Sequencing the Navel Orangeworm (NOW) Genome to Identify Genes Associated with Detoxification and Insecticide Resistance

Project No.: **11.ENTO1.Berenbaum**

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Objectives:

Our general immediate objective is to characterize the genome of the navel orangeworm using Illumina sequencing and manual annotation. Our specific objectives are a) sequencing and assembly, b) annotation, c) post-sequence use of genomic data.

A goal of this work is to characterize insect metabolizing enzymes (derived from the navel orangeworm gene) that may influence insecticide resistance. In doing this, resistance management plans can be developed, and as well, research is being done on manipulating the rate of detoxification of these enzymes and using the phytochemicals present in almond to do this.

Interpretive Summary:

The availability of the genome of the navel orangeworm will allow us, and other investigators, to determine which genes are upregulated, or turned on, by pesticides (including pyrethroids and the new ryanodine agonists Altacor, Belt), and hence likely to be responsible for their metabolism and detoxification. The steps involved in this effort include: 1) to identify genes that encode the larval enzymes that allow them to feed on a wide range of hosts; 2) to characterize specific chemicals in hostplants that stimulate antennal, tarsal, or ovipositor receptors that are used in hostfinding; 3) to identify olfactory receptors that allow adult females to identify oviposition sites, to identify olfactory or gustatory receptors that allow larvae to recognize and accept hostplants; and 4) to continue to conduct and integrate bioassays on interactions between phytochemicals and insecticides into mechanistic studies of specific detoxification enzymes with the goal of identifying specific inhibitors of insecticide-metabolizing enzymes, which can be used by growers to preserve efficacy and delay resistance acquisition.

Materials and Methods:

The sequencing and annotation of the navel orangeworm (NOW) genome is well underway. Using high-throughput ILLUMINA sequencing, we obtained two lanes of 180 bp-insert paired-

end library reads and two lanes of paired-end reads from a 1.5 kb-insert shotgun library. After quality-trimming reads and error correction with QUAKE[1] we assembled the reads using SOAPdenovo[2]. The preliminary 368 Mb assembly consists of 134,000 scaffolds and contigs, with an average 2.7kb scaffold size and a scaffold N50 of 16.6 kb. The estimated contig coverage depth is 36X. To improve this assembly, we are constructing a 5kb mate-pair library for longer range scaffolding.

While the genome assembly is being improved with libraries from longer inserts, we have begun the process of manual gene annotation, specifically seeking detoxification and chemosensory genes. To complement this effort, we carried out larval midgut Illumina RNAseq, which can inform an eventual automated genome annotation. We assembled the reads using Trinity[3], a denovo transcriptome assembler.

Results and Discussion:

With a projected genome size of 400 Mb, we now have determined that the NOW genome is comparable in size to the other two available lepidopteran genomes (the 432 Mb *Bombyx mori* and 269 Mb of *Heliconius melpomene).* It appears to be highly monomorphic, which should greatly aid assembly and annotation efforts.

We formatted a BLAST database from the 186,000 assembled transcripts and are now querying the database with annotated lepidopteran cytochrome P450 detoxification genes. Using both the genomic and transcriptomic data will allow us to hone in on the NOW P450s that are relevant to pesticide and plant detoxification for further study.

Research Effort Recent Publications:

No publications have yet been produced from this project but we anticipate being able to submit a manuscript soon.

References Cited:

1 Kelley, DR, Schatz MC, and Salzburg SL. QUAKE: quality-aware detection and error correction of sequencing errors. 2010. Genome Biol. 11:R116.

2 Ruiqiang Li, Chang Yu, Yingrui Li, Tak-Wah Lam, Siu-Ming Yiu, Karsten Kristiansen and Jun Wang. De novo assembly of human genomes with massively parallel short read sequencing. 2010. Genome Res 20: 265-272

3 Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A. Full-length transcriptome assembly from RNA-seq data without a reference genome. 2011. Nat Biotechnol 29-7.