Transcriptome analysis of western corn rootworm larvae and eggs

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Objectives

❑ The genome of haploid western corn rootworm (WCR), *Diabrotica virgifera virgifera*, is one of the largest among beetle species (~2.58 GB). In order to identify the gene sets expressed in their larval stages (when most damaging to corn) and to contribute to improving the genome assembly, we have sequenced and assembled transcriptomes from egg, neonate, and third-instar larval stages of WCR using next-generation technologies.

Methods

❑Using Illumina paired-end as well as 454 Titanium sequencing technologies, in total ~700gigabases were sequenced from cDNA prepared from eggs (15,162,017 Illumina reads), neonates (721,697,288 Illumina reads), midguts of third instar larvae (44,852,488 Illumina reads and 414,742 454 reads).

❑*de novo* assembly was performed using four different short read assemblers: Newbler (v2.5), Mira (v3.4.0), Velvet/Oasis (v1.2.03), and Trinity (rel 2013-02-25).

❑Hybrid assembly using both Illumina and 454 reads was also performed.

❑The Trinity assembly using the pooled Illumina datasets had the longest average length of contigs and N50, and the most hit against the known protein sequences. We chose this assembly as the most inclusive combined WCR transcriptome. Table 1 summarizes the assembly statistics.

In order to search the gamma-aminobutyric acid type A ($GABA_A$) receptor and gustatory receptors from the WCR transcriptome, their sequences from *Drosophila melanogaster* (Robertson et al., 2003) and *Tribolium castaneum* (Tribolium Genome Sequencing et al., 2008) were used as queries with NCBI BLAST (tblastn, ver. 2.2.29+) (Altschul et al., 1997).

Table 1. Summary of the WCR transcriptome assembly using pooled data. See Eyun et al., 2014 for more details for the WCR transcriptome assembly.

Samples	Egg, neonate, and third-instar larval midgut
Number of paired-end reads before filtering	$1{,}462.2\times10^6$ ($144{,}690\times10^6$ bp)
Number of paired-end reads after filtering	781.7×10^6 ($77{,}393\times10^6$ bp)
Assembly program used	Trinity (2013-02-25)
Total number of contigs	163,871
Average contig length (range)	914 bp (201–31,064 bp)
N50 length	1,396 bp

Results & Discussion

❑Our preliminary analysis identified 54 or more gustatory receptors from WCR larval/egg transcriptome. $GABA_A$ receptor gene sequence was also identified.

❑Among the gustatory receptor genes, three corresponding to CO_2 receptor genes (*Gr1*, *Gr2*, and *Gr3*) were compared against the draft genomic sequences (Figs, 1, 2, and 3). Five, six, and seven introns were identified from these *Gr* genes. Intron numbers and locations for these genes are not always conserved between WCR and *Tribolium*.

We also identified introns in the $GABA_A$ receptor gene in WCR (Fig 4). Nine introns were identified. Locations of all but one intron are conserved between WCR and *Tribolium*.

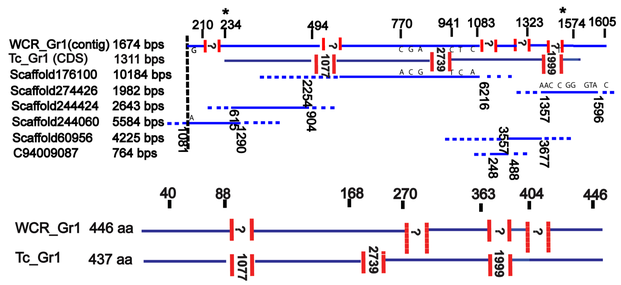

Figure 1. Alignment of the WCR contig sequence corresponding to the Gr1 (CO_2 receptor) gene against the scaffold sequences from the draft genome as well as the *T. castaneum* gene (Acc#XP_973273.1). The intron boundaries are indicated by the red bars with the intron lengths. The coding region in the contig is indicated by the region between the *. The unaligned regions of scaffolds are indicated by dotted blue lines. Polymorphic sites found between the contig and the genomic sequences are also indicated.

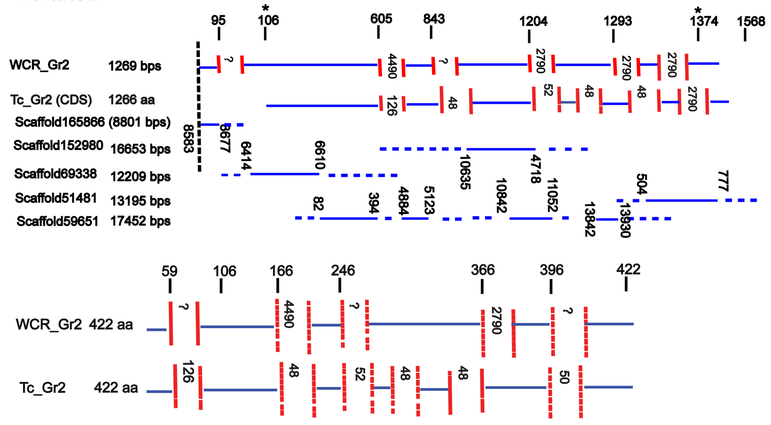

Figure 2. Alignment of the WCR contig sequence corresponding to the Gr2 (CO_2 receptor) gene against the scaffold sequences from the draft genome as well as the *T. castaneum* gene (Acc# EFA02924.1).

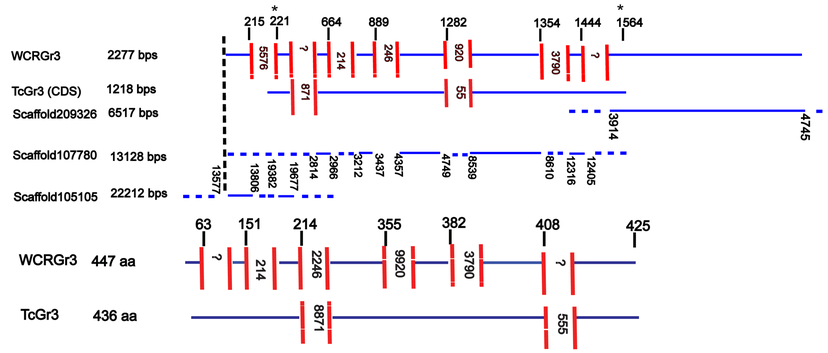

Figure 3. Alignment of the WCR contig sequence corresponding to the Gr3 (CO_2 receptor) gene against the scaffold sequences from the draft genome as well as the *T. castaneum* gene (Acc#NP_001107764.1).

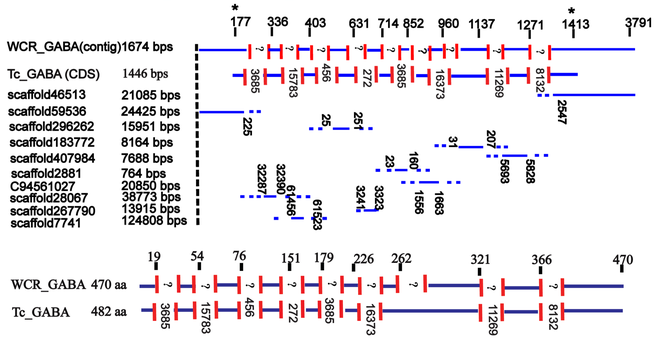

Figure 4. Alignment of the WCR contig sequence corresponding to $GABA_A$ receptor gene against the scaffold sequences from the draft genome as well as the *T. castaneum* gene (Acc# NP_001107764.1).

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