Abstract

Diet, nutrition, and obesity are important topics of current research. While many insect genome and/or transcriptome models are based on dietary specialists, the lady beetle *Coleomegilla maculata*, a common New World species, is highly omnivorous. *C. maculata* feeds on plants, fungi, insects and other arthropods; its diet frequently includes conspecific cannibalism. This study reports and discusses the first nutritionally based *C. maculata* transcriptomes. These transcriptomes were prepared from highly inbred specimens provided limited diets, after adult eclosion, of either pollen only or eggs of a soft bodied hemipteran insect only. Selected sequences from the transcriptomes were compared to verify basic genetic similarity of the sampled individuals. Differentially expressed genes associated with these diets were identified to aid with studies of omnivore diet and nutrition. Selected transcriptome sequences described herein are filed with the National Center for Biotechnology Information (NCBI), GenBank Bioproject PRJNA236444.

Key words: insect nutrition, omnivory, digestion, RNA stability, gene expression, Coccinellidae, biological control.

Introduction

North American agroecosystems are highly manipulated, particularly in the United States. Nonetheless, certain key insects persist within these ecosystem, acting as pests (consumers of or damaging to crops), as commensals, and as beneficials. One key beneficial insect found in many U.S. agroecosystems is the lady beetle *Coleomegilla maculata* (Coccinellidae: Coleoptera). *C. maculata* is a widely distributed [1] native North American species complex [2] and is generally accepted as an ecological indicator [3] and used in agricultural and ecological research as a representative non-target organism [4, 5]. Given its importance in agricultural ecology, the biology of *C. maculata* has been well studied. Studies of the diet of *C. maculata* have evaluated nutrition, focusing on pollen [6, 7], prey [8], and aiming at artificial diet development [9]. In the present work, gene expression in otherwise identical inbred specimens of this species of lady beetle are compared after restricted dietary intake, during the imaginal (adult) stage, of either pollen or insect eggs. The genetic resources described herein will facilitate further research on diet and digestive function in omnivorous organisms.

Results and Discussion

While *C. maculata* is accepted as an ecologically important lady beetle, it has not been widely utilized as a biological control agent. Some species of the family Coccinellidae are used as biological control in the US, such as *Hippodamia convergens* and *Adalia bipunc-
**Harmonia axyridis**, an introduced but invasive lady beetle in the US, has recently been modified to produce transgenic research organisms, and will be useful as a genetic model [11]. *C. maculata* has characters that make it a preferred model, including ease of maintenance [12] and visible phenotypic mutant strains ([13] and unpublished results). The genome size of *C. maculata* is relatively small, estimated at 0.19 pg [14]; based on standard conversion, assuming a diploid genome:

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\text{Genome size (Mbp) = 978 X DNA content (pg)}
\]

this is roughly equivalent to 186 Mbp [15] so sequencing the full genome of this insect should be achievable. A reference transcriptome of *C. maculata* has been published to the internet (http://2ei.univ-perp.fr/?page_id=89, accessed 22 July 2014). The transcripts provided by the study described herein will facilitate further molecular genetic, biochemical, and physiological investigation. Because the insects used for this study were highly inbred and also siblings, the sequences represent a limited number of alleles, and do not represent the wider populations of the species in Mississippi or the broader environment. On the other hand, the sequences obtained may be used for a baseline to determine population genetics and identify variability in the species and between subspecies.

Assemblies of the total RNA yielded 33,833 assembled sequences from the pollen fed treatment and 34,167 assembled sequences from the insect egg fed treatment. The combined sequences assembled into 43,151 sequences. Average sequence lengths were 1403, 1300, and 1456 respectively. While many of the treatment group sequences were identical or nearly so, there were representatives of unique sequences as shown in Figure 1. Because the assemblies were de novo, many unique and unclassified sequences were expected. To gain an insight on the similarities between the assemblies, sequences were analyzed by NCBI BLAST® (US National Library of Medicine) [16] using the tBLASTx “search translated nucleotide database using a translated nucleotide query” (database accessed 22 May 2013). The resulting BLAST spreadsheets were sorted and the longer and more similar (to NCBI accessions) sequences were examined. Sequences shorter than 500 nt and with expect values >1e-10 were discarded. The remaining sequences amounted to around 10% of the total assembled sequences: 3,376 from the pollen fed treatment, and 3,358 from the insect egg fed treatment (Table 1A, Table 1B). The sequences from both treatments were primarily similar to other insect sequences (95%, +/- 0.5%), and to RNA sequences (not specifically analyzed). The largest portion of similarities by insect order was to Coleoptera, as expected (49%), as shown by two pie charts, one for each treatment, in Figure 2.

The individual insects used for sequencing were inbred siblings, thus many identical sequences from the two samples were expected. The greatest number of similar sequences in GenBank came from the two beetle species *Tribolium castaneum* and *Dendroctonus ponderosae*. To validate the baseline similarity of our sequence sets, sixteen sequences that were most closely related to the same GenBank sequence, and at similar expect values and similar lengths, were chosen from each treatment and compared pairwise in BLAST (BLAST two sequences option). Results are shown in Table 2; all comparisons were at least 99% identical at the nucleotide level. These results support the assumption that

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**Figure 1.** Diagram of comparison of total contiguous sequences generated from adult insect samples fed either a diet of only insect eggs (IE) or only pollen (P).
the two sets of sequences represent nearly identical specimens, differing primarily in those transcripts of genes responding to diet provided to the adult insects. Also as expected, some sequences that were unique to the treatments were associated with the diet [17]. Among the pollen fed sequences were hits similar to plant sequences, and among the egg fed sequences were hits similar to the genus of the insect eggs, Lygus spp. Interestingly, some of the egg diet sequences were nearly identical (e-value 0) to a virus recently described from Lygus lineolaris [18]. It appears that the viable Lygus spp. eggs used as diet were carrying the virus, indicating that the virus was present in the laboratory colony and was able to resist degradation by the digestive system of C. maculata. The implications of this finding may be important for future pest control strategies aimed to utilize genetically modified or pathogenic viruses.

**Figure 2.** Individual transcriptomes of samples are very similar in overall characteristics, as expected for inbred sibling samples. Pie chart comparison of most similar (e<sub>-10</sub>) and longest (>500 nt) contiguous sequences generated from adult insect samples fed either a diet of only insect eggs (IE) or only pollen (P). Roughly 10% of the total sequences are represented.
Table 1A. Closest homologous sequences; quantities by taxonomy. Treatment: pollen diet, 3376 non-redundant sequences with expect value of <1.00e-10. Right hand columns are insect genera, left hand columns are non-insect. Sequences that differ from the insect egg diet treatment in quantity of hits are indicated by asterisk (*).

| Non-insect | Gene          | Hits | Non-specific higher taxonomic grouping |
|------------|---------------|------|----------------------------------------|
| * virus    | 5             | virus|                                        |
| Phyllocladus | 2              | Bacteria | Alphaproteobacteria, Rhizobiales |
| Simorhabdus | 1              | Bacteria | Alphaproteobacteria, Rhizobiales |
| Crinilium  | 1              | Bacteria | Cyanobacteria |
| Cronobacter | 1              | Bacteria | Gammaproteobacteria, Enterobacteriaceae |
| uncultured | 1              | Bacteria | unknown |
| Dectonymia | 1              | Eukarya | Actinobacteria |
| Entamoeba  | 1              | Eukarya | Ascomycota |
| * Babesia | 1 | Eukarya | Apicomplexa |
| * Neopora  | 1 | Eukarya | Actinobacteria |
| * Candida  | 2 | Eukarya | Fungi, Saccharomyces |
| Kluyveromyces | 1 | Eukarya | Fungi, Saccharomyces |
| * Leptoperlmata | 1 | Eukarya | Fungi, rot fungus |
| * Millironyma | 1 | Eukarya | Fungi, Saccharomyces |
| * Rhioporus | 1 | Eukarya | Fungi, beard mold |
| * Tetrapospora | 1 | Eukarya | Fungi, subgroup  |
| Nugeliers | 1 | Eukarya | Heterolobosea, Vahlkampfiidae |
| Hydra | 3 | Eukarya | Hydrozoa |
| * Coerperhadiis | 4 | Eukarya | Nematoda, Rhabditidae |
| Dieleyneus | 1 | Eukarya | Nematoda, Tylenchidae |
| Haemonomus | 1 | Eukarya | Nematoda, Haemonchidae |
| Trichinella | 1 | Eukarya | Nematoda, Trichinellidae |
| Trichoplus | 2 | Eukarya | Placozoa |
| * Dugensia | 2 | Eukarya | Planthallina, Geopanoidea |
| Schistosoma | 2 | Eukarya | Planthallina, Schistosomatidae |
| * Trichobilharzium | 2 | Eukarya | Planthallina, Schistosomatidae |
| * uncultered eskaryte | 2 | Eukarya | unknown |

Total 42 5% microbial: 1.24%

| Insect Genus | Hits | Order | Family | Order total | Percent |
|--------------|------|-------|--------|-------------|---------|
| Periplaneta  | 1    | Blattodea | Blattidae |     |         |
| * Blatella  | 2    | Blattodea | Ectobiidae |   |         |
| * Blattina  | 3    | Blattodea | Blattidae |   |         |
| * Dactariscus | 1 | Coleoptera | Botheideridae | | |
| Aprina | 1 | Coleoptera | Cerambycidae | | |
| Tetrapica | 1 | Coleoptera | unknown | | |
| Calliphorina | 1 | Coleoptera | Chrysomelidae | | |
| * Chrysoena | 10 | Coleoptera | Chrysoinae | | |
| * Coccinella | 1 | Coleoptera | Chrysomelidae | | |
| * Diabrotica | 2 | Coleoptera | Chrysomelidae | | |
| Gnorimeta | 1 | Coleoptera | Coleoptera | | |
| * Lepisterna | 2 | Coleoptera | Coleoptera | | |
| Phaedon | 2 | Coleoptera | Chrysomelidae | | |
| * Timarcha | 2 | Coleoptera | Chrysomelidae | | |
| * Adalia | 4 | Coleoptera | Coccinellidae | | |
| * Coccinella | 12 | Coleoptera | Coccinellidae | | |
| * Coleomegilla | 6 | Coleoptera | Coccinellidae | | |
| Halizia | 1 | Coleoptera | Coccinellidae | | |
| * Harmonia | 12 | Coleoptera | Coccinellidae | | |
| Hemospilida | 1 | Coleoptera | Coccinellidae | | |
| * Hypospodia | 2 | Coleoptera | Coccinellidae | | |
| Propylea | 1 | Coleoptera | Coccinellidae | | |
| Aphanius | 1 | Coleoptera | Coccinellidae | | |
| Carphoborus | 1 | Coleoptera | Coccinellidae | | |
| * Cercidium | 4 | Coleoptera | Coccinellidae | | |
| * Dendroctonus | 467 | Coleoptera | Coccinellidae | | |
| Hyneria | 1 | Coleoptera | Coccinellidae | | |
| Ips | 2 | Coleoptera | Coccinellidae | | |
| * Silphina | 1 | Coleoptera | Coccinellidae | | |
| Eretes | 1 | Coleoptera | Dytiscidae | | |
| * Melypteryx | 105 | Coleoptera | Coccinellidae | | |
| Agiotroba | 12 | Coleoptera | Coccinellidae | | |
| Luciola | 1 | Coleoptera | Luctridae | | |
| Georius | 2 | Coleoptera | Georissa | | |
| Hister | 1 | Coleoptera | Histeridae | | |
| Melolontha | 1 | Coleoptera | Melolonthidae | | |
| Myctophus | 2 | Coleoptera | Myctophagidae | | |
| Orthopterina | 2 | Coleoptera | Scaphiidae | | |
| Trachypius | 2 | Coleoptera | Scaphoidea | | |
| Microneta | 1 | Coleoptera | Tenebrionidae | | |
| * Tenebrio | 2 | Coleoptera | Tenebrionidae | | |
| * Tribolium | 1105 | Coleoptera | Tenebrionidae | | |
| Mylabris | 1 | Coleoptera | Mylabridae | | |
| * Acetes | 24 | Coleoptera | Coccinellidae | | |
| * Athetaedes | 32 | Coleoptera | Coccinellidae | | |
| * Cales | 24 | Coleoptera | Coccinellidae | | |
| Oedemerida | 1 | Coleoptera | Coleoptera | | |
| * Drosophila | 70 | Diptera | Drosophilidae | | |
| * Brachylora | 1 | Coleoptera | Melyridae | | |
| Musca | 1 | Diptera | Muscidae | | |
| Nemoptera | 1 | Diptera | Psychodidae | | |
| Oxyurus | 2 | Diptera | Stratiomyidae | | |
| * Anopheles | 4 | Diptera | Tabanidae | | |
| Bactrocera | 1 | Diptera | Tabanidae | | |
| * Ceratitis | 26 | Diptera | Tabanidae | | |
| Tiphia | 1 | Diptera | Tiphidae | | |
| Bemisia | 1 | Hemiptera | Aleyrodidae | | 86 2.55 |
| * Riptortus | 16 | Hemiptera | Alygidae | | |
| * Toxoptera | 3 | Hemiptera | Aleyrodidae | | |
| * Acaryioscanthus | 57 | Hemiptera | Aleyrodidae | | |
| Aphidoila | 1 | Hemiptera | Aleyrodidae | | |
| * Lathocoris | 1 | Hemiptera | Belostomatidae | | |
| Laodelphax | 2 | Hemiptera | Delphacidae | | |
| Beroea | 2 | Hemiptera | Beroeidae | | |
| * Macrocletus | 2 | Hemiptera | Macroceridae | | |
| * Diasphera | 1 | Hemiptera | Psyllidae | | |
| Apis | 224 | Hymenoptera | Apidae | | 95 | 14.3 |
| * Bombus | 96 | Hymenoptera | Apidae | | |
| * Glycyptotus | 1 | Hymenoptera | Bomboridae | | |
| * Lysiphlus | 7 | Hymenoptera | Braconidae | | |
| * Nasonia | 63 | Hymenoptera | Braconidae | | |
| Camponotus | 1 | Hymenoptera | Formicidae | | |
| * Megachile | 84 | Hymenoptera | Megachilidae | | |
| Osmia | 76 | Hymenoptera | Megachilidae | | 24 3.67 |
| * Bembix | 47 | Hymenoptera | Bombycidae | | |
| * Prionocystis | 1 | Hymenoptera | Cossidae | | |
| * Osmia | 7 | Hymenoptera | Crambidae | | |
| * Blaps | 1 | Hymenoptera | Gelechiidae | | |
| * Dendrolimus | 1 | Lepidoptera | Lasiocampidae | | |
| * Tais | 1 | Lepidoptera | Lecithoceridae | | |
| * Tilia | 1 | Lepidoptera | Lepidoptera | | |
| * Hecale | 1 | Lepidoptera | Noctuidae | | |

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Table 1B. Closest homologous sequences; quantities by taxonomy. Treatment: insect egg diet, 3358 non-redundant sequences with expect value of <1.00e-10. Right hand columns are insect genera, left hand columns are non-insect. Sequences that differ from the pollen diet treatment in quantity of hits are indicated by asterisk (*).

| Non-insect Genus | Phylum | Non-specific higher taxonomic grouping |
|------------------|--------|----------------------------------------|
| Anguilla         | 2      | Teleostei (bony fishes) Anguillidae (eel) |
| Anoplopoma      | 1      | Teleostei (bony fishes) sablefish     |
| Cotus            | 1      | Teleostei (bony fishes) Cottidae (sculpins) |
| * Danio          | 1      | Teleostei (bony fishes) Cyprinidae    |
| Maylandia        | 2      | Teleostei (bony fishes) Cichlidae     |
| * Notobranchius  | 6      | Teleostei (bony fishes) killifish     |
| Salmo            | 1      | Teleostei (bony fishes) Salmonidae (salmon) |
| Simocypridella   | 3      | Teleostei (bony fishes) Cyprinidae    |
| Takifugu         | 4      | Teleostei (bony fishes) Tetraodontidae (puffers) |
| Tetraodon        | 1      | Teleostei (bony fishes) Tetraodontidae (puffers) |
| * Labeo          | 1      | Actinopterygii (ray-finned fishes) Araceae lice |
| * Carassius       | 1      | Actinopterygii (ray-finned fishes) Brissitaeae |
| * Scardinius      | 1      | Actinopterygii (ray-finned fishes) Chlorophyta green algae |
| Cucurnis         | 1      | Actinopterygii (ray-finned fishes) Cucurbitaceae melons |
| * Glycine        | 4      | Actinopterygii (ray-finned fishes) Fabaceae soybean |
| Jatropa          | 1      | Actinopterygii (ray-finned fishes) Euphorbiaceae spurge |
| * Oryza          | 1      | Actinopterygii (ray-finned fishes) Poaceae rice |
| * Fragaria       | 1      | Actinopterygii (ray-finned fishes) Rosaceae strawberry |
| * Selaginella     | 1      | Actinopterygii (ray-finned fishes) Trachophyta spike moss |
| Total            | 132    | % non-insect metazoans: 3.91% |

(continued to right)
| Animal Group | Species | Host | Taxonomy | Notes |
|-------------|---------|------|----------|-------|
| Lepeophtheirus | 3 | Arthropoda | Copepoda | Caligidae (fish lice) |
| Tachypoecus | 1 | Arthropoda | Limulidae | horsehoe crab |
| Branchiosteorhiza | 3 | Cephalochorda | Branchiostomatidae | lancelets, amphioxus |
| Ichthyophthirius | 1 | Chondrichthyes | Ciliophora | Oligotrichinae |
| * Saccoglossus | 5 | Eukaryota | Hemichordata | Hemitrachelids (sipuncula) |
| Anoplophora | 1 | Eukaryota | Nematoda | Pentamerida (nematoids) |
| * Patinopenaeus | 1 | Eukaryota | Mollusca | Bivalvia (pelecypods) |
| * Bithynia | 4 | Mollusca | Gastropoda | Bithyniidae (snails) |
| Amphipredon | 1 | Ostracoda | Amphipoda | Sponges |
| * Seboides | 1 | Ostracoda | Tetrarhabdida | Abbreviated key |
| Rana | 1 | Echinodermata | Amphibia | Ranidae (frogs) |
| * Xenopus | 5 | Echinodermata | Amphibia | Pipidae (frogs) |
| Melongrana | 5 | Echinodermata | Aves | Turdidae |
| * Taeniopygia | 1 | Echinodermata | Aves | Finch |
| Discopyge | 1 | Echinodermata | Chondrichthyes | Nacridae (ray) |
| * Bo | 2 | Echinodermata | Mammalia | Bovidae (cattle) |
| Cephalopyge | 1 | Echinodermata | Mammalia | Capriniidae (goat) |
| Cavia | 2 | Echinodermata | Mammalia | Rodentia (guinea pigs) |
| Centipedium | 1 | Echinodermata | Mammalia | Rhinocerotidae (rhinoceros) |
| Dasyopus | 1 | Echinodermata | Mammalia | Erinaceidae (shrews) |
| Micrura | 1 | Echinodermata | Mammalia | Primatidae |
| Monodelphis | 1 | Echinodermata | Mammalia | Didelphidae (opposum) |
| * Mus | 2 | Echinodermata | Mammalia | Rodentia (mouse) |
| Neomus | 2 | Echinodermata | Mammalia | Primatidae (gibbon) |
| Ochoton | 1 | Echinodermata | Mammalia | Pika |
| Odocoileus | 5 | Echinodermata | Mammalia | Odocoileidae (deer) |
| * Oryctolagus | 1 | Echinodermata | Mammalia | Leporidae (rabbits) |
| Ouloemur | 2 | Echinodermata | Mammalia | Primates |
| * Ovis | 1 | Echinodermata | Mammalia | Sheep |
| Rattus | 2 | Echinodermata | Mammalia | Rodentia (rat) |
| * Cricetulus | 3 | Eutheria | Mammalia | Dasyuridae (Tasmanian devil) |
| Trichinus | 4 | Eutheria | Mammalia | Trichidae (manatee) |
| * Anolis | 1 | Eutheria | Mammalia | Squamata |
| Chrysemys | 1 | Eutheria | Mammalia | Testudines |
| Gallus | 1 | Aves | Acipenseridae | Phasianidae (fowl) |
| Anguilla | 2 | Aves | Teleostei | Anguillidae (eel) |
| Anguipoma | 3 | Aves | Teleostei | Saurelidae (sailfish) |
| Cottus | 1 | Aves | Teleostei | Cottidae (sculpins) |
| Dorion | 2 | Aves | Teleostei | Ophidiidae (conger) |
| * Scaphirhynchus | 5 | Aves | Teleostei | Blennidae (lagoon fish) |
| * Salmo | 1 | Aves | Teleostei | Salmonidae (salmon) |
| Sinocypridichthys | 3 | Aves | Teleostei | Cyprinidae |
| Takifugu | 4 | Aves | Teleostei | Tetraodontidae (puffers) |
| Teredon | 1 | Aves | Teleostei | Tetraodontidae (puffers) |
| Cucumis | 1 | Aves | Cucurbitaceae | melons |
| Jatropha | 1 | Aves | Euphorbiaceae | spurge |
| * Psychophis | 1 | Aves | Orchidacea | orchid |
| Total | 122 | | | 3.63% |

* indicates a difference in presence (present in one sample but not the other) or a difference in quantity of transcripts found.
† Non-insect taxa are highly variable, so generally recognizable taxonomic names are listed. Taxonomic identifications are from NCBI Taxonomy Browser, [http://www.ncbi.nlm.nih.gov/Taxonomy/](http://www.ncbi.nlm.nih.gov/Taxonomy/) in the NCBI Taxonomy Browser database.

Metazoans are included in the "microbial" portion of the spreadsheet (ie; fungi); those categorized in % microbial may be associated with symbions or food, rather than insect.
Table 2. Selected sequences (16) from treatment samples that appear identical, and their actual nt identities. Abbreviations incorporated into sequence IDs: Insect egg (IE); pollen.

Table 3. Longest 15 sequences unique in treatments. P: pollen diet, IE: insect egg diet.

The fifteen longest unique sequences from each diet treatment are listed in Table 3. While the unique sequences in pollen-fed transcripts appear to be related to carbohydrate cleavage, which seems logical for animals digesting plant materials, the unique sequences from the egg-fed did not appear to match any obvious category of transcript, other than the insect virus [18] mentioned earlier.

Further analyses of the transcripts reported here will provide insight on genes that are linked to differential metabolism of plant and animal based diets. The sequences identified here, after validation among further representatives of this species, will be used to measure quantitative changes in gene expression between insects utilizing multiple sources of foods, and those that are deprived of specific dietary components. Potential projects including varying diet at different stages of insect development; this species is known to utilize different diet components at different stages of development [19-21]. Another possibility is to evaluate genetic responses to specific prey, as C. maculata has been shown to utilize prey of specific sizes and species [22]. This will help us understand how to produce high quality generalist predator bio-

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logical control agents, and assure conservation of beneficial insects in our constantly changing world environment. The details of the genes expressed by this omnivorous insect, and their correlation with diet and nutrition, will provide insights into nutritional health of other omnivores including humans.

Methods

Insect culture

Lady beetles used to establish laboratory colonies were collected from fields surrounding the USDA-ARS and Mississippi State University Delta Research and Extension Center in Stoneville, MS, 38776. Insect cultures were maintained in the National Biological Control Laboratory at Stoneville, MS without wild specimen introgression from August 2010 through the time of RNA sample collection in March 2013. Insects were reared as larvae in Petri dishes of sizes ranging from 35 to 250mm with mesh glued into one side of the dish for ventilation, and as adults in 5.25 x 5.25 inch cages [12]. Temperature was generally maintained at 24°C for 16 lighted hours and 19°C for 8 dark hours in Percival (Perry, IA) E30B growth chambers. Larvae and adults were fed ad libitum a combination of pollen, Daphnia, Brewer’s yeast, honey, and eggs of laboratory cultures of Lygus spp [12]. Water was provided in 1.5 ml microcentrifuge tubes with caps removed and plugged with cotton.

Inbreeding for homozygosity

Inbreeding of beetle stock was performed by isolating individual gravid females from the primary wild type colony, collecting and rearing all eggs from individual females, and continuing culture using only offspring of the most fertile and fecund female. From the resulting culture the inbreeding step (female selection) was repeated for a total of six isofemale (I6) selection steps.

Sample preparation

Insect specimens from a single egg mass collected from the I6 colony were fed standard diet as larvae. Surviving individuals were isolated after pupation and provided either pollen alone or Lygus eggs alone upon adult eclosion. Total RNA was isolated from whole individual adults six days after adult eclosion. Specimens were spray washed with reverse osmosis (RO) water to remove any food particles. After one hour isolation (resting from wash and away from food) specimens were briefly anesthetized with carbon dioxide. Insects were transferred to sample tubes and crushed whole in RNA extraction buffer using blue Kontes® (Kimble Chase, Thermo Fisher Scientific, Waltham, MA) pestle. Total RNA was extracted using USB PrepEase total RNA kit following manufacturer instructions (Affymetrix, Santa Clara, CA). Samples were measured using a NanoDrop 1000 (Thermo Fisher Scientific) spectrophotometer, and the samples with highest final concentration and highest 260/230 ratio were chosen for sequencing. RNA samples were kept in ultralow freezer set for -75°C until shipping.

Transcript Sequencing

Total RNA samples were shipped on dry ice to the University of Washington High-Throughput Genomics Center, WTC East, Suite 600, 2211 Elliott Ave., Seattle, WA 98121-1692. Illumina (Illumina, Inc., San Diego, CA, USA) RNA-seq library construction, 36 bp single end multiplex quality control library testing, and 76 bp paired end multiplex 4x sequencing was followed by Trinity (Broad Institute, MA) contiguous sequence assembly [23]. Sequences were assembled by diet treatment (eggs only or pollen only) and as a combined group assembly. Assembled sequences were limited by request to >200nt. Assembled sequences were provided to the USDA ARS Genomics and Bioinformatics Research Unit (GBRU) in Stoneville, MS for further analysis.

Sequence Analyses

Assembled sequences were analyzed by NCBI BLAST® (US National Library of Medicine) [16] using the tBLASTx “search translated nucleotide database using a translated nucleotide query” (database accessed 22 May 2013). BLAST results were analyzed by diet treatment (eggs only or pollen only) and as a combined group. Spreadsheet results were sorted by Hit Score and only those sequences in the two treatment groups meeting the combined criteria of >500 nucleotide (nt) and E (expect) score ≤E-10 were used for further analysis.

Because the sequenced samples were derived from two individual insects that differed only in their adult diet, the transcriptomes were expected to be nearly identical for those sequences representing genes that were not associated with diet. To verify that assemblies represented genetically similar individuals as expected, sixteen sequences were chosen based on apparent identity by length of assembled sequence and closest tBLASTx hit. Sequences were chosen from the group most similar to Tribolium castaneum sequences and from the group most similar to Dendroctonus ponderosae sequences, with the assumption that sequences from other beetles would represent conserved transcripts. Sequences from pollen-fed
(P) and insect egg-fed (IE) were compared at the nucleotide level using BLAST.

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Competing Interests

The author has declared that no competing interest exists.

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