

Wheat germ oil in larval diet influences gene expression in adult oriental fruit fly

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ABSTRACT

Culture medium supplemented with wheat germ oil (WGO) causes physiological reactions, such as increased fecundity and mobility, in some insects. Although the impact of WGO on insect physiology is important, the mechanisms of these actions are poorly understood. In this paper, we test the hypothesis that the addition of WGO to medium developed for larval oriental fruit flies modulates gene expression in the corresponding adults. We separately reared larvae of *Bactrocera dorsalis* on diets lacking or supplemented with WGO, and analyzed for expressed proteins in the resulting adult males and females by 2D-electrophoresis. Analysis of the gels revealed significant changes in expression levels of >70 proteins, 64 of which were identified by mass spectrometric analysis on MALDI-TOF/TOF. Apparent changes in expression levels for 6 of these proteins were confirmed by quantitative real-time PCR, showing that the changes in mRNA expression were reflected in changes in protein expression. These findings support the hypothesis that one mechanism of WGO actions in insect nutrition is the modulation of gene expression.

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1. Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel; Tephritidae) extensively damages a broad host range of cultivated fruits and is widely spread in tropical Asia, Hawaii, Guam, the Mariana Islands and Tahiti. Yield losses and fruit quality degradation negatively impact fruit commercialization in these areas and have led to implementation of area-wide integrated management programs, including a sterile insect technique (SIT) component. SIT requires continuous mass-rearing, sterilization and release of adult fruit flies. A major improvement in the cost-effective rearing of the oriental fruit fly included the replacement of mill feed bulking agent with a sponge cloth that enabled rearing on a liquid diet (Chang et al., 2006). The addition of wheat germ oil (WGO) to the liquid diet improved rearing efficiency by significantly increasing larval development, pupal recovery, percentage of adult fliers, mating, egg production and hatch (Chang and Vargas, 2007; Chang, 2009).

The fatty acid composition of WGO includes 42–59% linoleic acid (18:2n – 6), 12–28% oleic acid, 11–19% palmitic acid, 2–11% α-linolenic acid (18:3n – 3), and stearic acid 1%, and 0.14% of vitamin E. Proportions vary somewhat due to the plant-based source of the product (Kahlon, 1989). The influence of WGO in insect nutritional studies was first reported by Fraenkel and

Blewett (1946). The improving effects of WGO on insect performance derive from its high content of nutritionally essential and nonessential fatty acids, and from physiologically active tocopherols. Subsequent experiments with pure fatty acids demonstrated that polyunsaturated fatty acids (PUFAs), including oleic acid (18:1n – 9), linoleic acid (18:2n – 6), alpha linolenic acid (18:3n – 3), and gamma linolenic acid (18:3n – 6) are essential nutrients for many insect species (Dadd, 1985). Mosquitoes are peculiar because they specifically require 20:4n – 6, which can be derived from dietary 18:2n – 6 in most insects. A few insect species are unique among animals because they are able to synthesize 18:2n – 6 *de novo* (de Renobales et al., 1987).

Based on the improved adult performance resulting from the addition of WGO to the liquid diet and recent advances in human nutritional research (de Roos and McArdle, 2008), we generated the hypothesis that one mode of WGO action in insect development is through its influence on gene expression. We report here that the presence of WGO in larval diet substantially alters expression of genes encoding a range of proteins in the corresponding adults.

2. Materials and methods

2.1. Insects and sample collection

Newly collected eggs (<6 h) of oriental fruit fly, *B. dorsalis* (Hendel) were provided by the Tropical Crop and Commodity

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Protection Research Unit of the USDA's Agricultural Research Service (ARS) in Honolulu, HI. The fruit fly colony has been maintained at 25 °C, 65% relative humidity, and 12D:12L for more than 360 generations. *B. dorsalis* larvae were reared on a liquid diet devoid of, or separately supplemented with, WGO (0.66%, v/v) (Chang et al., 2006; Chang and Vargas, 2007). Adults from both larval diets were maintained on a mixture of sugar:protein hydrolysate (hydrolyzed protein) (3:1, w/w) (Chang et al., 2004). Whole males and females were collected at 11-day post-eclosion, respectively. Samples were placed in cryogenic vials (5 ml) and stored at –80 °C before protein analysis.

2.2. Sample preparation

Adult samples were homogenized 3 × in a 5 ml tube with 1 ml of 10 mM Tris–HCl (pH 7.0) containing protease inhibitors (final dilution = 1:100; Sigma, St. Louis, #P8340 for Mammalian Cell and Tissue Extracts) using a Tissue Master™ (Omni Intl., Marietta, GA) for 15 s, held on ice for 30 s and then homogenized again. Homogenates were centrifuged twice at 15,294 × g for 15 min at 4 °C. The resulting infranatants were transferred to new vials on ice. Protein content was determined using the Pierce Micro BCA Protein Assay Kit, with BSA as a quantitative standard (Rockford, IL). Volumes (ca.40 μl) containing 0.5 mg protein were gently mixed with 200 μl of rehydration solution (9 M Urea, 3% (w/v) CHAPs, 100 mM DTT, 0.2% Bio-Lyte, 0.001% bromophenol blue) before immediate use. Three independent biological replicates were processed for each treatment.

2.3. 2D-electrophoresis

Ten microliters of 2D gel protein standards (Bio-Rad, Hercules, CA, #161-0320) were added to each sample tube. IPG strips (pH 3–10; 11 cm; Bio-Rad, #163-2014) were rehydrated overnight with these sample solutions. Iso-electric focusing was performed with a Protean IEF cell system (Bio-Rad) using the standard protocol and a pre-set linear volt ramp program (8000 V and 50 μA/strip max., 35,000 vH). The focused strips were stored at –80 °C for later use.

For the second dimension the IPG strips were equilibrated (15 min/buffer: 6 M urea, 2% SDS, 20% glycerol, 130 mM DTT, 0.375 M Tris–HCl, pH 8.7 [Buffer I]) followed by 6 M urea, 2% SDS, 20% glycerol, 135 mM iodoacetamide, 0.375 M Tris–HCl, pH 8.7 [Buffer II]) and subjected to SDS-PAGE using the Criterion Cell system (Bio-Rad, #165-6001) with a precast gel (8–16% Tris–HCl, Bio-Rad, #345-0105). Gels were stained with Coomassie Blue G-250 (BioSafe Stain; Bio-Rad) and analyzed using Delta 2D software (Decodon GmbH, Greifswald, Germany). Protein spots with densities that were significantly different between treatments (Student's *t*-test, $p < 0.05$), were removed using a 1.5 mm spot picker (The Gel Company, San Francisco, CA) and stored at –80 °C. One gel was run for each independent biological replicate.

2.4. MS/MS analysis

Proteins were digested with trypsin, extracted, then lyophilized and reconstituted with water in preparation for MS/MS analysis as previously described (Stanley et al., 2008). A portion of each protein was mixed with alpha-cyano-4-hydroxycinnamic acid matrix and applied to the MALDI target and analyzed as previously described (Stanley et al., 2008). The resulting sequence data, combined with observed MW and pI values, were used to establish protein identities. For those proteins that did not yield significant matches using Mascot (<http://www.matrixscience.com/home.html>), manual *de novo* sequence analysis and/or partial sequence tag analysis (i.e., sequences deduced by visual inspection of each spectrum) was performed. Sequences generated from these analyses were used to interrogate NCBI-BLAST for protein matches (using the PAM30 matrix and searching within "Insecta"). *E*-values and frequency of matches to a specific protein were the primary criteria for these determinations.

2.5. Quantitative real-time PCR

Total RNA was isolated from fly tissues using the TRIzol Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. RNA concentrations were measured using a spectrophotometer (absorbance = 260 nm). The extracted total RNA was further

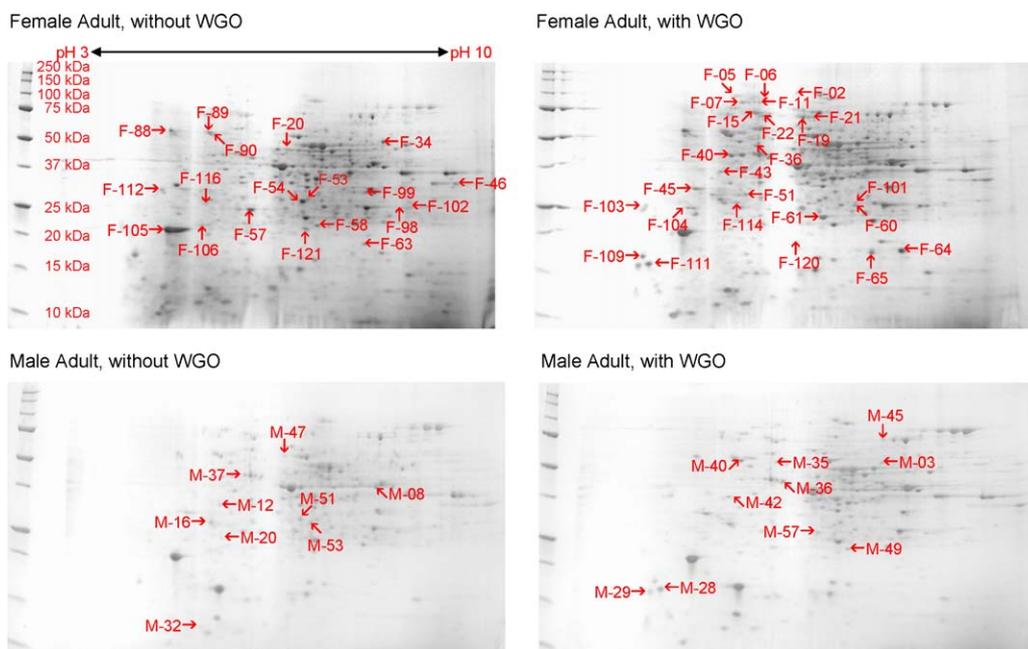


Fig. 1. Representative 2D gels showing the influence of dietary WGO on protein expression in 11-day-old male and female adult *B. dorsalis* reared on diet with, and without WGO. The labeled spots indicated in this image were selected for extraction and MS/MS analysis.

Table 1
Proteins from male adult *Bactrocera dorsalis* homogenates identified using Mascot.

Spot no.	Putative protein with accession no. (species)	Calculated mass ^a	Calculated pI ^a	MOWSE protein score ^b	Total ion score ^c	No. peptides ^d	% Protein coverage ^e	E-value ^f	Peptide sequence (with highest ion score) ^g
M-03 ^h	Glutathione reductase family member <i>Musca domestica</i> gi 1848294	53896	6.34	160	134	9	27%	1.7e–10	ILLNVTGHIPTTAAEFTR (110)
M-08	Aldolase CG6058-PF, isoform F <i>Drosophila melanogaster</i> gi 24650276	42931	6.97	416	359	12	38%	4.1e–36	NTPSYQSILENANVLAR (140)
M-12 ^h	CG10527-PA <i>Drosophila melanogaster</i> gi 20130199	31822	4.69	81	74	3	12%	0.014	WYDENVITVGR (74)
M-16	14-3-3 zeta CG17870-PD, isoform D <i>Drosophila melanogaster</i> gi 24652322	28324	4.77	321	222	21	64%	1.3e–26	SVTETGVLSNEER (73)
M-20	GK17930 <i>Drosophila willistoni</i> gi 195441965	27626	4.57	257	230	7	36%	3.3e–20	YLFAYGGIEYEDVR (114)
M-28	G119058 <i>Drosophila mojavensis</i> gi 195121923	14484	4.75	126	112	3	16%	4.2e–07	ATSPDSTDVIIVK (74)
M-29 ^h	G119058 <i>Drosophila mojavensis</i> gi 195121923	14484	4.75	106	94	3	16%	4.2e–05	ATSPDSTDVIIVK (66)
M-35	ATP synthase subunit beta vacuolar <i>Aedes aegypti</i> gi 157111261	55466	5.38	743	577	27	68%	8.4e–69	IPIFSAAGLPHNEIAAQICR (108)
M-36	Succinyl-CoA synthetase beta chain <i>Culex quinquefasciatus</i> gi 170039565	48729	7.10	140	131	8	15%	1.7e–08	FDDNAEFR (60)
M-37	PREDICTED: similar to Actin-87E isoform 1 <i>Apis mellifera</i> gi 66509780	42157	5.30	563	449	20	60%	8.2e–51	SYELPDGQVITIGNER (118)
M-40	beta-Tubulin at 56D CG9277-PB, isoform B <i>Drosophila melanogaster</i> gi 24655737	50571	4.76	771	613	25	68%	1.3e–71	GHYTEGAELVDSVLDVVR (154)
M-42	GF11475 <i>Drosophila ananassae</i> gi 194767665	33100	4.80	156	87	20	58%	4.2e–10	LAFVEDELEVAEDR (45)
M-45	PREDICTED: similar to Aconitase CG9244-PB <i>Tribolium castaneum</i> gi 91088677	86232	8.20	278	233	19	28%	2.6e–22	NDANPATHSFVTSPELVTSIAGR (115)
M-49	Thioredoxin peroxidase 1 CG1633-PA, isoform A <i>Drosophila melanogaster</i> gi 17157991	21952	5.52	192	169	4	37%	1.1e–13	DYGVLEETGIPFR (119)
M-51 ^h	Arginine kinase <i>Schistocerca americana</i> gi 3183060	40333	5.87	255	212	14	33%	5.3e–20	LGFLTFCPTNLGTTLR (87)
M-53	Glyceraldehyde-3-phosphate dehydrogenase <i>Glossina morsitans morsitans</i> gi 74950265	35868	6.96	352	294	12	36%	1.1e–29	VPTPNVSVVDLTVR (125)
M-57	Heat shock-like protein, similar to heat shock 70 kDa proteins <i>Ceratitis capitata</i> gi 662802	71435	5.36	301	221	25	36%	1.3e–24	TVTNAVITVPAYFNDSQR (120)

^a As reported by Mascot.

^b MOWSE protein scores >75 are significant ($p < 0.05$) using the Metazoa database.

^c Ion scores >45 are significant ($p < 0.05$).

^d The number of peptide sequences identified by Mascot that contributed to the MOWSE score.

^e The percent of the protein sequence that is accounted for by the matching peptide sequences.

^f E-value generated by Mascot.

^g Sequences of the peptides with the highest ion scores. The individual ion scores are shown in parenthesis.

^h Results were confirmed using *de novo* sequence analysis (see Table 2) because of inconsistencies within the Mascot results, low MOWSE scores, and/or inconsistencies between molecular weight or pI values of the observed proteins versus those of the putative proteins.

digested with RNase-free DNase I (Promega, Madison, WI, # M6101). The first-strand cDNA was synthesized from 3 µg total RNA using Oligo(dT) primers and superscript II reverse transcriptase (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol.

Real-time qPCR amplification and analysis was performed on the 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, CA). Final sample volume was brought to 25 µl using SYBR Green

Supermix™ (Applied Biosystems). The real-time PCR program used was: hold at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, then 60 °C for 1 min. The specificity of the SYBR Green PCR signal was further confirmed by melting curve analysis and agarose gel electrophoresis. The mRNA expression was quantified using the comparative CT (cross-threshold, the PCR cycle number that crosses the signal threshold) method (Livak and Schmittgen, 2001). The cDNA was amplified using gene-specific primers. The primers for

Table 2

Proteins from male adult *Bactrocera dorsalis* homogenates identified by *de novo* sequence analysis.

Spot no.	Putative protein with accession no. (species)	Calculated mass ^a	Calculated pI ^a	No. peptides ^b	E-value ^c	Peptide sequence ^d
M-03	Thioredoxin reductase-1, isoform B <i>Drosophila melanogaster</i> gi 24640551	64323	8.11	4	2e-09	YSPLXXXVEYGLTSDDLFSLDREPGK
M-12	CG10527 <i>Drosophila melanogaster</i> gi 20130199	31556	4.69	2	5e-07	QKPEVAEVPTGILDAGEFR
M-29	J20027 <i>Drosophila virilis</i> gi 195384110	14422	4.66	2	1e-07	KALDYLLSLPPAQR
M-32	AGAP002850-PA <i>Anopheles gambiae</i> str. PEST gi 158290457	17025	4.47	1	0.015	HHATALGITVPYPLLVDVA
M-47	G123295 <i>Drosophila mojavensis</i> gi 195108923	55136	6.77	2	0.29	IAQEEIFGPFDRGAR
M-51	Arginine kinase <i>Drosophila melanogaster</i> gi 857565	29741	6.73	2	2e-06	TFLVWCNEEDHLR

^a Determined using the Expasy Compute PI/MW tool, http://ca.expasy.org/cgi-bin/pi_tool.

^b The number of sequences deduced using *de novo* sequence analysis that resulted in the same protein identity or function.

^c E-values were generated by BLASTp searches using PAM30, within "Insecta". Values presented here are those of the peptides with the most significant E-values.

^d Sequences were determined by *de novo* sequence analysis from observations of the original spectra. Only the peptide sequences with the lowest E-values are shown. Note: all sequences are those of deduced partial sequence tags (therefore Lys/Arg residues not shown on C-terminal).

Table 3

Quantity^a and function of proteins identified by MS/MS from male adults whose larvae fed on diet with (+WGO) or without wheat germ oil (-WGO), respectively.

Spot No.	Protein Function	+WGO/-WGO ratio	t-Test p-value
<i>Energy and metabolism</i>			
M-08	Involved in glycolysis.	0.37	0.029
M-35	Involved in solute transport and pH regulation in organelles.	1.84	0.055
M-36	Involved in the TCA cycle.	2.18	0.010
M-45	Involved in the TCA cycle.	2.55	0.021
M-47	1-Pyrroline-5-carboxylate dehydrogenase: a mitochondrial inner membrane, involved in the biosynthesis of proline and ornithine.	0.48	0.011
M-51	Important for maintenance of ATP levels.	0.30	0.002
M-53	Involved in glycolysis and gluconeogenesis.	0.44	0.029
<i>Signal transduction</i>			
M-16	Numerous effects on intracellular signaling.	0.63	0.018
<i>Protein structure and signal transduction</i>			
M-57	Involved in protein folding and signal transduction.	0.81	0.034
<i>Cell protection</i>			
M-03	Serves as an antioxidant, may have redox regulatory roles.	2.03	0.008
M-20	Glutathione S-transferase: involved in cellular detoxification.	0.19	0.008
M-49	Reduces hydrogen peroxide, peroxyxynitrite, and organic hydro-peroxides levels in the cell.	1.99	0.006
<i>Cell structure and function</i>			
M-40	Major component of microtubules.	1.72	0.016
<i>Development and gene regulation</i>			
M-12	Farnesoic acid O-methyltransferase: important in the production of juvenile hormone.	0.52	0.009
<i>Mobility or exoskeleton</i>			
M-28	Insect cuticle protein: chitin-binding protein.	6.54	0.003
M-29	Insect cuticular protein: chitin-binding protein.	120.35	0.025
M-37	Involved in the formation of filaments and muscle contraction.	0.75	0.002
M-42	Tropomyosin: regulates muscle contraction in striated muscle; role is unclear in smooth muscle and non-muscle tissues.	5.14	0.025
<i>Lipid storage and metabolism</i>			
M-32	Niemann-Pick type C2 (Npc2) (a lysosomal protein), contains a cholesterol/lipid binding site.	0.20	0.015

^a As determined by Delta 2D software (DECODON GmbH, Greifswald, Germany).

Table 4
Proteins from female adult *Bactrocera dorsalis* homogenates identified using Mascot.

Spot no.	Putative protein with accession no. (species)	Calculated mass ^a	Calculated pI ^a	MOWSE protein score ^b	Total ion score ^c	No. peptides ^d	% Protein coverage ^e	E-value ^f	Peptide sequence (with highest ion score) ^g
F-02	GA13011 <i>Drosophila pseudoobscura pseudoobscura</i> gi 198470320	105924	5.85	101	60	18	23%	0.00013	WYQTGAFLPFFR (60)
F-05	GE23773 <i>Drosophila yakuba</i> gi 195503625	90210	4.89	429	357	24	28%	2.1e−37	GVVDSDDLPLNVS R (106)
F-06	GA15351 <i>Drosophila pseudoobscura pseudoobscura</i> gi 198457521	89564	5.20	395	175	51	61%	5.2e−34	VTQGFSGADLTEICQR (86)
F-07	Heat shock protein 83 <i>Ceratitis capitata</i> gi 76780421	81976	4.92	558	415	33	48%	2.6e−50	HFSVEGQLEFR (91)
F-15	GF20248 <i>Drosophila ananassae</i> gi 194762782	72394	5.22	604	510	29	35%	6.5e−55	VTHAVVTPAYFNDAQR (119)
F-19	GJ12940 <i>Drosophila virilis</i> gi 195375499	33133	4.88	299	221	18	40%	2.1e−24	DLVETVAKGDYDIGAAFDGDGR (174)
F-22	GI18088 <i>Drosophila mojavensis</i> gi 195118495	68563	5.18	749	552	35	63%	2.1e−69	EASIVTIGITLSEYFR (119)
F-34	Aconitate hydratase <i>Glossina morsitans morsitans</i> gi 110611272	69852	7.81	316	261	14	24%	4.1e−26	IPFNVTGSEQIR (107)
F-36	GF20295 <i>Drosophila ananassae</i> gi 194762612	47083	5.22	422	202	32	73%	1e−36	TLEFIEVQEEYIKDEQR (107)
F-40	Heat shock-like protein, similar to heat shock 70 kDa proteins <i>Ceratitis capitata</i> gi 662802	71435	5.36	934	810	23	38%	6.5e−88	WLDANQLAEKEEYHR (155)
F-43	GF11475 <i>Drosophila ananassae</i> gi 194767665	33100	4.80	330	207	23	57%	1.6e−27	KLAFVEDELEVAEDR (104)
F-45	Gasp CG10287-PA, isoform A <i>Drosophila melanogaster</i> gi 24644504	29372	4.73	639	498	16	63%	2.1e−58	NEEVANGFSCPAAGELANAGSFSR (125)
F-46	GH18558 <i>Drosophila grimshawi</i> gi 195036830	35690	9.21	293	229	10	36%	8.2e−24	DDLFNVNAGIIR (101)
F-51	GI15982 <i>Drosophila mojavensis</i> gi 195114108	18905	8.96	289	268	6	32%	2.1e−23	NFIDVGAGVDEYR (120)
F-53	PREDICTED: similar to H(+)-transporting ATPase isoform 1 <i>Nasonia vitripennis</i> gi 156536963	26175	7.77	225	164	14	54%	5.2e−17	LELIAQQLVPEIR (100)
F-54	CJ20492 <i>Drosophila virilis</i> gi 195382366	35502	8.26	358	297	10	34%	2.6e−30	VPTPNVSVVDLTVR (145)
F-57	GE10635 <i>Drosophila yakuba</i> gi 195504079	39850	7.60	332	292	9	32%	1e−27	NTPSYQSILENANVLAR (128)
F-58	Phosphoglycerate mutase <i>Aedes aegypti</i> gi 157112126	28595	6.34	172	133	9	32%	1e−11	FLGDEETVR (63)
F-60	Glycerol-3-phosphate dehydrogenase <i>Ceratitis capitata</i> gi 1945505	28946	7.68	440	341	18	64%	1.7e−38	AEGGGIDLISHIITR (126)
F-61	Chain A, triose phosphate isomerase <i>Caenorhabditis elegans</i> gi 24159115	30049	6.60	148	133	4	28%	2.6e−09	KPDIDGFLVGGASLKPDFVKIINAR (133)
F-63	GH20332 <i>Drosophila grimshawi</i> gi 195027848	22244	8.54	368	315	9	47%	1.2e−30	VTDKVVFFDITIGGEPAGR (144)
F-88	GK13913 <i>Drosophila willistoni</i> gi 195451659	46501	4.39	166	128	11	29%	5.3e−12	HEQNIDCGGGYVK (90)

Table 4 (Continued)

Spot no.	Putative protein with accession no. (species)	Calculated mass ^a	Calculated pI ^a	MOWSE protein score ^b	Total ion score ^c	No. peptides ^d	% Protein coverage ^e	E-value ^f	Peptide sequence (with highest ion score) ^g
F-90	beta-Tubulin at 56D CG9277-PB, isoform B <i>Drosophila melanogaster</i> gi 24655737	50571	4.76	639	422	32	74%	2.1e-58	GHYTEGAELVDSVLDVVR (152)
F-99	Aldolase CG6058-PF, isoform F <i>Drosophila melanogaster</i> gi 24650276	42931	6.97	370	311	11	28%	1.6e-31	NTPSYQSILENANVLAR (146)
F-101	PREDICTED: similar to putative phosphoglycerate mutase <i>Tribolium castaneum</i> gi 91092672	28816	6.97	143	99	11	46%	8.4e-09	QLIAAHGNSLR (99)
F-104	Probable Elongation factor 1-beta <i>Drosophila melanogaster</i> gi 13124189	24289	4.40	94	81	4	24%	0.0006	HIASFEEAER (67)
F-105 ^h	Calcium-binding protein <i>Drosophila melanogaster</i> gi 2345094	21467	4.71	92	NS	11	70%	0.0011	NS
F-106	GK17930 <i>Drosophila willistoni</i> gi 195441965	27626	4.57	97	63	8	42%	0.00032	YLFAYGGIEYEDVR (63)
F-109	Calmodulin (CaM) <i>Pyuridae</i> gen. sp. gi 122063215	16801	4.06	405	307	11	89%	5.2e-35	VFDKDGNGFISAAELR (102)
F-111 ^h	G119058 <i>Drosophila mojavensis</i> gi 195121923	14484	4.75	167	155	2	10%	3.3e-11	KATSPDSTDVIVK (80)
F-112	Gasp CG10287-PB, isoform B <i>Drosophila melanogaster</i> gi 161078060	27103	4.58	416	330	14	66%	4.1e-36	NEEVANGFSCPAAGELANAGSFSR (125)
F-114	Heat shock-like protein, similar to heat shock 70 kDa proteins <i>Ceratitis capitata</i> gi 662802	71435	5.36	197	133	24	43%	3.3e-14	QTQFTFTYSNDQPGVLIQVYEGER (57)
F-116	20S Proteasome alpha 5 subunit <i>Ceratitis capitata</i> gi 27525440	26675	4.97	309	239	11	42%	2.1e-25	HIGCVTSLGTADAR (74)
F-121	Cj10377 <i>Drosophila virilis</i> gi 195395068	33951	8.05	127	119	6	22%	3.3e-07	TATPEQAQEVHAYLR (116)

^a As reported by Mascot.

^b MOWSE protein scores >75 are significant ($p < 0.05$) using the Metazoa database.

^c Ion scores >45 are significant ($p < 0.05$). NS = no significant ions but total protein score still significant.

^d The number of peptide sequences identified by Mascot that contributed to the MOWSE score.

^e The percent of the protein sequence that is accounted for by the matching peptide sequences.

^f E-value generated by Mascot.

^g Sequences of the peptides with the highest ion scores. The individual ion scores are shown in parenthesis.

^h Results were confirmed using *de novo* sequence analysis (see Table 5) because of inconsistencies within the Mascot results, low MOWSE scores, and/or inconsistencies between molecular weight or pI values of the observed proteins versus those of the putative proteins.

protein spot F22 were 5'-CTGTCCAAGTACTCCAATC-3' and 5'-GGCATGTTGGAGGTGTGGC-3'; primers for protein spot F36 were 5'-AAGTGATCATGGCCACCAA-3' and 5'-GCCAGAACAATGTAGCG-GTT-3'; primers for protein spot F40 were 5'-TCGTACTGCTTCAA-CATGAAG-3' and 5'-TGGTACAGCTTGGTATGAT-3'; primers for protein spot F43 were 5'-ATGAGAACAACCGTATGTGCA-3' and 5'-TCCAGCTCGTCTTCAACGAAG-3'; primers for protein spot F19 were 5'-GACGGCGATCGCAACATGAT-3' and 5'-TCCACGGCAGAGGCAG-TGG-3'; primers for protein spot M35 were 5'-GCCATCGGC-GAGGGCATGA-3' and 5'-AGATACGCAGCAGCTGCCAGC-3'; primers for protein spot M40 were 5'-TCCAGGAGCTGTCAAGCGC-3' and 5'-TCGGCGTCTCGTCCGGCGT-3'. To normalize the cDNA, the primers for the housekeeping gene actin (5'-TCCATCATGAAGTCCGACGT-3' and 5'-AGAAGCACTTCCGGTGGACGA-3') were also used to amplify cDNA from the samples.

3. Results

Adult female *B. dorsalis* initiated egg laying at 11-day post-eclosion. Representative 2D protein gels from male and female

adults reared from diet with, and without, WGO in their larval stages showing differences in protein expression are presented in Fig. 1. Over 500 spots per gel for each treatment were detected in gel analysis. Of those, 17 proteins in the male samples and 38 proteins in the female samples that were selected for in-gel digestion and analysis by MALDI-TOF MS/MS resulted in putative protein identification.

About half of the proteins that changed expression levels were up-regulated for both males and females. Identification of the 17 proteins from males is displayed in Tables 1–3 and the 38 proteins from females are presented in Tables 4–6. Several of the same protein functional categories were affected for both males and females. Two notable differences are the increased expression of proteins associated with carbohydrate metabolism and proteins associated with protein structure and signal transduction. Expression of these genes changed considerably for females, but not males.

For males, larval media supplemented with WGO up-regulated gene expression by 1.84-fold (M-35) to 120.35-fold (for protein M-29). Dietary WGO also led to down-regulated expression of some

Table 5
Proteins from female adult *Bactrocera dorsalis* homogenates identified by *de novo* sequence analysis.

Spot no.	Putative protein with accession no. (<i>species</i>)	Calculated mass ^a	Calculated pI ^a	No. peptides ^b	E-value ^c	Peptide SEQUENCE ^d
F-20	PREDICTED: similar to maltase 1 <i>Tribolium castaneum</i> gi 91081575	65305	4.66	3	7e–07	YYLHQFTSAQPDLNFR
F-64	23 kDa heat shock protein beta <i>Ceratitis capitata</i> gi 195954354	18927	5.74	3	1e–05	VQIQQTGPAHLNVK
F-98	Phosphoglyceromutase <i>Drosophila melanogaster</i> gi 111145319	28672	6.68	1	0.009	FDVAHTSVLTR
F-103	ESCL, isoform B <i>Drosophila melanogaster</i> gi 85726419	20576	5.11	1	0.70	DEEAESEATDAAPAEVVKR
F-105	Sarcoplasmic calcium-binding protein 1 <i>Drosophila melanogaster</i> gi 62862484	21647	4.71	6	4e–05	YSWDNRVDFVVR
F-111	J20027 <i>Drosophila virilis</i> gi 195384110	14422	4.66	2	1e–07	KALDYLLSLPPAQRKR
F-120	GJ13834 <i>Drosophila virilis</i> gi 195378863	20656	5.40	2	2e–04	LPLLLSLADDLNR

^a Determined using the ExPASy Compute PI/MW tool, http://ca.expasy.org/cgi-bin/pi_tool.

^b The number of sequences deduced using *de novo* sequence analysis that resulted in the same protein identity or function.

^c E-values were generated by BLASTp searches using PAM30, within "Insecta". Values presented here are those of the peptides with the most significant E-values.

^d Sequences were determined by *de novo* sequence analysis from observations of the original spectra. Only the peptide sequences with the lowest E-values are shown.

Note: all sequences are those of deduced partial sequence tags (therefore Lys/Arg residues not shown on C-terminal).

genes by 20% (M-32) to 80% (M-57). For females, WGO supplementation led to up-regulation of gene expression by 1.28-fold (F116) to 17.93-fold (F-05). Expression of three genes encoding proteins increased by 15.8-fold (F-02), 17.9-fold (F-05) and 9.8-fold (F-19) in females. WGO also led to down-regulation of genes in females, by 20% (F-57) to 74% (F-105). Seventeen down-regulated genes were identified as shown in Table 6.

We designed primers to genes corresponding to six proteins that changed in expression on analysis of 2D gels. Genes were selected on the basis of their overall expression level and efficiency of design. We used these primers to confirm parallel changes in protein spot densities and mRNA expression for proteins F-22, F-36, F-40, F-43, M-35, and M-40 (Fig. 2). As for M29, the Mascot data for this protein was inconsistent (the ID data was inconsistent, see footnote 'h' of Table 1) until later when we determined the protein ID by *de novo* sequence analysis. The end result was that M29 had the same protein identification as M28, a protein for which we were unable to design suitable primers.

4. Discussion

The data reported in this paper support the hypothesis that one mechanism of WGO action in insect nutrition is through its influence on gene expression. Dietary polyunsaturated fatty acids (PUFAs) also influence gene expression in vertebrate systems, sometimes altering expression of many genes. In human peripheral blood mononuclear cells of healthy elderly people, for a single example, dietary supplement with a combination of eicosapentaenoic acid (20:5n–3) and docosahexaenoic acid (22:6n–3) (DHA) changed expression of 1040 genes after 26 weeks (Bouwens et al., 2009). We used 11-day-old adults because preliminary studies showed no difference in protein expression levels in newly eclosed adults (Chang and Coudron, 2007). The absence of gene expression differences in newly eclosed adult oriental fruit flies (Chang and Coudron, 2007) and the present finding of substantial differences in 11-day-old adults indicate a time frame during which the impact of WGO in the larval diet is expressed in the adult stage. We defer

further speculation until after our study of the timing of gene expression changes. We registered up-regulation and down-regulation of gene expression as changes in the expression of 17 identified proteins in adult males and 38 identified proteins in adult females.

WGO influenced expression of more genes in females than in males, but also exerted larger influences on expression changes in females which we speculate is due to ovarian development events. We now turn to a discussion of a few selected genes, those that decreased in expression by at least 50% or increased by at least 2-fold.

Dietary WGO led to enhanced expression of genes encoding enzymes needed for energy production in adult males and females. For males this included the TCA cycle enzymes succinyl-CoA synthetase (M-36) and aconitase, an iron-sulfur protein that catalyzes isomerization of citrate to isocitrate via *cis*-aconitate (M-45). For females this included ATPase (F-06), ATP synthase (F-22) and phosphoglucomutase, which in glycolysis converts glucose-1-phosphate to glucose-6-phosphate (F-19). Expression of genes encoding proteins associated with carbohydrate metabolism, including glycosyl hydrolases, which hydrolyze complex sugars into smaller ones (F-02) and maltase (F-20) also increased. These differences between the increased gene expression levels in males and females, i.e., the increased TCA cycle activity for males and increased ATP production and carbohydrate metabolism in females, presumably reflect the different energy needs of mature male and female flies. We infer therefore, that WGO increased TCA cycle activity for males and glycolysis for females. With further investigation it may be possible to correlate these increases in gene expression with traits that are desirable for the rearing and performance of insects. These include reported increases in adult flight capabilities, mating, and in egg production and hatch in oriental fruit flies reared on media supplemented with WGO (Chang and Vargas, 2007).

WGO led to increased expression of genes encoding cuticular and musculature proteins in males and females. These include chitin-binding proteins (M-28, M-29, F-111) and the muscle contraction protein tropomyosin (M-42, F-43). In males, WGO

Table 6Quantity^a and function of proteins identified by MS/MS from Female Adults whose larvae fed on diet with (+WGO) or without wheat germ oil (–WGO), respectively.

Spot no.	Protein function	+WGO/–WGO ratio	t-Test p-value
<i>Energy metabolism</i>			
F-06	ATPase: associated with a wide variety of cellular activities.	3.94	0.013
F-19	Phosphoglucosmutase 1: important in glycolysis and glycoprotein synthesis.	9.76	0.011
F-22	V/A-type ATP synthase: couples ATP hydrolysis to the build up of an H ⁺ gradient; membrane-associated.	2.24	0.003
F-34	Mitochondrial aconitase A catalytic domain: enzyme in the TCA cycle.	0.66	0.032
F-46	Malate dehydrogenase, mitochondrial: enzyme in the TCA cycle.	0.55	0.020
F-53	ATP synthase subunit.	0.64	0.026
F-54	Glyceraldehyde 3-phosphate dehydrogenase: plays a role in glycolysis and gluconeogenesis.	0.49	0.024
F-57	Fructose-1,6-bisphosphate aldolase: plays a role in glycolysis and gluconeogenesis.	0.20	0.003
F-58	Phosphoglycerate mutase: 8 th reaction in glycolysis.	0.53	0.009
F-60	Essential for the production of energy used in insect flight.	0.36	0.014
F-61	Plays an important role in several metabolic pathways and is essential for efficient energy production.	0.73	0.021
F-98	Phosphoglyceromutase: involved in glycolysis.	1.88	0.004
F-99	Aldolase: involved in glycolysis.	0.74	0.033
F-101	Phosphoglycerate mutase: involved in glycolysis.	0.46	0.037
F-121	Triosephosphate isomerase (TIM): plays an important role in several metabolic pathways, essential for efficient energy production.	0.43	0.001
<i>Carbohydrate metabolism</i>			
F-02	Glycosyl hydrolases family 31.	15.85	0.003
F-20	Domains include: maltase 1, trehalose-6-phosphate hydrolase and α -amylase.	4.43	0.015
<i>Protein structure and function</i>			
F-36	ATP dependent 26S proteasome regulatory subunit: involved in posttranslational modification, protein turnover, and acts as a chaperone.	1.96	0.021
F-63	Cyclophilin-type peptidylprolyl cis-trans isomerase: involved in protein folding, can modulate protein function.	1.97	0.018
F-64	Acts as chaperone that protects proteins against heat-induced denaturation and aggregation.	3.95	0.0001
F-88	Calreticulin: Ca ²⁺ binding/storage chaperone involved in protein folding and quality-control pathways.	1.37	0.034
F-104	Catalyzes an important step in the elongation cycle of protein biosynthesis.	2.59	0.002
F-116	Central enzyme of nonlysosomal protein degradation in both the cytosol and nucleus.	1.28	0.005
<i>Protein structure and signal transduction</i>			
F-05	HSP90: involved in protein folding/stabilization, refolding of denatured proteins, and signal transduction.	17.93	0.002
F-07	Contributes to various cellular processes including signal transduction, protein folding, protein degradation.	4.67	0.012
F-15	Hsp70 protein: involved in protein folding and signal transduction; ATP dependent.	1.80	0.003
F-40	Involved in protein folding and signal transduction; ATP dependent.	1.40	0.006
F-114	Involved in protein folding and signal transduction; ATP dependent.	1.32	0.022
F-120	α -crystallin-type small(s) Heat Shock Protein: stress-induced protein chaperone that prevents aggregation and assists in protein refolding.	13.93	0.011
<i>Signal transduction</i>			
F-105	Participates in numerous cellular regulatory processes such as metabolism, muscle contraction, growth, and immune responses.	0.74	0.051
F-109	Calcium-binding protein: participates in numerous cellular regulatory processes.	0.68	0.056
<i>Cell structure</i>			
F-90	Major component of microtubules.	1.59	0.006
<i>Cell protection</i>			
F-106	Glutathione S-transferase: involved in cellular detoxification.	0.38	0.008
<i>DNA replication and repair</i>			
F-51	dUTPase: hydrolyses dUTP to dUMP and pyrophosphate.	1.79	0.013
<i>Mobility, digestion, or exoskeleton</i>			
F-43	Tropomyosin: in striated muscle, regulates muscle contraction; in smooth muscle and non-muscle tissues, exact role is unclear.	4.36	0.049
F-45	Chitin-binding peritrophin-A: found in midgut peritrophic matrices.	0.52	0.011
F-105	Important for proper muscle functioning.	0.74	0.051
F-111	Insect cuticular protein; chitin-binding protein.	2.53	0.007
F-112	Chitin-binding peritrophin-A domain: often associated with midgut peritrophic matrix proteins.	0.38	0.003
<i>Gene regulation</i>			
F-103	Extra sex combs-like protein: involved in gene regulation of post-embryonic development.	5.34	0.028

^a As determined by Delta 2D software.

supplementation led to increased expression of genes for cell protection proteins, specifically glutathione reductase and thioredoxin reductase-1 (M-03). For females, dietary WGO led to enhanced expression of genes for heat shock proteins (F-05, F-07, F-11, F-64), chaperone proteins (F-36 and F-63), protein elongation factor (F-104) and the development-related protein Extra Sex Combs-like protein (ESCL; F-103). ESCL is a member of the polycomb Group genes, responsible for transient repression of

homeotic gene expression (Sathe and Harte, 1995). These increased expression levels, i.e., structural and signal transduction, may reflect higher activity associated with increased reproduction compared to adults of larvae reared without WGO.

WGO also led to decreases in gene expression, enhancing the verisimilitude of our finding that dietary WGO influences gene expression. For adult males and females reared on the diet enriched with WGO we recorded decreased expression of genes for

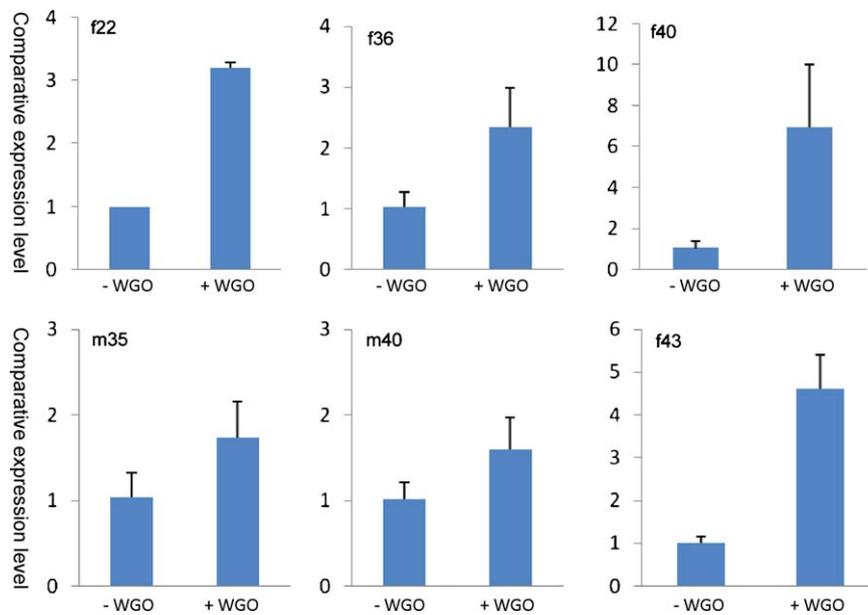


Fig. 2. WGO influence on mRNA levels in male (m) and female (f) adult *B. dorsalis*. Gene-specific primers were generated based on sequence data from selected proteins and used to determine mRNA levels using quantitative PCR. Histogram bars represent mean \pm SE.

aldolases (M-08, F-57), glyceraldehyde 3-phosphate dehydrogenase (GAPDH; M-53, F-54) and glutathione S-transferase or GST (M-20, F-106). Aldolase A, the protein detected in female homogenates acts in glycolysis, breaking fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, mostly in muscle. The F isoform from males is an F-actin binding protein, probably involved in regulating cell structure. GAPDH is best known for its role in glycolysis but has also been implicated in transcription activation (Tarze et al., 2007). GST is important in detoxification processes. We speculate that decreased expression indicates an elevated need for these proteins in males and females reared on lipid-deficient diets.

As is true for most analyses of protein expression, the biological significance of all gene changes affected by lipid-deficient or lipid-replete culture media is outside the scope of a single paper. Nonetheless, our finding that a gene encoding an insect chitin-binding protein (M-29) increased in expression by 120-fold in adult males reared on media supplemented with WGO has profound implications for understanding insect biology at the basic and applied levels. Chitin, a polymer of N-acetyl- β -D-glucosamine, is a major component of insect cuticle and an integral component of insect peritrophic matrices. The biological role of chitin is to serve as a mechanically strong scaffolding. It is associated with various proteins, cuticle proteins and peritrophic matrix proteins. Because of its functions in cuticle and peritrophic matrices, chitin undergoes a nearly constant and tightly regulated flux of degradation and synthesis (Merzendorfer and Zimoch, 2003). The chitinases, peritrophic matrix proteins and some other proteins associated with chitin are chitin-binding proteins, featuring characteristic chitin-binding domains (CBDs). Chitinases have one, two or three CBDs, while peritrophic matrix proteins have 2–5 CBDs (Shen and Jacobs-Lorena, 1999). Protein M-29 may be a cuticular chitin-binding protein, although it could as well be a peritrophic matrix component. In either case, it is clear that expression of the gene encoding this protein depends on meeting requirements for essential lipid, particularly PUFAs. While PUFAs are not major components of chitin–protein matrices, one aspect of the biological significance of nutritionally essential PUFAs is related to expression of a gene encoding a chitin-binding protein. We infer from the very large increase in expression of a gene encoding protein M-29 that essential

PUFA nutrition is linked to structural aspects of chitin–protein matrices. With respect to applied insect science, a major thrust in global biological control programs is to mass-rear biologically fit beneficial insects in a cost-effective manner. Expression of genes encoding sensitive biomarker proteins, such as M-29, may be applied to efficient formulation of high quality culture media for selected beneficial insect species (Coudron et al., 2006).

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