


Research Paper

Characterization of Adult Transcriptomes from the Omnivorous Lady Beetle *Coleomegilla maculata* Fed Pollen or Insect Egg Diet

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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-*

tata [10]. *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:

$$\text{Genome size (Mbp)} = 978 \times \text{DNA content (pg)}$$

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

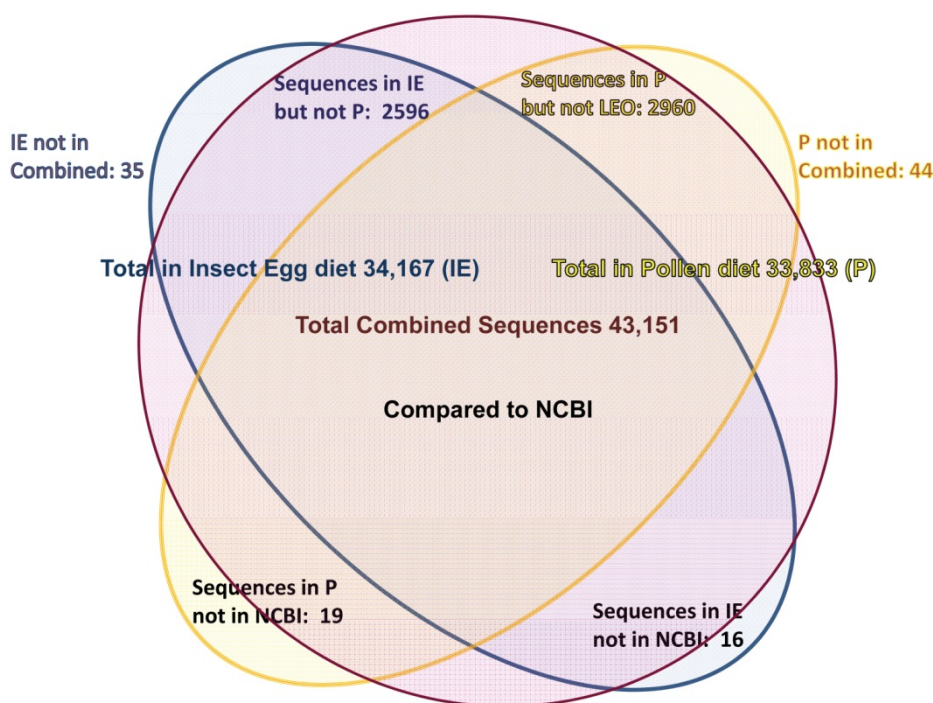
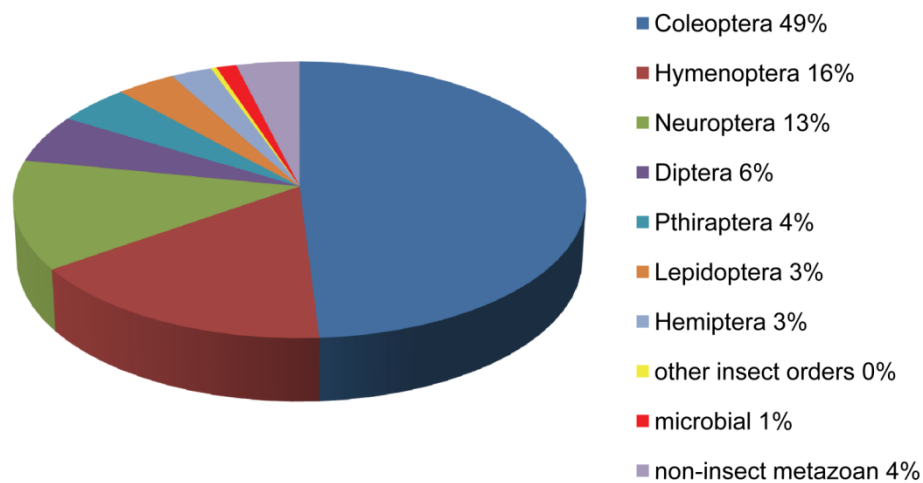


Figure 1. Diagram of comparison of total contiguous sequences generated from adult insect samples fed either a diet of only insect eggs (IE) or only pollen (P).

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.

Insect egg (IE) diet treatment: sequences with closest similarity to NCBI accessions (score E-10 or better)



Pollen (P) diet: sequences with closest similarity to NCBI accessions (score E-10 or better)

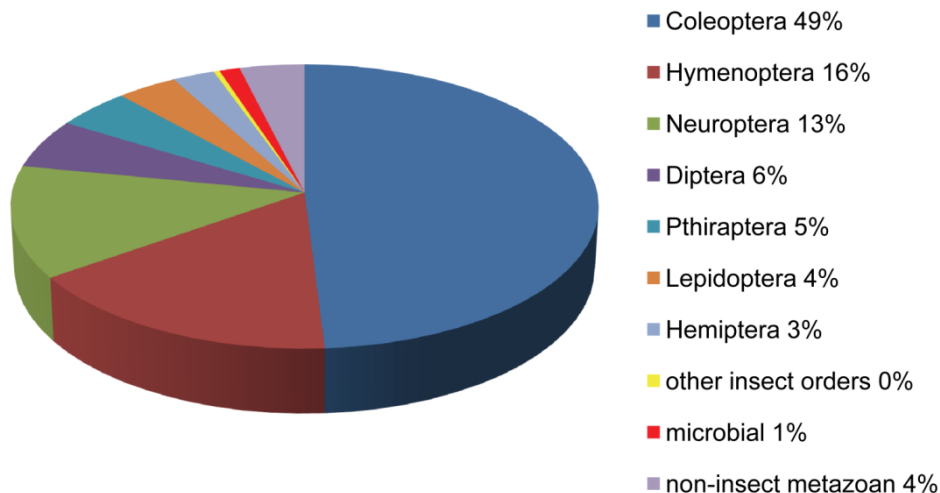


Figure 2. Individual transcriptomes of samples are very similar in overall characteristics, as expected for inbred sibling samples. Pie chart comparison of most similar (expect $<e-10$) and longest (>500 nt) contiguous sequences generated from adult insect samples fed either a diet of only insect eggs (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 <math> < 1.00e-10 </math>. 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				(continued from left)					
† Genus	hits	Non-specific higher taxonomic grouping		Insect Genus	hits	Order	Family	Order total	Percent
* virus	5	virus		Periplaneta	1	Blattodea	Blattidae		
Phyllobacterium	2	Bacteria	Alphaproteobacteria Rhizobiales	* Blattella	2	Blattodea	Ectobiidae		
Sinorhizobium	1	Bacteria	Alphaproteobacteria Rhizobiales	Biphyllus	3	Coleoptera	Biphylidae	1664	49.29%
Crinalium	1	Bacteria	Cyanobacteria	* Dastarcus	1	Coleoptera	Bothriidae		
Cronobacter	1	Bacteria	Gammaproteobacteria Enterobacteriaceae	Apriona	1	Coleoptera	Cerambycidae		
uncultured bacteria	1	Bacteria	unknown	Tetraopes	1	Coleoptera	Cerambycidae		
Dictyostelium	1	Eukaryota	Amoebozoa Mycetozoa	Callosobruchus	1	Coleoptera	Chrysomelidae		
Entamoeba	1	Eukaryota	Amoebozoa Entamoebidae	* Chrysomela	10	Coleoptera	Chrysomelidae		
* Babesia	1	Eukaryota	Apicomplexa	* Crioceris	1	Coleoptera	Chrysomelidae		
* Neospora	1	Eukaryota	Apicomplexa	* Diabrotica	2	Coleoptera	Chrysomelidae		
* Candida	2	Eukaryota	Fungi Saccharomycetes	Gonioctena	1	Coleoptera	Chrysomelidae		
Kluyveromyces	1	Eukaryota	Fungi Saccharomycetes	* Leptinotarsa	2	Coleoptera	Chrysomelidae		
* Leptosphaeria	1	Eukaryota	Fungi rot fungus	Phaedon	2	Coleoptera	Chrysomelidae		
Milleromyza	1	Eukaryota	Fungi Saccharomycetes	Timarcha	2	Coleoptera	Chrysomelidae		
* Rhizopus	1	Eukaryota	Fungi bread mold	* Adalia	4	Coleoptera	Coccinellidae		
* Tetrapispora	1	Eukaryota	Fungi Saccharomycetes	* Coccinella	12	Coleoptera	Coccinellidae		
Naegleria	1	Eukaryota	Heterolobosea	* Coleomegilla	6	Coleoptera	Coccinellidae		
Hydra	3	Eukaryota	Hydrozoa	Halyzia	1	Coleoptera	Coccinellidae		
* Caenorhabditis	4	Eukaryota	Nematoda Rhabditidae	* Harmonia	12	Coleoptera	Coccinellidae		
Ditylenchus	1	Eukaryota	Nematoda Tylenchina	Henosepilachna	1	Coleoptera	Coccinellidae		
Haemonchus	1	Eukaryota	Nematoda Haemonchidae	* Hippodamia	2	Coleoptera	Coccinellidae		
Trichinella	1	Eukaryota	Nematoda Trichinellidae	Propylea	1	Coleoptera	Coccinellidae		
Trichoplax	2	Eukaryota	Placozoa	Aphanarthrum	1	Coleoptera	Curculionidae		
* Dugesia	2	Eukaryota	Platyhelminthes Geoplanoidea	Carphoborus	1	Coleoptera	Curculionidae		
Schistosoma	2	Eukaryota	Platyhelminthes Schistosomatidae	* Curculio	4	Coleoptera	Curculionidae		
* Trichobilharzia	1	Eukaryota	Platyhelminthes Schistosomatidae	* Dendroctonus	467	Coleoptera	Curculionidae		
* uncultured eukaryote	2	Eukaryota	unknown	Hypera	1	Coleoptera	Curculionidae		
Total	42		% microbial: 1.24%	Ips	2	Coleoptera	Curculionidae		
† Non-insect Genus		Non-specific higher taxonomic grouping, common name		Sitophilus	1	Coleoptera	Curculionidae		
Platynereis	1	Annelida	Polychaeta segmented worm	Eretes	1	Coleoptera	Dytiscidae		
Urechis	1	Annelida	Polychaeta spoonworm	* Meladema	1	Coleoptera	Dytiscidae		
* Amblyomma	2	Arthropoda	Acari Ixodidae	Agriotes	1	Coleoptera	Elaterridae		
* Ixodes	8	Arthropoda	Acari Ixodidae	Luciola	1	Coleoptera	Elaterridae		
Metaseiulus	3	Arthropoda	Acari Phytoseiidae (mites)	Georissus	2	Coleoptera	Georissidae		
Phrynus	1	Arthropoda	Arachnida Phryniidae (whip spiders)	Hister	1	Coleoptera	Histeridae		
Latrodectus	1	Arthropoda	Araneae Theridiidae (widow spiders)	Mylabris	1	Coleoptera	Meloidae		
* Lepeophtheirus	2	Arthropoda	Copepoda Caligidae (fish lice)	Mycetophagus	2	Coleoptera	Mycetophagidae		
Tachyplesus	1	Arthropoda	Limulidae horseshoe crab	Onthophagus	2	Coleoptera	Scarabaeidae		
Branchiostoma	3	Cephalochordata	Branchiostomidae lancelets, amphioxus	Trypoxylus	1	Coleoptera	Scarabaeidae		
Ichthyophthirius	1	Ciliophora	Oligohymenophorea Ophryoglenina	Microdera	1	Coleoptera	Tenebrionidae		
* Nematostella	1	Cnidaria	Actiniaria sea star	* Tenebrio	2	Coleoptera	Tenebrionidae		
* Strongylocentrotus	3	Echinodermata	Euechinozoa	* Tribolium	1105	Coleoptera	Tenebrionidae		
* Saccoglossus	4	Hemichordata	Harrimaniidae	Myopa	1	Diptera	Conopidae	190	5.63%
Chlamys	1	Mollusca	Bivalvia Pectinidae (scallop)	* Aedes	24	Diptera	Culicidae		
* Sepia	1	Mollusca	Decapodiformes cuttlefish	* Anopheles	32	Diptera	Culicidae		
* Bithynia	3	Mollusca	Gastropoda Bithyniidae (snail)	* Culex	24	Diptera	Culicidae		
* Plakortis	1	Porifera	Homoscleromorpha sponges	Ochlerotatus	1	Diptera	Culicidae		
* Amphimedon	2	Porifera	Niphathidae sponges	* Teleopsis	1	Diptera	Diopsidae		
Suberites	1	Porifera	Tetractinomorpha	* Drosophila	70	Diptera	Drosophilidae		
Ciona	1	Tunicata	Enterogona	* Bicellaria	1	Diptera	Hybotidae		
Rana	1	Vertebrata	Amphibia Ranidae (frogs)	Musca	1	Diptera	Muscidae		
* Xenopus	3	Vertebrata	Amphibia Pipidae (frogs)	Nemopalpus	1	Diptera	Psychodidae		
Meleagris	3	Vertebrata	Aves turkey	Oxyera	2	Diptera	Stratiomyidae		
Taeniopygia	3	Vertebrata	Aves finch	* Anastrepha	4	Diptera	Tephritidae		
Discopyge	1	Vertebrata	Chondrichthyes Narcinidae (ray)	Bactrocera	1	Diptera	Tephritidae		
Bos	2	Vertebrata	Mammalia Bovidae (cattle)	* Ceratitis	26	Diptera	Tephritidae		
Canis	2	Vertebrata	Mammalia Canidae (dog, wolf)	Tipula	1	Diptera	Tipulidae		
Cavia	2	Vertebrata	Mammalia Rodentia (guinea pigs)	Bemisia	1	Hemiptera	Acyrodidae	86	2.55%
Ceratotherium	1	Vertebrata	Mammalia Rhinocerotidae (rhinoceros)	* Riptortus	16	Hemiptera	Alydidae		
* Cricetulus	1	Vertebrata	Mammalia Cricetidae (hamster)	* Toxoptera	3	Hemiptera	Aphididae		
Dasyus	1	Vertebrata	Mammalia armadillo	* Acyrthosiphon	57	Hemiptera	Aphididae		
* Loxodonta	1	Vertebrata	Mammalia elephant	Aphis	1	Hemiptera	Aphididae		
Macaca	1	Vertebrata	Mammalia Primate	* Lethocerus	1	Hemiptera	Belostomatidae		
Monodelphis	1	Vertebrata	Mammalia Didelphidae (opossum)	Laodelphax	2	Hemiptera	Delphacidae		
* Mus	1	Vertebrata	Mammalia Rodentia (mouse)	* Buena	2	Hemiptera	Notonectidae		
Nomascus	2	Vertebrata	Mammalia Primate (gibbon)	* Maconellicoccus	2	Hemiptera	Pseudococcidae		
Ochotona	1	Vertebrata	Mammalia pika	* Diaphorina	1	Hemiptera	Psyllidae		
* Odobenus	3	Vertebrata	Mammalia Odobenidae (walrus)	* Apis	224	Hymenoptera	Apidae	552	16.35%
Orcinus	1	Vertebrata	Mammalia Delphinidae (dolphins)	* Bombus	96	Hymenoptera	Apidae		
* Ornithorhynchus	8	Vertebrata	Mammalia Monotremata (platypus)	* Glyptapanteles	1	Hymenoptera	Braconidae		
* Otlemur	1	Vertebrata	Mammalia Primate	* Lysiphlebus	7	Hymenoptera	Braconidae		
* Rattus	1	Vertebrata	Mammalia Rodentia (rat)	* Nasonia	63	Hymenoptera	Braconidae		
* Saimiri	2	Vertebrata	Mammalia squirrel monkey	Camponotus	1	Hymenoptera	Formicidae		
* Sarcophilus	2	Vertebrata	Mammalia Dasyuridae (Tasmanian devil)	* Megachile	84	Hymenoptera	Megachilidae		
Trichechus	3	Vertebrata	Mammalia Trichechidae (manatee)	* Osmia	76	Hymenoptera	Megachilidae		
Anoplopoma	1	Vertebrata	Percomorpharia	* Bombyx	47	Lepidoptera	Bombycidae	124	3.67%
* Anolis	4	Vertebrata	Sauria Iguanidae	* Prionoxystus	1	Lepidoptera	Cossidae		
* Charina	1	Vertebrata	Sauria Serpentes (snakes)	* Ostrinia	7	Lepidoptera	Crambidae		
Chrysemys	1	Vertebrata	Sauria Testudines	* Biston	1	Lepidoptera	Geometridae		
Gallus	1	Vertebrata	Sauria Phasianidae (fowl)	* Dendrolimus	1	Lepidoptera	Lasiocampidae		
				* Tisis	1	Lepidoptera	Lecithoceridae		
				* Belipia	1	Lepidoptera	Limacodidae		
				* Helicoverpa	1	Lepidoptera	Noctuidae		

Anguilla	2	Vertebrata	Teleostei (bony fishes)	Anguillidae (eel)	* Heliothis	1	Lepidoptera	Noctuidae		
Anoplopoma	1	Vertebrata	Teleostei (bony fishes)	sablefish	Spodoptera	25	Lepidoptera	Noctuidae		
Cottus	1	Vertebrata	Teleostei (bony fishes)	Cottidae (sculpins)	Euphydryas	2	Lepidoptera	Nymphalidae		
* Danio	1	Vertebrata	Teleostei (bony fishes)	Cyprinidae	* Heliconius	12	Lepidoptera	Nymphalidae		
Maylandia	2	Vertebrata	Teleostei (bony fishes)	Cichlidae	Poladryas	1	Lepidoptera	Nymphalidae		
* Nothobranchius	6	Vertebrata	Teleostei (bony fishes)	killifish	* Papilio	11	Lepidoptera	Papilionidae		
Salmo	1	Vertebrata	Teleostei (bony fishes)	Salmonidae (salmon)	Antheraea	1	Lepidoptera	Saturniidae		
Sinocyclocheilus	3	Vertebrata	Teleostei (bony fishes)	Cyprinidae	Manduca	1	Lepidoptera	Sphingidae		
Takifugu	4	Vertebrata	Teleostei (bony fishes)	Tetraodontidae (puffers)	* Monochamus	4	Lepidoptera	Sphingidae		
Tetraodon	1	Vertebrata	Teleostei (bony fishes)	Tetraodontidae (puffers)	Neococytius	1	Lepidoptera	Sphingidae		
* Lactuca	1	Plant	Asteraceae	lettuce	Edosa	1	Lepidoptera	Tineidae		
* Camelina	1	Plant	Brassicaceae		Leucomele	1	Lepidoptera	Tineidae		
* Scenedesmus	1	Plant	Chlorophyta	green algae	Tinea	1	Lepidoptera	Tineidae		
Cucumis	1	Plant	Cucurbitaceae	melons	* Argyresthia	1	Lepidoptera	Yponomeutidae		
* Glycine	4	Plant	Fabaceae	soybean	* Zelleria	1	Lepidoptera	Yponomeutidae		
* Jatropha	1	Plant	Euphorbiaceae (spurges)		* Chrysopa	431	Neuroptera	Chrysopidae	431	12.77%
* Oryza	1	Plant	Poaceae	rice	* Locusta	2	Orthoptera	Acrididae		
* Fragaria	1	Plant	Rosaceae	strawberry	* Schistocerca	1	Orthoptera	Acrididae		
* Selaginella	1	Plant	Tracheophyta	spike moss	Dianemobius	1	Orthoptera	Gryllidae		
					* Gryllus	3	Orthoptera	Gryllidae		
					* Pediculus	154	Pthiraptera	Pediculidae	154	4.56%
Total	132		% non-insect metazoans:	3.91%	Xenopsylla	1	Siphonaptera	Pulicidae		
					Dipseudopsis	1	Trichoptera	Dipseudopsidae	12	0.36%
(continued to right)					Total insect hits	3213			3213	95.17%

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				(continued from left)				
Non-insect Genus	hits	Non-specific higher taxonomic grouping	Insect Genus	hits	Order	Family	Order total	Percent
* virus	6	virus	Periplaneta	1	Blattodea	Blattidae		
Phyllobacterium	2	Bacteria	* Blattella	1	Blattodea	Ectobiidae		
Sinorhizobium	1	Bacteria	Biphyllus	3	Coleoptera	Biphyllidae	1648	49.08%
Crinalium	1	Bacteria	Apriona	1	Coleoptera	Cerambycidae		
Cronobacter	1	Bacteria	Tetraopes	1	Coleoptera	Cerambycidae		
* uncultured bacteria	1	Bacteria	Callosobruchus	1	Coleoptera	Chrysomelidae		
Dictyostelium	1	Eukaryota	Chrysomela	12	Coleoptera	Chrysomelidae		
Entamoeba	1	Eukaryota	Diabrotica	1	Coleoptera	Chrysomelidae		
* Tetrahymena	1	Eukaryota	Gonioctena	1	Coleoptera	Chrysomelidae		
* Strongylocentrotus	4	Eukaryota	* Labidomera	1	Coleoptera	Chrysomelidae		
* Euglena	1	Eukaryota	Leptinotarsa	3	Coleoptera	Chrysomelidae		
* Candida	3	Eukaryota	Phaedon	2	Coleoptera	Chrysomelidae		
Kluyveromyces	1	Eukaryota	Timarcha	2	Coleoptera	Chrysomelidae		
Millerozyma	1	Eukaryota	* Adalia	3	Coleoptera	Coccinellidae		
* Tetrapisispora	2	Eukaryota	* Coccinella	9	Coleoptera	Coccinellidae		
Naegleria	1	Eukaryota	* Coleomegilla	4	Coleoptera	Coccinellidae		
Hydra	3	Eukaryota	* Epilachna	1	Coleoptera	Coccinellidae		
* Dicyema	1	Eukaryota	Halyzia	1	Coleoptera	Coccinellidae		
* Caenorhabditis	6	Eukaryota	* Harmonia	11	Coleoptera	Coccinellidae		
Ditylenchus	1	Eukaryota	Henosepilachna	1	Coleoptera	Coccinellidae		
Haemonchus	1	Eukaryota	* Hippodamia	3	Coleoptera	Coccinellidae		
Trichinella	1	Eukaryota	Propylea	1	Coleoptera	Coccinellidae		
Trichoplax	2	Eukaryota	Aphanarthrum	1	Coleoptera	Curculionidae		
Dugesia	1	Eukaryota	Carphoborus	1	Coleoptera	Curculionidae		
Schistosoma	2	Eukaryota	* Curculio	3	Coleoptera	Curculionidae		
Ciona	1	Eukaryota	* Dendroctonus	468	Coleoptera	Curculionidae		
			Hypera	1	Coleoptera	Curculionidae		
Total	47	% microbial:	Ips	2	Coleoptera	Curculionidae		
			Sitophilus	1	Coleoptera	Curculionidae		
Non-insect Genus		Non-specific higher taxonomic grouping	* Dermestes	1	Coleoptera	Dermestidae		
Platynereis	1	Annelida	Eretes	1	Coleoptera	Dytiscidae		
Urechis	1	Annelida	Agriotes	1	Coleoptera	Elateridae		
* Mesenchytraeus	1	Annelida	Luciola	1	Coleoptera	Elateridae		
* Amblyomma	1	Arthropoda	Georissus	2	Coleoptera	Georissidae		
* Ixodes	7	Arthropoda	Hister	1	Coleoptera	Histeridae		
* Metaseiulus	3	Arthropoda	Mylabris	1	Coleoptera	Meloidae		
Phrynus	1	Arthropoda	Mycetophagus	2	Coleoptera	Mycetophagidae		
Latrodectus	1	Arthropoda	* Holotrichia	1	Coleoptera	Scarabaeidae		
			Onthophagus	2	Coleoptera	Scarabaeidae		

* Lepeoptheirus	3	Arthropoda	Copepoda	Caligidae (fish lice)	Trypoxylus	1	Coleoptera	Scarabaeidae	
Tachypleus	1	Arthropoda	Limulidae	horseshoe crab	* Necrophila	1	Coleoptera	Silphidae	
Branchiostoma	3	Cephalochorda	Branchiostomidae	lanceleto, amphioxus	Microdera	1	Coleoptera	Tenebrionidae	
Ichthyophthirius	1	Ciliophora	Oligohymenophorea	Ophryoglenina	* Tenebrio	1	Coleoptera	Tenebrionidae	
* Saccoglossus	5	Eukaryota	Hemichordata	Harrimaniidae	* Tribolium	1092	Coleoptera	Tenebrionidae	
Anoplopoma	1	Eukaryota	Vertebrata	Percomorpharia	* Dalmannia	1	Diptera	Conopidae	192 5.72%
Chlamys	1	Mollusca	Bivalvia	Pectinidae (scallop)	Myopa	1	Diptera	Conopidae	
* Patinopecten	1	Mollusca	Bivalvia	Pectinidae (scallop)	* Aedes	26	Diptera	Culicidae	
Bithynia	4	Mollusca	Gastropoda	Bithyniidae (snail)	* Anopheles	31	Diptera	Culicidae	
Amphimedon	1	Porifera	Niphathidae	sponges	* Culex	22	Diptera	Culicidae	
Suberites	1	Porifera	Tetractinomorpha		Ochlerotatus	1	Diptera	Culicidae	
Rana	1	Vertebrata	Amphibia	Ranidae (frogs)	* Teleopsis	2	Diptera	Diopsidae	
* Xenopus	5	Vertebrata	Amphibia	Pipidae (frogs)	* Drosophila	77	Diptera	Drosophilidae	
Meleagris	3	Vertebrata	Aves	turkey	Musca	1	Diptera	Muscidae	
Taeniopygia	3	Vertebrata	Aves	finch	Nemopalpus	1	Diptera	Psychodidae	
Discopyge	1	Vertebrata	Chondrichthyes	Narcinidae (ray)	Oxycera	2	Diptera	Stratiomyidae	
Bos	2	Vertebrata	Mammalia	Bovidae (cattle)	* Anastrepha	1	Diptera	Tephritidae	
Canis	2	Vertebrata	Mammalia	Canidae (dog, wolf)	Bactrocera	1	Diptera	Tephritidae	
Cavia	2	Vertebrata	Mammalia	Rodentia (guinea pigs)	* Ceratitis	24	Diptera	Tephritidae	
Ceratotherium	1	Vertebrata	Mammalia	Rhinocerotidae (rhinoceros)	Tipula	1	Diptera	Tipulidae	
Dasytus	1	Vertebrata	Mammalia	armadillo	Bemisia	1	Hemiptera	Acyrodidae	98 2.92%
Macaca	1	Vertebrata	Mammalia	Primate	* Riptortus	17	Hemiptera	Alydidae	
Monodelphis	1	Vertebrata	Mammalia	Didelphidae (opossum)	* Toxoptera	2	Hemiptera	Aphididae	
* Mus	2	Vertebrata	Mammalia	Rodentia (mouse)	* Acyrthosiphon	60	Hemiptera	Aphididae	
Nomascus	2	Vertebrata	Mammalia	Primate (gibbon)	Aphis	1	Hemiptera	Aphididae	
Ochotona	1	Vertebrata	Mammalia	pika	Laodelphax	2	Hemiptera	Delphacidae	
Odobenus	5	Vertebrata	Mammalia	Odobenidae (walrus)	* Oncopeltus	1	Hemiptera	Lygaeidae	
Orcinus	1	Vertebrata	Mammalia	Delphinidae (dolphins)	* Lygus	9	Hemiptera	Miridae	
Ornithorhynchus	7	Vertebrata	Mammalia	Monotremata (platypus)	Buenoa	1	Hemiptera	Notonectidae	
* Oryctolagus	1	Vertebrata	Mammalia	Leporidae (rabbits)	* Maconellicoccus	3	Hemiptera	Pseudococcidae	
Otolemur	2	Vertebrata	Mammalia	Primate	Diaphorina	1	Hemiptera	Psyllidae	
* Ovis	1	Vertebrata	Mammalia	sheep	* Apis	221	Hymenoptera	Apidae	548 16.32%
Rattus	2	Vertebrata	Mammalia	Rodentia (rat)	* Bombus	92	Hymenoptera	Apidae	
Sarcophilus	3	Vertebrata	Mammalia	Dasyuridae (Tasmanian devil)	* Ascogaster	1	Hymenoptera	Braconidae	
Trichechus	4	Vertebrata	Mammalia	Trichechidae (manatee)	* Glyptapanteles	3	Hymenoptera	Braconidae	
* Anolis	3	Vertebrata	Sauria	Iguanidae	* Lysiphlebus	8	Hymenoptera	Braconidae	
Chrysemys	1	Vertebrata	Sauria	Testudines	* Nasonia	66	Hymenoptera	Braconidae	
Gallus	1	Vertebrata	Sauria	Phasianidae (fowl)	Camponotus	1	Hymenoptera	Formicidae	
Anguilla	2	Vertebrata	Teleostei (bony fishes)	Anguillidae (eel)	* Megachile	79	Hymenoptera	Megachilidae	
Anoplopoma	1	Vertebrata	Teleostei (bony fishes)	sablefish	* Osmia	77	Hymenoptera	Megachilidae	
Cottus	1	Vertebrata	Teleostei (bony fishes)	Cottidae (sculpins)	* Bombyx	46	Lepidoptera	Bombycidae	113 3.37%
* Danio	2	Vertebrata	Teleostei (bony fishes)	Cyprinidae	* Ostrinia	6	Lepidoptera	Crambidae	
Maylandia	2	Vertebrata	Teleostei (bony fishes)	Cichlidae	* Promalactis	1	Lepidoptera	Elachistidae	
* Nothobranchius	5	Vertebrata	Teleostei (bony fishes)	killifish	* Palaeomicra	1	Lepidoptera	Micropterigidae	
* Salmo	1	Vertebrata	Teleostei (bony fishes)	Salmonidae (salmon)	* Helicoverpa	3	Lepidoptera	Noctuidae	
Sinocyclocheilus	3	Vertebrata	Teleostei (bony fishes)	Cyprinidae	Spodoptera	25	Lepidoptera	Noctuidae	
Takifugu	4	Vertebrata	Teleostei (bony fishes)	Tetraodontidae (puffers)	Euphydryas	2	Lepidoptera	Nymphalidae	
Tetraodon	1	Vertebrata	Teleostei (bony fishes)	Tetraodontidae (puffers)	* Heliconius	11	Lepidoptera	Nymphalidae	
Cucumis	1	Plant	Cucurbitaceae	melons	Poladryas	1	Lepidoptera	Nymphalidae	
Jatropha	1	Plant	Euphorbiaceae (spurges)	orchid	* Papilio	8	Lepidoptera	Papilionidae	
* Psychopsis	1	Plant	Orchidaceae		Antheraea	1	Lepidoptera	Saturniidae	
					Manduca	1	Lepidoptera	Sphingidae	
Total	122		% non-insect metazoans:	3.63%	* Monochamus	3	Lepidoptera	Sphingidae	
					Neococytius	1	Lepidoptera	Sphingidae	
(continued to right)					* Edosa	1	Lepidoptera	Tineidae	
					Leucomela	1	Lepidoptera	Tineidae	
					* Tinea	1	Lepidoptera	Tineidae	
					* Protohermes	1	Megaloptera	Corydalidae	
					* Chrysopa	434	Neuroptera	Chrysopidae	434 12.92%
					* Locusta	1	Orthoptera	Acrididae	
					* Schistocerca	2	Orthoptera	Acrididae	
					Dianemobius	1	Orthoptera	Gryllidae	
					* Gryllus	4	Orthoptera	Gryllidae	
					* Pediculus	145	Pthiraptera	Pediculidae	145 4.32%
					Xenopsylla	1	Siphonaptera	Pulicidae	
					Dipseudopsis	1	Trichoptera	Dipseudopsidae	13 0.39%
					Total	3191			3191 94.74%

* 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/Browser/wwwtax.cgi>

Metazoans are included in the "microbial" portion of the spreadsheet (ie. fungi); those categorized in % microbial may be associated with symbionts 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.

Tribolium sequence pairs:				Sequence ID				length gi and description (RNA)				Bit Score Expect nt Identities			
Sequence ID	length	gi	Expect	Bit Score	Expect	Sequence ID	length	gi	Expect	Bit Score	Expect	nt Identities			
IEcomp8421_c0_seq1	2902	gij189233647 refl	3E-64	258	3E-64	Pcomp8751_c0_seq4	2917	gij189233647 refl XM_967463.2 PREDICTED: Tribolium castaneum similar to beta-arrestin 1 (LOC661293), mRNA	258	3E-64	6	2902/2902, 100%			
IEcomp6954_c0_seq1	3236	gij189233777 refl	1E-35	163	1E-35	Pcomp8809_c0_seq1	3268	gij189233777 refl XM_970744.2 PREDICTED: Tribolium castaneum similar to Mif CG17469-PB (LOC659688), mRNA	163	1E-35	3	3192/3192, 100%			
IEcomp7095_c0_seq1	6235	gij189233793 refl	2E-14	93.7	2E-14	Pcomp3394_c0_seq1	6173	gij189233793 refl XM_001814833.1 PREDICTED: Tribolium castaneum similar to Gb1-cadherin (LOC661970), mRNA	93.7	2E-14	4	6096/6174, 99%			
IEcomp14202_c0_seq1	1597	gij189233859 refl	3E-13	87.7	3E-13	Pcomp14830_c0_seq1	1591	gij189233859 refl XM_967562.2 PREDICTED: Tribolium castaneum similar to succinyl-coa synthetase beta chain (LOC661403), mRNA	87.7	3E-13	13	1584/1586, 99%			
IEcomp10244_c0_seq3	2101	gij189233868 refl	3E-51	214	3E-51	Pcomp6797_c0_seq2	2098	gij189233868 refl XM_963541.2 PREDICTED: Tribolium castaneum similar to AGAP007416-PA (LOC657053), mRNA	214	3E-51	9	2096/2096, 100%			
IEcomp12989_c0_seq1	2756	gij189233948 refl	7E-34	157	7E-34	Pcomp13520_c0_seq1	2727	gij189233948 refl XM_001815112.1 PREDICTED: Tribolium castaneum similar to CG11526 CG11526-PA (LOC661304), mRNA	157	7E-34	16	2703/2720, 99%			
IEcomp2432_c0_seq1	3559	gij189234001 refl	4E-14	91.7	4E-14	Pcomp7193_c0_seq2	3612	gij189234001 refl XM_967366.2 PREDICTED: Tribolium castaneum similar to CG10249 CG10249-PD (LOC661190), mRNA	91.7	4E-14	1	3559/3559, 100%			
IEcomp2367_c0_seq1	1500	gij189234377 refl	9E-29	139	9E-29	Pcomp21956_c0_seq1	1517	gij189234377 refl XM_001815988.1 PREDICTED: Tribolium castaneum similar to PCI domain containing 2 (LOC100142368), mRNA	139	9E-29	15	1494/1494, 100%			
IEcomp22006_c0_seq1	1683	gij189234523 refl	5E-12	83.8	5E-12	Pcomp5895_c0_seq1	1612	gij189234523 refl XM_967649.2 PREDICTED: Tribolium castaneum similar to zinc finger protein, putative (LOC661494), mRNA	83.8	5E-12	14	1609/1609, 100%			
Dendroctonus sequence pairs:				Sequence ID				length updated similarity (protein)*				Bit Score Expect			
Sequence ID	length	gi (per analysis)	Expect	Bit Score	Expect	Sequence ID	length	gi	Expect	Bit Score	Expect	nt Identities			
IEcomp9964_c0_seq1	3242	gij332375734 gbl	4E-91	347	4E-91	Pcomp9907_c0_seq1	3227	gi1085467 XP_970131.1 340 aa PREDICTED: guanine nucleotide-binding protein subunit beta-1	347	4E-91	8	3224/3227, 99%			
IEcomp9135_c0_seq1	1474	gij459305579 gbl	1E-134	490	1E-134	Pcomp9568_c0_seq1	1476	gi1087239 XP_975511.1 PREDICTED: eukaryotic initiation factor 4A-III [Tribolium castaneum] 404 aa	490	1E-134	7	1462/1465, 99%			
IEcomp5768_c0_seq1	1484	gij459307330 gbl	2E-32	151	2E-32	Pcomp13212_c0_seq2	1479	gi189241344 PREDICTED: obg-like ATPase 1 [Tribolium castaneum] Sequence ID: ref XP_969865.2 Length: 399	151	2E-32	2	1472/1479, 99%			
IEcomp11734_c0_seq2	1416	gij459311962 gbl	3E-50	210	3E-50	Pcomp11908_c0_seq4	1376	gi1088003 XP_973796.1 PREDICTED: putative RNA-binding protein Luc7-like 2 [Tribolium castaneum]	210	3E-50	11	1369/1378, 99%			
IEcomp14296_c0_seq1	1161	gij459314268 gbl	1E-11	81.8	1E-11	Pcomp14860_c0_seq1	1186	gi1080555 XP_967130.1 PREDICTED: clathrin light chain isoform X1 [Tribolium castaneum]	81.8	1E-11	18	1161/1161, 100%			
IEcomp13735_c0_seq1	838	gij459323274 gbl	7E-25	125	7E-25	Pcomp7175_c0_seq1	847	gi288869512 NP_001165863.1 XP_970587 ribosomal protein S3 [Tribolium castaneum]	125	7E-25	17	836/837, 99%			
IEcomp14748_c0_seq1	1270	gij459324930 gbl	2E-14	91.7	2E-14	Pcomp15141_c0_seq1	1286	gi642911142 XP_008200598.1 PREDICTED: protein disulfide-isomerase A6 [Tribolium castaneum]	91.7	2E-14	19	1265/1265, 100%			

* Sequences most similar to *Dendroctonus ponderosae* were not identified as predicted mRNA/proteins, because of the annotation status of the genome. To provide better insight these sequences were individually analyzed by BLASTx and the closest annotated description is provided. Bit Scores and Expect values refer to the tBLASTx result, listed in the "gi (per analysis)" column.

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

Unique sequences in pollen diet sample			Putative transcript type			Unique sequence in insect egg diet sample			Putative transcript type		
Sequence ID	length			Sequence ID	length						
1	Pcomp7841_c0_seq1	2080	Glycoside hydrolase	IEcomp11484_c0_seq1	2167	uncharacterized hypothetical protein					
2	Pcomp12927_c0_seq2	1918	Synaptic vesicle protein	IEcomp13279_c0_seq1	1935	uncharacterized hypothetical protein					
3	Pcomp7836_c0_seq1	1683	Fatty acid binding protein	IEcomp4164_c0_seq1	1910	putative transposon					
4	Pcomp49463_c0_seq1	1645	Reverse transcriptase	IEcomp13213_c0_seq1	1889	unknown					
5	Pcomp529_c0_seq1	1146	7 transmembrane receptor	IEcomp11074_c0_seq1	1775	unknown					
6	Pcomp26787_c0_seq1	1144	unidentifiable	IEcomp11853_c0_seq1	1607	unknown					
7	Pcomp38644_c0_seq1	1049	organic anion transporter	IEcomp6564_c0_seq1	1583	Ankyrin repeat protein					
8	Pcomp39179_c0_seq1	982	Apolipoprotein precursor	IEcomp9557_c0_seq1	1575	unknown					
9	Pcomp57928_c0_seq1	982	unknown	IEcomp4213_c0_seq1	1450	Lygus lineolaris virus 1					
10	Pcomp6831_c0_seq1	975	uncharacterized protein	IEcomp13459_c0_seq21	1332	Drosophila A virus structural protein					
11	Pcomp33_c0_seq1	964	Synaptic vesicle protein	IEcomp13259_c0_seq4	1276	RhoGAP15B					
12	Pcomp9099_c0_seq1	959	unknown	IEcomp35607_c0_seq1	1091	copa-type polyprotein					
13	Pcomp59166_c0_seq1	956	Glycoside hydrolase	IEcomp2540_c0_seq2	1078	HARBI1-like nuclease					
14	Pcomp57181_c0_seq1	947	transposable element-like	IEcomp27681_c0_seq1	1016	unidentifiable					
15	Pcomp4200_c0_seq1	828	unidentifiable	IEcomp31819_c0_seq1	1016	Cytochrome P450 18A1					

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 include 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-

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 anaesthetized 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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