack of dopamine to act a protein cross linking agent.

Here I probe the effects on development of the red flour beetle, *Tribolium castaneum*, by knocking down Armet in late (determined by mass) larvae. Tribolium is the model RNAi system for insects, owing to the systemic effect of injection of dsRNAs. We demonstrate that depletion of Armet leads to an arrest in development during the eclosion from pupa to adult. It is possible that a loss of dopaminergic neurons is causing an inability for these insect to properly cross-link their adult cuticle.
Material & Methods

Synthesis of dsRNA

The T-7 promoter PCR based method was used for preparation of dsRNAs (Tomoyasu et al 2005). For TcArmet dsRNAs the primers with the following sequence were used: 5’- TAA TAC GAC TCA CTA TAG GGC TTC ACT GTT CTA TTC GCAA G -3’ and 5’- TAA TAC GAC TCA CTA TAG GGA GTT CGG TAT GTT TAT GCT TCA -3’ (forward and reverse respectively). The product created was 486 bp in length and began at position 18 of the open reading frame and terminated at position 504. For TcVer dsRNAs the T7 template for vermilion was provided by Dr. Muthukrishnan.

PCR products were purified using the Qiaquick PCR Purification Kit (Qiagen, Valencia, CA). dsRNAs were synthesized using the Ampliscribe™ T7-Flash™ Kit (Epicentre biotechnologies, Madison, WI). dsRNAs were purified by adding phenyl chloroform, extracting with chloroform, and precipitating with 3M sodium acetate and isopropyl alcohol. dsRNA pellets were washed with 75% ethanol in DEPC treated deionized distilled water, and resuspended in DEPC treated deionized distilled water. dsRNA concentrations were confirmed using a Nanodrop 1000 (Thermoscientific, Wilmington, DE).

Injection

Larvae weighing 2 mg (late instar larvae) were selected for injection. Larvae were injected with 200 ng of dsRNA using a pulled glass capillary needle. Capillaries (outer diameter of 1.0 mm,
inner diameter of 0.5 mm, Sutter Instruments, Navato, CA) were pulled using a Model P-87 capillary puller and filament (Sutter Instruments, Navato, CA).

**RNA Extraction**

RNA from injected late instar larvae was extracted 48 hrs after injection using the TriZol reagent (Invitrogen, Grand Island, NY), followed by extraction with chloroform, and precipitation with 3M sodium acetate and isopropyl alcohol. RNA was converted to cDNA using Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen, Grand Island, NY).

**qRT-RT-PCR**

Quantitative real time reverse transcription polymerase chain reaction was employed to create an age dependent expression profile of Armet with respect to housekeeping gene RPS6. Insects from early larvae (<2 mg), late larvae (>2 mg), prepupating, pupa, newly emerged adults, and adults aged 1 week were selected for expression analysis. Also, qRT-RT-PCR was used to quantify the knockdown levels for dsArmet and dsVermilion. 2 mg larvae were injected with dsArmet or dsVermilion and RNA was extracted 48 hours post injection. For RPS6 the forward and reverse primers 5’-AGA TAT ATG GAA GCA TCA TGA AGC-3’ and 5’-CGT CGT CTT CTT CTC TGC TCA AAT TG-3’ respectively were used. For Armet the forward and reverse primer used were 5’AAG ACT GCC AAG GGT GCA T-3’ and 5’-CAT GCA CAT TCA CTG GGA AC-3’ respectively. qRT-RT-PCR was conducted using a Bio-Rad iCycler iQ Real-Time PCR machine (Hercules, CA). Bio-Rad SYBER Green SsoFast™ Evagreen SuperMix was used.
to fluorescently label PCR products. Melting temperature of 94°C Celsius, annealing temperature of 55°C Celsius, and extension temperature of 68°C Celsius were used for 40 cycles of PCR. Results were analyzed using ExPCRT program (http://www.expertqpcr.com) based on the ΔΔCt method.

Results

To determine the level of Armet transcript in different developmental stages, qRT-RT-PCR was used. 3 biological replicates of 5 pooled insects were measured for their Armet transcript level relative to ribosomal protein S6 (RPS6) transcript. Early instar larvae, late instar larvae, prepupa, pupa, newly emerged adults, and adults aged 1 week were probed for their Armet transcript level in Figure 12. Armet transcript level reaches its peak levels during the late instar larvae, newly emerged adults, and 1 week old adult stages.

To understand the role of Armet in development of *T. castaneum* dsRNA mediated depletion of Armet’s transcript was employed. Late instar larvae were injected with 200ng of either double stranded RNA encoding the gene Armet (dsArmet) or Vermilion (dsVER). Three sets of injections were made and development of larvae to adult stage was monitored. The numbers of living insects with respect to days after injection are reported in Figures 1, 4, and 7. Insects injected with dsVER had a higher number of surviving insects in all three replicates. The developmental stages of all three replicates were also monitored. The number of insects in each developmental stage is provided in Figures 2, 3, 5, 6, 8, and 9. In all three cases Tribolium injected with dsVER were able to progress through pupation and eclosion into adulthood, and showed the typical phenotype of white or pigmentless eyes. The insects injected with dsVER
were able to progress through pupation, but development was arrested at the pharate adult stage and they were unable to undergo eclosion to adulthood in the majority of injected insects.

Pictures of Tribolium injected with either dsVER or dsArmet are provided in Figure 10 and 11. In Figure 10 insects injected with either dsVER or dsArmet are presented. The dsVER insect shows pigmentless eyes and was able to undergo eclosion to adulthood. The insect injected with dsArmet was able to progress to late pupa stage but arrested before eclosion to adulthood. In Figure 11 three insects injected with dsArmet are shown at various levels of pupa development. The insect on the left and in the middle are living injected insects that had tanned normally and are just prior to the lethal pupa phenotype. The insect on the right depicts a dead dsArmet injected insect that was unable to eclose to adult.

To assess the efficiency of the dsRNA mediated knockdown of Armet. qRT-RT-PCR measurements were taken for Armet in 2 biological replicates of 5 pooled insects each. Injected larvae were harvested for mRNA content as larvae 48 hours post injection. Relative expression levels of Armet transcript are displayed in Figure 13. Insects injected with dsArmet but no dsVER showed lower relative Armet transcript level.

Armet exists in insects and all vertebrates. An alignment of Homo sapiens, Mus musculus, Drosophila melanogaster, Acyrthosiphon pisum, and Tribolium castaneum is provided in Figure 14. The structure of the T. castaneum Armet gene is presented in Figure 15. TcArmet contains 3 exons and 2 introns. The final mRNA is 763 bp in length.
Discussion

Knocking out Armet in *D. melanogaster* showed lethal effects during the first instar larval stages (Palgi et al 2009). I have shown by depleting Armet transcript level in late instar larvae that Tribolium their development during the progression of pupa to adult. Although these two results are not comparable at developmental levels, the Armet transcript is crucial for Tribolium development. By measuring the insects for dopamine content and probing at the morphology of the insect brain by dissection and microscopy, a connection to the results seen in *Drosophila* could be made. By injecting adults and allowing them to reproduce and transfer the knock down to their offspring by way of parental RNAi we would be able to compare early embryogenesis and larva development in *T. castaneum* to that of *D. melanogaster* (Bucher et al 2002).

I present a phenotype that is easily interpreted visually, the insects are able to progress from last instar to pupae, but die when attempting to emerge. However, there is no evidence here to elucidate the molecular mechanism of dsArmet killing. It is possible that death is occurring, as in the case with *D. melanogaster*, from the degradation of dopaminergic neurons in the brain. Protein secretion & cross-linking by the cuticle epithelium is an event of importance during eclosion that may require the presence of Armet. Although I injected much earlier, Armet may not be crucial until this step in development. Secretion by the midgut and epithelium is a tightly regulated process involving the secretion of numerous factors, possibly causing ER stress, to proceed correctly (Chaudhari et al 2011). It is important that a future developmental expression profile be created for TcArmet in individual tissues. Also, injected beetles be dissected and/or sectioned at the late pupae stage and first be assayed with microscopy for morphological
differences. Additionally, using immunohistochemistry to determine the state of known secreted factors during development will allow us to indentify the molecular basis for dsArmet killing.

It might also be possible to tease out a weak phenotype by injecting lower concentrations of dsRNA as was done in the case of *Laccase-2* (Arakane et al 2005). It was shown that high concentrations of injected dsRNA for *Laccase-2* resulted in a lethal phenotype and failure to undergo cuticle tanning or sclerorization. At lower levels of injected dsRNAs the insects were able to survive and show a similar but weaker phenotype. By injecting smaller concentrations of dsArmet, insects may be able to proceed beyond the pupa stage and eclose to adult. In this situation a greater number of phenotypes could be visually assessed with living specimens.

### Conclusion

Armet is differentially expressed at the various stages of *T. castaneum* development. Depletion of TcArmet transcript in late instar larvae leads to a late pupa lethal phenotype. The likely cause of death is due to a lack of dopaminergic neurons surviving the development of the adult brain, and an inability for the insects to properly form their adult cuticle.
Figure 2.1 Survival curves after injection of dsVER RNA or dsArmet RNA (#1)
9 Insects were injected with 200 ng of either dsVER RNA (blue) or dsArmet RNA (Red).
#1 denotes the first replication of this experiment.
Figure 2.2 Number of insects in various developmental stages after dsVER RNA injections (#1)

9 Insects were injected with 200 ng of dsVER RNA. The number of insects in developmental stages: larvae (blue), Pre-pupa (red), pupa (green), and adult (purple). #1 denotes the first replication of this experiment.
Figure 2.3 Number of insects in various developmental stages after dsArmet RNA injection (#1)

9 Insects were injected with 200 ng of dsArmet RNA. The number of insects in developmental stages: larvae (blue), Pre-pup (red), pupa (green), and adult (purple).
Figure 2.4 Survival curves after injection of dsVER RNA or dsArmet RNA (#2)

10 Insects were injected with 200 ng of either dsVER RNA (blue) or dsArmet RNA (Red). One dsArmet injected insect was not counted due to injection related death. #2 denotes the first replication of this experiment.
Figure 2.5 Number of insects in various developmental stages after dsVer RNA injection (#2)

10 Insects were injected with 200 ng of dsVER RNA. The number of insects in developmental stages: larvae (blue), Pre-pupa (red), pupa (green), and adult (purple). #2 denotes the second replication of this experiment.
Figure 2.6 Number of insects in various developmental stages after dsArmet RNA injection (#2)

9 Insects were injected with 200 ng of dsArmet RNA. The number of insects in developmental stages: larvae (blue), Pre-pupa (red), pupa (green), and adult (purple). #2 denotes the second replication of this experiment.
Figure 2.7 Graph representing live tribolium injected with either dsVER RNA or dsArmet RNA (#3)

9 Insects were injected with 200 ng dsVER RNA (blue) and 15 with dsArmet RNA (Red). #3 denotes the third replication of this experiment.
Figure 2.8 Number of insects in various developmental stages after dsVER RNA injection (#3)

9 Insects were injected with 200 ng of dsVER RNA. The number of insects in developmental stages: larvae (blue), Pre-pupa (red), pupa (green), and adult (purple).
Figure 2.9 Number of insects in various developmental stages after dsArmet RNA injection (#3)

Insects were injected with 200 ng of dsArmet RNA. The number of insects in developmental stages: larvae (blue), Pre-pupa (red), pupa (green), and adult (purple). #3 denotes the third replication of this experiment.
Figure 2.10 Larvae injected with dsVER RNA or dsArmet RNA

Insects were injected with 200 ng of dsVER or dsArmet and allowed to develop normally. Insects injected with dsVER lack of pigment in the eyes of pupa. Insects injected with dsArmet showed normal eye development but their development arrested at late pupa stage.

Pupa Lethal Phenotype

dsVer RNA             dsArmet RNA
Figure 2.11 dsArmet RNA injected larvae
Left: Living dsArmet injected insect showing normal tanning prior to lethal phenotype. Middle: Living dsArmet injected insect showing normal tanning just prior to lethal phenotype. Right: Dead dsArmet injected insect after lethal phenotype.

Pupa Lethal Phenotype
Figure 2.12 Armet transcript levels at several stages of development

RT-PCR expression profile of Armet including Early instar larvae (YL), late instar larvae (LL), prepupal (PP), pupal (P), young adult (YA), and 1 week aged adult beetles (LA) relative to RPS6. At each stage 3 biological replicates (n=3) pooled from 5 insects were used. Larvae weighing less than 2 mg were selected for being early instar larvae (YL). Larvae greater than 2 mg were designated as late instar larvae (LL). Insects identified as prepupal (PP) due to the signature elongated banana prepupal shape. Pupa (P). Adult’s newly emerged from pupa were pooled for young adult (YA). Adults aged 1 week were designated late adults (LA). Armet transcript level measurements were done with respect to RPS6 transcript level. Error bars represent the standard error of each sample.
Figure 2.13 Armet transcript levels after injection with dsVER RNA or dsArmet RNA. Graph of qRT-PCR data for dsArmet and dsVER knock-down beetles relative to RPS6. Duplicate measurements (n=2) were conducted on pools of 5 larvae. Larvae injected with 200 ng of dsArmet or dsVER were harvested as larvae 48 hrs post injection. Armet transcript level was measured relative to RPS6.
Figure 2.14 Alignment of Armet amino acid sequences from Homo sapiens, Mus musculus, Drosophila melanogaster, Acyrthosiphon pisum, and Tribolium castaneum

ClustalW multiple sequence alignment for Armet sequences. Consensus sequence: “*” identical amino acid, “:” conserved amino acid, “.” semi-conserved amino acid.

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<td><strong>H. sapiens</strong></td>
<td>MRWATQGLAVALASLVP--GSRALRPGDECVISLGRFYQDLKDRDVTFSATI 56</td>
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**D. melanogaster**

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
<th>Length</th>
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<tbody>
<tr>
<td><strong>D. melanogaster</strong></td>
<td>ETAFKKFCQAQK-NKEHRFCYLGLESATGILNLMKPLSMPAEKICEKLKKDAQ 112</td>
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<tr>
<td><strong>A. pism</strong></td>
<td>EEQFKKYCLSTKIDKEKRLCYLGLEDATGLISEMSKPLSPIKEKCKLKKMDAQ 113</td>
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<tr>
<td><strong>T. castaneum</strong></td>
<td>ESKFRDCKNTR-NKENRCYLGLESATGILNLMKPLSMPAEKICEKLKKDAQ 109</td>
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<tr>
<td><strong>M. musculus</strong></td>
<td>EEELIKFCREAT-GKENRLCYIGDATKINVEKICLKDKDSQ 112</td>
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<tr>
<td><strong>H. sapiens</strong></td>
<td>ENELIKFCREAT-GKENRLCYIGDATKINVEKICLKDKDSQ 115</td>
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**D. melanogaster**

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<tbody>
<tr>
<td><strong>D. melanogaster</strong></td>
<td>ICDLRYEQIDLSVDKLRLKYVLKRLKILNDWDESCDGMLEKDFIKRIELKPKYSRE 172</td>
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<tr>
<td><strong>A. pism</strong></td>
<td>VCDIKYDEIDWKTVALKMVNLKILNLWGEIDCMEKDTYIREEKLPSYVKEE 173</td>
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<tr>
<td><strong>T. castaneum</strong></td>
<td>ICELKYDKIQIDLSVDKLRLKILNDWGEQCGCIEKSEISKREELKHHHT--E 167</td>
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<tr>
<td><strong>M. musculus</strong></td>
<td>ICELKYDKIQIDLSVDKLRLKILNDWGEQCGCIEKSEISKREELKHHHT--E 172</td>
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<tr>
<td><strong>H. sapiens</strong></td>
<td>ICELKYDKIQIDLSVDKLRLKILNDWGEQCGCIEKSEISKREELKHHHT--E 175</td>
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**D. melanogaster**

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<td><strong>D. melanogaster</strong></td>
<td>L 173</td>
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<tr>
<td><strong>A. pism</strong></td>
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<tr>
<td><strong>T. castaneum</strong></td>
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<tr>
<td><strong>M. musculus</strong></td>
<td>ASARTDL 179</td>
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<tr>
<td><strong>H. sapiens</strong></td>
<td>ASARTDL 182</td>
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Figure 2.15 Alignment of Armet exons against the genomic sequence of *T. castaneum*

Tribolium Armet consists of 3 exons and 2 introns.

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<th>Chromosome</th>
<th>TACTTAAATAAGTCATTAAGACAGTCACAATTTCATAGAGCCCACCTTCCACACTAAAGC 60</th>
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<td>TcArmet</td>
<td>----------------------------------------------------------------</td>
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<tr>
<td>Chromosome</td>
<td>CTTGAGGAGACGTGGGACACAGCTCAGGCTGCAACAGTGTACCCACAAAATCCAAATTC 120</td>
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<tr>
<td>TcArmet</td>
<td>----------------------------------------------------------------</td>
</tr>
<tr>
<td>Chromosome</td>
<td>ACCGTTCTGTCAAATCAATTCTTTTAATAACTGTCAGTGTTGAAACAGGCAATAGTTAAT 180</td>
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<tr>
<td>TcArmet</td>
<td>ATTTTTATACGTGTCAGTGTTGAAACAGGCCAATAGTTAAT 43</td>
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<tr>
<td>Chromosome</td>
<td>TATTTTTGATAAAAAGTGTAACAATCAGTGATTTTTTACGAAATAAAAGCTCAAGAGT 240</td>
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<td>TcArmet</td>
<td>TATTTTTGATAAAAAGTGTAACAATCAGTGATTTTTTACGAAATAAAAGCTCAAGAGT 97</td>
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<tr>
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<tr>
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<td>TcArmet</td>
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<tr>
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<tr>
<td>Chromosome</td>
<td>ATCCAATTTTCGAGACTATTGTAAAACACAGGAGAAAACACAGGAGATCGATTTTGTAAAGCAG 540</td>
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<tr>
<td>TcArmet</td>
<td>ATCCAATTTTCGAGACTATTGTAAAACACAGGAGAAAACACAGGAGATCGATTTTGTAAAGCAG 309</td>
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Chromosome      CAATTTGGATTTCTCAAGTTTTCACAAAAATAAAAAATGCAATAAAAATTTCAGTGTTACTA 600
TcArmet         -- --------------------------------------------------------

Chromosome      CTTGGGAGGCTTTGGAAGAAATGCACTGGTTTATTTGGGCAGAATGCTAAAACGCTTTTC 660
TcArmet         CTTGGGAGGCTTTGGAAGAAATGCACTGGTTTATTTGGGCAGAATGCTAAAACGCTTTTC 377

Chromosome      ATGGTCAATGCAATCCGAAAAACCTCCATTTGAAAGAAGAAAAGGCCCACAAATCTG 720
TcArmet         ATGGTCAATGCAATCCGAAAAACCTCCATTTGAAAGAAGAAAAGGCCCACAAATCTG 437

Chromosome      CGAATTGCGCGCTACGACGTCGCAATCGATTTAAAGACAGTTGATTTGAAGAAACTCAAAGT 780
TcArmet         CGAATTGCGCGCTACGACGTCGCAATCGATTTAAAGACAGTTGATTTGAAGAAACTCAAAGT 497

Chromosome      GAGGGATTTGAAGAAGATTATCAACGACTGGGGCGAAGACTGCCAAGGGTGCATCGAAAA 840
TcArmet         GAGGGATTTGAAGAAGATTATCAACGACTGGGGCGAAGACTGCCAAGGGTGCATCGAAAA 557

Chromosome      AAGCGAGTTTGCAATCAAATGACGAGAATTGAAGCTTTTAAACATACCGAACTTTAAGTGTG 900
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Chromosome      TTGTTACTAGTGAAATATTGGTTACAGATTGTTTATTAGGATTGTATTTAT 960
TcArmet         TTGTTACTAGTGAAATATTGGTTACAGATTGTTTATTAGGATTGTATTTAT 677

Chromosome      CAGTGAATGTGCAATTATAACTTTTTATTTTATTAGGATTGTATTTAT 1020
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Chromosome      ATTTGTAATAAAACATGAAAAGAATTTTTATTAAAAATGATACATTTTTATTA 1080
TcArmet         ATTTGTAATAAAACATGAAAAGAATTTTTATTAAAAATGATACATTTTTATTA 763

Chromosome      CATTACTATCTACATCCAGGGGGGGCCCTGAATAAAAACATTTTTATTTTTATTA 1140
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Chromosome      GATTATTTGAAACTCTCTTTTCTCTCAAGCGTCTAGTACAGTG 1181
TcArmet         GATTATTTGAAACTCTCTTTTCTCTCAAGCGTCTAGTACAGTG 898


Mutti N, Park Y, Reese J, Reeck G. RNAi knockdown of a salivary transcript leading to lethality in the pea aphid, acyrthosiphon pisum. JIS 2006;6(38).


