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Larval dietary wheat germ oil influences age-specific protein expression in adults of the oriental fruit fly

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ABSTRACT

Changes in essential dietary components alter global gene expression patterns in animals. We reported on a proteomics study designed to identify molecular markers of deficiencies in culture media developed for the oriental fruit fly, *Bactrocera dorsalis*. In that study, we found significant changes in expression of 70 proteins in adults of larvae reared on media lacking wheat germ oil (WGO), compared to media supplemented with WGO. Of these, a gene encoding an insect chitin-binding protein was expressed at about 120-fold higher levels in adult males reared on media supplemented with WGO. We inferred it may be feasible to develop the gene as a molecular marker of dietary lipid deficiency. The work was focused, however, on analysis of 11 day old adults. We have no information on expression of the chitin-binding protein, nor on any other proteins at other adult ages. In this paper we address the idea that the whole animal proteome changes dynamically with age. We reared separate groups of fruit fly larvae on media with and without WGO supplementation and analyzed protein expression in adult males and females age 0, 4, 8 and 12 days old using 2D electrophoresis. Gel densitometry revealed significant increases (by >2-fold) and decreases (by >50%) in expression levels of 29 proteins in females and 10 in males. We identified these proteins by mass spectrometry on MALDI TOF/TOF and bioinformatic analyses of the protein sequences. Two proteins, peroxiredoxin (26-fold increase) and vitellogenin 1 (15-fold increase) increased in expression in day 8 females. The key finding is that most changes in protein expression occurred in day 8 females. We infer that the fruit fly proteome changes with adult age. The natural changes in proteome with adult age is a crucial aspect of developing these and other proteins into molecular markers of lipid deficiency in fruit flies and possibly other insect species.

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1. Introduction

Polyunsaturated fatty acids (PUFAs) are essential nutrients for most insect species (Dadd, 1985). There are important exceptions to the generalization that insects require dietary PUFAs. The fruit fly, *Drosophila melanogaster*, and possibly other dipterans, are able to live through several generations without dietary PUFAs (Rapport et al., 1984). Mosquitoes have a PUFA requirement, however, it is met by very small, vitamin quantities of dietary arachidonic acid (20:4n – 6) and not by C18 PUFAs (Dadd, 1985). House crickets, *Acheta domesticus*, and an unknown number of other insect species are apparently free of essential fatty acid requirements because they express a Δ-12 desaturase that catalyzes the conversion of oleic acid (18:1n – 9) into linoleic acid ($^{\Delta\text{-}9,12}$18:2n – 6; de Renobales et al., 1987). Symptoms of PUFA deficiency include juvenile mortality, wing deformation and monstrous failure at adult eclosion (Dadd, 1985). In other cases, PUFA deficiency symptoms do not appear until a second or subsequent generation (Dadd, 1985). Because essential fatty acid deficiencies do not appear early in developing insects, optimizing the lipid components of culture media for mass rearing typically requires long-term research programs. The goal of proteomic analyzes is to attenuate the lengthy research programs.

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a widespread pest of fruit and vegetable agriculture throughout the Pacific islands and tropical Asia. Current programs to manage this pest include use of a sterile insect technique (SIT), which is based on the ability to mass-produce sterile adults in a cost-efficient manner (Parker, 2005). Cost-effective mass rearing requires an understanding of nutritionally essential dietary components and appropriate sources to supply these components. Dietary PUFAs are apparently essential for *B. dorsalis* because supplementing a liquid diet with wheat germ oil (WGO; composed of five fatty acids, which vary in proportions, 16:1[11–19%], 18:0 [1%], 18:1[12–28%], 18:2n – 6[42–59%] and 18:3n – 3[2–11%], and vitamine E) led to substantial improvements in life parameters, including larval development, pupal recovery, proportions of flight-capable adults,

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mating, egg production and egg hatch rates (Chang and Vargas, 2007; Chang, 2009). Because these improvements in biological performance emerge from underlying changes in gene expression, we investigated the hypothesis that one mechanism of the WGO influence on fly performance parameters is via its influence on gene expression, as seen in studies in mammalian nutrition (de Roos and McArdle, 2008). Experiments designed to test our hypothesis revealed that rearing *B. dorsalis* larvae on WGO-supplemented media led to substantial changes in protein expression in the corresponding adults (Chang et al., 2010), and the third instar larvae and F_1 eggs (Coudron et al., 2011). We infer that the essential nutrients provided in WGO, PUFAs and possibly vitamin E, influence the cellular proteome in developing and adult oriental fruit flies.

These reports were focused, however, on analyzes of protein expression in juvenile and adult insects at a single time point in their developmental excursion. While it is understood that protein expression changes during development, it is not clear whether the changes in protein expression associated with WGO supplementation is fixed throughout the life cycle or varies as a function of age. In this paper we address this important issue by analyzing the influence of dietary WGO on gene/protein expression in adult male and female fruit flies at several points in adulthood. Here we report that the influence of dietary WGO on protein expression varies among adult ages.

2. Material and methods

2.1. Insects and sample collection

Newly laid colony eggs (<6 h) of oriental fruit fly, *B. dorsalis* were provided by the Tropical Crop and Commodity Protection Research Unit of the USDA's Agricultural Research Service (ARS) in Honolulu, Hawaii and maintained as previously described (Chang et al., 2010). In nature females lay about 1200–1500 eggs over their lives (Weems et al., 2010). Their life spans in culture exceed 95 days, however, we kept the flies up to no more than 20 days in this work. *B. dorsalis* larvae were reared throughout their development to pupation on a liquid diet supplemented with WGO (0.66%, v:v), or in separate groups without WGO (Chang and Vargas, 2007). Adults from both larval diets were maintained on a mixture of sugar:protein hydrolysate (3:1, wt:wt) (Chang et al., 2004). Adult females and males, age 0, 4, 8 and 12 days post-emergence were placed in cryogenic vials (5 ml) and stored at −80 °C until processing for protein analysis. All adults were maintained as virgins until age 12 days. At age 12 days, one group was set up for mating and individuals were collected while mating. All other fruit flies were unmated. The gender and mating status are indicated in the protein codes: M = males; F = females; MT and FT identify mated adults.

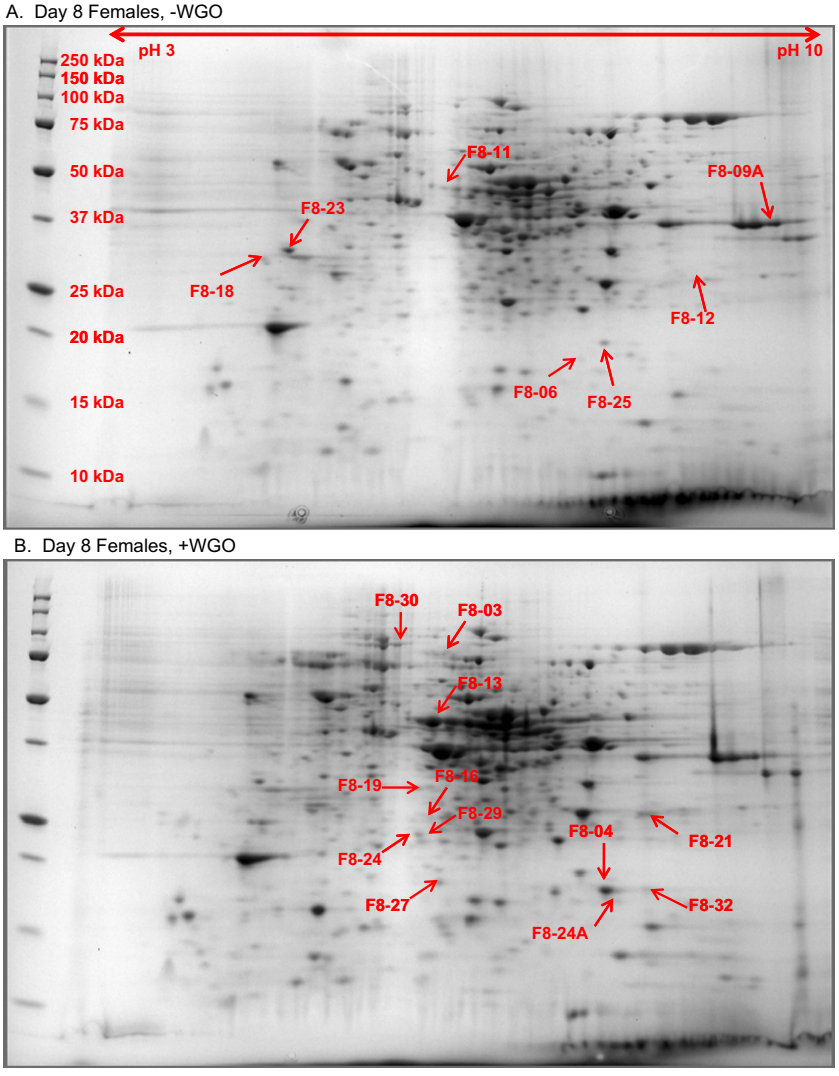

Fig. 1. Representative 2D gels showing the influence of supplementing basal culture medium with WGO on protein expression in 8 day old male and female adult fruit flies, *B. dorsalis*. (A) adults from larval diets without WGO supplementation; (B) adults from larval diets with WGO supplementation. The protein preparation and electrophoresis protocols are detailed in M & Ms. Ladders on the left of each gel represent molecular weight markers. The numbered protein spots are identified in the Tables.