 doi: 10.1111/j.1365-294X.2005.02654.x

Gene expression patterns accompanying a dietary shift in *Drosophila melanogaster*

L. D. CARSTEN, T. WATTS and T. A. MARKOW
Department of Ecology and Evolutionary Biology, 310 Biosciences West, University of Arizona, Tucson, AZ 85721, USA

Abstract

The ability of many organisms to switch to new hosts can be critical to their survival in the wild. However, the genetic mechanisms underlying such shifts are poorly understood. In this study, we used complementary DNA (cDNA) microarrays to ask if changes in gene expression are observed in response to a dietary shift in *Drosophila melanogaster*, a dietary generalist. We found significant and repeatable differential expression in a number of genes related to metabolic function and stress, suggesting that a functional genomics approach will be useful in seeking loci involved in the ability of flies to utilize different resources.

Keywords: diet, dietary shifts, *Drosophila*, ecological genomics, functional genomics, gene expression, microarray

Received 1 March 2005; revision accepted 20 May 2005

Introduction

While functional genomic techniques are frequently used to address a variety of questions in molecular disciplines (Gibson & Mackay 2002; Reinke & White 2002), they also have great untapped potential to address ecological questions, such as the architecture of complex traits (Gibson 2002; Glazier *et al*. 2002; Ffrench-Constant *et al*. 2003). Elucidating the links between genotype, gene expression, phenotype, and environment should help us better understand how an organism adjusts to variable or novel conditions. For instance, the ability to switch to new food sources can help organisms survive in fluctuating environments, but little is known about the underlying genetic and molecular mechanisms involved in such shifts. Regulation of gene expression may be one way in which an organism can make these switches. Because different food sources can vary substantially in nutritional content, suites of genes associated with various biochemical pathways are predicted to be expressed differentially in response to dietary shifts.

Drosophila melanogaster provides an ideal model system in which to examine genetic changes associated with dietary shifts. This fly is a dietary generalist (Soliman & van Herrewege 1988), and thus the genome likely codes for biochemical pathways that would facilitate a broad diet. In addition, the *D. melanogaster* genome is well annotated, which allows identification of genes and gene families that may be up- or down-regulated in response to different diets. A number of studies have addressed the role of specific genes in the metabolic pathways of *Drosophila*, such as lipid or glycogen metabolism (Hader *et al*. 2003; Montooth *et al*. 2003; Teixeira *et al*. 2003; Wisotzkey *et al*. 2003). In addition, previous microarray studies have identified distinct patterns of gene expression under conditions of food restriction (Zinke *et al*. 2002) and sugar ingestion (Zinke *et al*. 1999).

While previous work has revealed genes and gene expression patterns related to metabolism, no study to our knowledge has elucidated regulatory changes associated with dietary shifts. In this study, we used complementary DNA (cDNA) microarrays to explore how gene expression patterns change in response to moving *D. melanogaster* larvae from cornmeal medium to pure bananas. These two food sources are known to differ chemically as well as in their effects on the elemental compositions of the bodies of the flies reared on them (Markow *et al*. 1999). In particular, the macromolecular content of these foods is varied: pure bananas contain a higher percentage of carbohydrates, and a lower percentage of proteins and lipids, than the standard cornmeal medium (Banana: protein 4.3%, lipid 1.3%, carbohydrate 91.1%; cornmeal medium: protein 10.1%, lipid 2.0%, carbohydrate 85.7%. Amounts were calculated from values of ingredients posted at the US Department of Agriculture website, http://www.nal.usda.gov/fnic/foodcomp/search/). This study attempts to link changes

Correspondence: Laura Carsten, Fax: 520-621-9190; E-mail: carsten@email.arizona.edu

© 2005 Blackwell Publishing Ltd

in expression patterns with differences between these food sources. Our ability to detect statistically significant changes in expression patterns indicates that this approach will be useful in understanding the genetic mechanisms underlying diet breadth, an ecologically relevant trait.

Materials and methods

Fly rearing and RNA extraction

Flies used in this study were derived from a multifemale collection at Iraklion, Crete, Greece, in August 2002 and reared in the laboratory for several generations. Three replicates were performed. At the start of each replicate, we collected 300 newly eclosed adults, separated them by sex, and stored them at low density in shell vials with standard cornmeal media for 3 days. Sexually mature males and females were placed together and allowed to mate. Mated females were allowed to oviposit overnight in chambers containing Petri dishes filled with cornmeal medium (http://flyfood.arl.arizona.edu/cornmeal.php3). Hatching first instar larvae were reared for 36 h on cornmeal medium in Petri dishes and then transferred to one of two conditions: (i) a control Petri dish consisting of cornmeal medium, or (ii) an experimental Petri dish containing pure mashed banana. After 24 h, larvae were plucked from these dishes, washed, transferred to microcentrifuge tubes in groups of 20, and flash frozen in liquid nitrogen. All samples were stored at −80 °C until RNA extraction. RNA was extracted from each tube using a QIAGEN RNeasy Kit (QIAGEN Inc., standard protocol).

We used cDNA microarrays printed by the Genetic Analysis and Technology Core Custom Microarray Facility at the University of Arizona to assess expression levels. Printing material was amplified from BDGP Unigene Library version 1.0 containing 6000 ESTs. Products were purified using Millipore Multiscreen filtration plates and resuspended in 50% DMSO solution. Each product was printed in triplicate on Corning GAPSII aminosilane slides using a Virtek Chipwriter PRO microarray spotter.

Between 10 and 15 μg of RNA were used for each reaction. RNA was reverse transcribed and labelled with one of two fluorescent dyes, Cy3 or Cy5 (details of protocol at http://gatc.arl.arizona.edu/Services/Microarray/aminoallyllabelling.html). Hybridization was completed on a Genomic Solutions Gentac automated hybridization station. Labelled material was allowed to hybridize to the array for 16 h at 60 °C. Wash conditions were (i) low stringency wash twice at 50 °C for 40 s (1× SSC/0.2% SDS), (ii) medium stringency wash twice at 42 °C for 40 s (0.1× SSC/0.2% SDS), (iii) high stringency wash twice at 42 °C for 40 s (0.1× SSC). Hybridized arrays were scanned using the Applied Precision arrayWoRx^e slide scanner. We performed three independent hybridizations and three dye flips for a total of six arrays (2 treatments × 2 dyes × 3 replications = 12 samples, for 6 arrays). Each replicate consisted of an independent biological sample isolated on separate days.

Microarray analysis

To analyse differential expression between the two treatments, we conducted a mixed-model ANOVA test of relative fluorescence intensities (Wolfinger *et al*. 2001). This procedure fits the effects of interest to each gene across the entire experiment, which allows for direct contrasts between expression levels and obviates the need for a reference sample (Jin *et al*. 2001). The raw values were $\log_2$ transformed and normalized using a quantile normalization procedure (Bolstad *et al*. 2003). We used SAS (SAS Institute Inc.) to perform the mixed-model procedure with the following parameters:

$$\text{Normalized Intensity} = \text{Trt}_i + \text{Dye}_j + \text{Day}_k + \text{Set}_l$$

where Trt refers to the ith treatment, either cornmeal or banana food; Dye refers to the jth dye type, Cy3 or Cy5; Day to the kth biological replicate ($k = 1, 2, 3$); and Set to the lth set of three replicate spot sets per slide ($m = 1, 2, 3$). Trt and Dye were fixed effects, while Day and Set were random effects. Because a P value cut-off of 0.05 is not sufficient to detect false positive errors in this type of experiment (i.e. multiple tests), but the Bonferroni correction is likely too conservative for microarray analyses (Jin *et al*. 2001), we estimated q values from raw P values. The q value estimates a measure of significance for each gene based on the 'false discovery rate', or the rate at which genes that appear significant are actually not significant (Storey & Tibshirani 2003). Under this model, a gene associated with $q \leq 0.05$ is considered to be differentially expressed between treatments. Gene function for differentially expressed genes was assigned based on annotations in FlyBase.

Results

A number of genes showed significant differential expression when larval *Drosophila* were switched from a cornmeal medium to a banana diet. At the level of $q \leq 0.05$, there is support for the effect of treatment (food type) on 90 genes. In Fig. 1, we present a 'volcano plot' of statistical significance against treatment effect. A difference of one unit on the x-axis represents a twofold change in fluorescence intensity. Thus, genes further away from the zero value on that axis show higher differences in intensity of expression, while genes above the value 3 on the y-axis (corresponding to a P value of 0.001, which is approximately a q value of 0.05) show statistically significant differences in expression. The

© 2005 Blackwell Publishing Ltd, *Molecular Ecology*, 10.1111/j.1365-294X.2005.02654.x

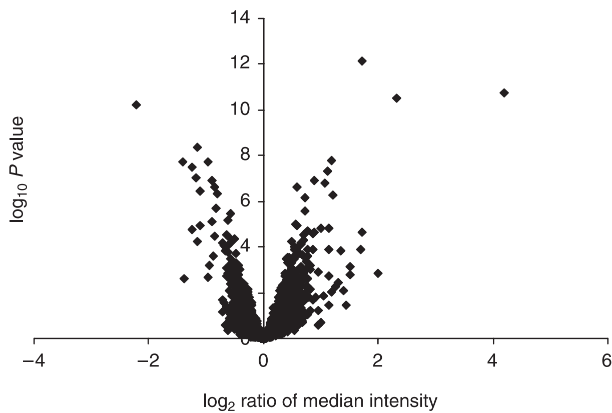

Fig. 1 Volcano plot of the 5620 genes examined in this study. Each spot represents a gene, with the $\log_{10}$ of the P value plotted against the $\log_2$ ratio of quantile-transformed median fluorescence intensity for each gene. Genes to the right of zero are down-regulated in the banana treatment, while genes to the left are up-regulated. A difference of one unit on the x-axis represents a twofold change in fluorescence intensity. Genes further away from the zero value on that axis show a higher difference in intensity of expression, while genes above the value 3 (equivalent to a q value ≤ 0.05) on the y-axis show statistically significant differences in expression.

other fixed effect, Dye, was not significant for any of the 5620 genes at $q \leq 0.05$. For the random effects in the model, Day and Set, there were no abnormal variance components evident (the highest variance for any effect in this subset was 1.16, compared with a high of 22.5 in the entire set of genes examined).

For the 90 genes significant for the main effect of interest, treatment, 40 genes were up-regulated (19 previously named and/or of known function), and 50 were down-regulated (36 previously named and/or of known function). These genes have diverse roles, largely related to metabolism and stress regulation (Table 1).

Of the 55 genes listed in Table 1, six (10.9%) have a role in carbohydrate metabolism/transport, 11 (20.0%) have a role in fatty acid/lipid metabolism/transport, and six (10.9%) have a role in protein metabolism. Additionally, eight genes (14.5%) have a role in stress regulation and/or defence. Of the six genes involved in carbohydrate metabolism or transport, three were up-regulated in response to the dietary shift from cornmeal medium to bananas, and three were down-regulated. Similarly, the results for genes related to protein metabolism were fairly evenly mixed according to treatment, with four up-regulated and two down-regulated in response to the dietary shift. In contrast, of the 11 genes involved in fatty acid/lipid metabolism and transport, three were up-regulated and eight were down-regulated in response to the shift. This suggests that there is little change in carbohydrate and protein metabolism and relatively less fatty acid/lipid metabolism occurring when these larvae use banana as a food source. The results also suggest that physiological stress may be reduced in response to this dietary shift; of the eight genes involved in stress regulation and/or defence, two were up-regulated while six, including a heat shock protein and three cytochrome P450 genes, were down-regulated.

Discussion

Significant changes in transcript levels for a number of *Drosophila melanogaster* genes are observed when same-age larvae are transferred to a new food source. Differential transcription of genes involved in normal metabolism is to be expected when these animals are asked to break down and incorporate foods of different chemical make-up. Because the different replicates came from biological samples reared on separate days, which could produce slight variability in age among larvae in overnight cultures, it was important to examine the degree of variability between replicates for the 'Day' effect. Because we fitted Day as a separate effect in our model, any variance arising between replicates should be accounted for in the model; however, looking at the variance associated with this effect directly also indicates that the Day effect is not significant for any of the differentially expressed genes. Thus, it appears that the changes in transcript levels that we observed are indeed due to differences in treatment rather than to any slight variability in larval age.

The patterns of gene regulation exhibited in response to the dietary shift to bananas may reflect differences in macromolecular content of these two food sources. In particular, bananas contain relatively more carbohydrates, and relatively fewer lipids, than cornmeal medium. The down-regulation of genes related to lipid/fatty acid metabolism when larvae were shifted to banana is consistent with these differences. We note that of the eight down-regulated fatty acid/lipid metabolism/transport genes, three are involved in fatty acid oxidation or catalysis, suggesting that fatty acid breakdown is reversed in the lipid-poor medium of banana. In addition, differences in transcript levels of stress-related genes suggest that larvae are less stressed in banana as opposed to cornmeal medium. Taken together, these results could indicate that larvae are preparing for energy storage when switched to a banana diet, which may indicate that banana is a preferred medium for *Drosophila* larvae.

Our results contrast somewhat with those of Zinke *et al*. (2002), who found that in contrast to starved larvae, sugar-fed animals up-regulated at least five enzymes that have a clear role in fatty acid biosynthesis. Instead, we found that larvae switched to a diet higher in carbohydrates tended to down-regulate fatty acid catabolism-related genes, and we found little evidence for increased lipid biosynthesis. However, Zinke *et al*. (2002) did find that sugar-fed larvae

© 2005 Blackwell Publishing Ltd, *Molecular Ecology*, 10.1111/j.1365-294X.2005.02654.x

Table 1 List of genes with expression changes in response to a dietary shift from cornmeal media to pure banana. Genes shaded grey were down-regulated, and unshaded genes were up-regulated. In total, 50 genes were down-regulated and 40 genes were up-regulated; this table shows the subset of 36 down-regulated and 19 up-regulated genes that have been named and/or have a known function

Annotation ID	$\log_2$ ratio	*P* value	*q* value	*Name*/Function	Biological process
CG4919	0.72	6.74E-07	1.90E-04	*Gclm*/glutathione gamma-glutamylcysteinyltransferase	glutathione biosynthesis
CG8782	0.56	2.31E-04	2.03E-02	*Oat*/ornithine-oxo-acid transaminase activity	amino acid biosynthesis
CG18730	1.13	1.34E-04	0.013	*Amy-p*/calcium ion binding, alpha-amylase activity, carb. metabolism	carbohydrate metabolism
CG8256	−0.68	8.33E-05	9.36E-03	*l(2)k05713*/glycerol-3-phosphate dehydrogenase	glycerol metabolism
CG15096	−0.63	1.76E-04	0.017	carb transport/metabolism, phosphate/cation transport	carbohydrate metabolism
CG1869	0.81	6.04E-04	4.24E-02	chitin binding	polysaccharide metabolism
CG10072	−0.60	3.93E-05	5.66E-03	*sgl*/UDP-glucose 6-dehydrogenase	heparan sulphate proteoglycan biosynthesis, polysaccharide chain biosynthesis
CG9466	2.32	3.15E-11	5.91E-08	alpha mannosidase	carbohydrate metabolism
CG9441	0.43	5.12E-04	0.037	*Pu*/GTP cyclohydrolase I activity	tetrahydrobiopterin biosynthesis
CG10800	−0.62	6.61E-06	1.55E-03	*Rca1*/mitosis regulation	regulation of mitosis
CG32019	0.68	2.06E-04	1.84E-02	*bt*/myosin-light-chain kinase activity	mesoderm development
CG5112	0.50	4.99E-04	3.64E-02	fatty acid amide hydrolase activity	nitrogen compound metabolism
CG7400	0.79	7.29E-04	4.71E-02	*Fatp*/long-chain fatty acid transporter	fatty acid metabolism
CG3415	0.49	4.31E-04	0.032728	estradiol 17-beta-dehydrogenase activity	fatty acid biosynthesis
GH23546	−0.49	6.18E-04	4.28E-02	*desat 1*/fatty acid biosynthesis stearoyl-CoA desaturase	fatty acid biosynthesis
CG5009	0.53	9.66E-05	1.06E-02	palmitoyl-CoA oxidase activity, fatty acid beta-oxidation	fatty acid beta-oxidation
CG3902	0.73	3.03E-04	2.45E-02	short branched chain acyl-CoA dehydrogenase	acyl-CoA metabolism
CG17597	0.77	1.30E-04	1.32E-02	acyl-CoA metabolism, lipid transport, fatty acid beta-oxidation	fatty acid beta-oxidation
CG2196	−0.58	3.32E-04	0.026	cation transport, coenzyme and prosthetic group metabolism	cation transport
CG6207	−0.51	4.17E-05	5.87E-03	*GlcAT-P*/glycoprotein biosynthesis	glycosphingolipid biosynthesis
CG8946	0.65	6.15E-05	7.86E-03	*Sply*/sphinganine-1-phosphate aldolase	sphingolipid catabolism
CG17320	0.69	5.08E-05	6.97E-03	*ScpX*/sterol carrier protein X-related thiolase	phospholipid transport
CG10962	1.72	7.16E-13	4.02E-09	oxidoreductase	metabolism
CG10184	0.40	3.39E-04	2.61E-02	threonine aldolase activity	amino acid catabolism
CG10138	−0.54	7.50E-04	0.047	*PpD5*/protein amino acid dephosphorylation	protein amino acid dephosphorylation
CG6483	−0.90	1.15E-07	5.06E-05	*Jon65Aiii*/serine-type endopeptidase	proteolysis and peptidolysis
CG10472	−0.59	6.60E-04	4.37E-02	serine-type endopeptidase	proteolysis and peptidolysis
CG6457	−0.98	1.78E-08	1.26E-05	*yip7*/chymotrypsin activity	proteolysis and peptidolysis
CG2145	0.50	5.48E-05	7.33E-03	serine-type peptidase	proteolysis and peptidolysis
CG9672	1.20	5.62E-07	1.66E-04	serine-type endopeptidase, carboxypeptidase	proteolysis and peptidolysis
CG13094	−2.21	5.89E-11	8.28E-08	*Dh31*/neuropeptide hormone activity	fluid secretion
CG17027	0.65	7.54E-04	0.047	inositol-1(or 4)-monophosphatase activity	intracellular signalling cascade
CG31811	0.56	1.00E-05	2.17E-03	*cenG1A*/AFR GTPase activator	small GTPase mediated signal transduction
CG2849	−0.57	6.69E-05	8.17E-03	*Rala*/GTPase activity	negative regulation of JNK cascade
CG9434	−1.15	6.07E-05	7.86E-03	*Fst*/response to cold	response to cold
CG2736	0.64	1.94E-04	0.018	scavenger receptor activity, defence response	defence response
CG1633	−0.68	1.05E-04	1.13E-02	*Jafrac 1*/thioredoxin peroxidase	cell redox homeostasis; defence response
CG4533	0.70	2.97E-05	4.50E-03	*l(2)efl*/heat shock protein	heat shock
CG3466	0.54	2.77E-04	0.023	*Cyp4d2*/electron transporter activity, oxidoreductase activity	electron transport; stress response
CG8453	0.76	2.02E-05	3.55E-03	*Cyp6g1*/cytochrome P450 microsome membrane	electron transport; stress response
CG4486	1.12	5.02E-08	2.82E-05	*Cyp9b2*/cytochrome P450-9b2, oxidoreductase activity	electron transport; stress response
CG11140	0.53	1.23E-04	1.30E-02	*Aldh-III*/aldehyde dehydrogenase activity, response to toxin	defence response
CG17058	1.00	1.48E-05	2.87E-03	*Peritrophin-A*/chitin binding, structural	chitin metabolism
CG7298	−1.15	4.07E-09	4.58E-06	structural constituent of peritrophic membrane	chitin metabolism

Table 1 *Continued*

Annotation ID	$\log_2$ ratio	*P* value	*q* value	*Name*/Function	Biological process
CG17052	1.72	2.11E-05	0.004	structural constituent of peritrophic membrane (*sensu* Insecta)	chitin metabolism
CG6933	−0.85	3.53E-05	5.22E-03	structural constituent of peritrophic membrane	chitin metabolism
CG4818	0.59	1.15E-05	2.32E-03	structural constituent of cuticle	unknown
CG1763	1.13	1.56E-05	2.92E-03	*nod*/kinesin motor	meiotic spindle organization and biogenesis
CG14228	0.43	3.05E-04	0.024	*Mer*/protein binding, endocytosis	endocytosis
CG4178	4.20	1.86E-11	5.23E-08	*Lsp1*/nutrient reservoir activity	transport
CG11064	−0.95	6.24E-04	4.28E-02	*RfaBp*/fatty acid binding	lipid transport
CG6544	0.58	2.33E-07	8.19E-05	*fau*	unknown
CG3767	0.85	2.64E-05	4.24E-03	*JhI-26*	unknown
CG4084	−0.52	6.00E-04	0.042	*l(2)not*/G-protein coupled receptor activity	unknown
CG8666	0.72	2.76E-06	7.04E-04	*Tsp39D*/cell-cell adhesion	cell-cell adhesion

Annotation ID refers to the annotation on FlyBase. $\log_2$ ratio refers to the $\log_2$ ratio of median fluorescence intensity for each gene. *P* values associated with the significance of each gene are shown for comparison with *q* values, or the rate at which genes that appear significant are actually not significant. Function and biological process are also supplied.

down-regulated four lipase genes involved in fat breakdown, which is similar to our findings. In addition, one of the genes up-regulated in the Zinke study in response to sugar, *sug*, was also up-regulated in the present study in a response to a shift to bananas, although this effect was only marginally significant ($q = 0.06$). This was the only gene that showed similar patterns of regulation between the two studies; however, this is not surprising, as a contrast between sugar and starvation alone may induce different changes than a contrast between two foods containing different nutrients and secondary compounds.

Sug is induced in sugar-fed larvae largely in the midgut, where it appears to repress fat catabolism prior to activation of the fatty acid synthesis pathway (Zinke *et al*. 2002). This characterization is consistent with the results of the present study, which indicate that fat catabolism is repressed in response to a shift to the banana diet.

Overall, these trends indicate that although different food sources may be of different value to growing *D. melanogaster* larvae, these larvae can facilitate switches between food sources through differential expression of genes related to stress and metabolism. Previous studies have shown that rates of lipid and protein metabolism change in various *Drosophila* species under stressful conditions found in natural settings, such as desiccation and low food availability (Marron *et al*. 2003). The results presented here provide a first glimpse at the potential genetic mechanisms that allow such metabolic responses to variable food conditions.

Several projects are currently underway to sequence the genomes of ecologically diverse *Drosophila* species (for specifics, see http://www.genome.gov/Pages/Research/Sequencing/BACLibrary/DrosophilaBAC.pdf). With the availability of complete genome sequences for additional species, our approach could be expanded to examine differences in gene expression when species with dietary specializations are switched to different foods. Comparing these results with those obtained from *D. melanogaster*, a dietary generalist, may shed light on the genetic mechanisms involved in dietary specialization and perhaps speciation.

Acknowledgements

We thank Cheryl Vanier for generous assistance with statistical analysis. Luciano Matzkin and Michael Goodisman provided helpful statistical advice and comments on experimental design and analysis. We thank Michael Wells for discussions regarding gene function and metabolism. We also thank Caitlin Cameron, Brian Coullahan, and Susan Miller for technical work and advice on the microarrays. Grants to Therese Markow from the NIH and NSF funded this work.

References

Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*, **19**, 185–193.

Ffrench-Constant R, Waterfield N, Daborn P *et al*. (2003) *Photorhabdus*: towards a functional genomic analysis of a symbiont and pathogen. *FEMS Microbiology Reviews*, **26**, 433–456.

Gibson G (2002) Microarrays in ecology and evolution. *Molecular Ecology*, **11**, 17–24.

Gibson G, Mackay TF (2002) Enabling population and quantitative genomics. *Genetical Research*, **80**, 1–6.

Glazier AM, Nadeu JH, Aitman TJ (2002) Finding genes that underlie complex traits. *Science*, **298**, 2345–2349.

Hader T, Muller S, Aguilera M *et al.* (2003) Control of triglyceride storage by a WD40/TPR-domain protein. *EMBO Reports*, **4**, 511–516.

Jin W, Riley RM, Wofinger RD *et al.* (2001) The contributions of sex, genotype, and age to transcriptional variance in *Drosophila melanogaster*. *Nature Genetics*, **29**, 389–395.

Markow TA, Raphael B, Breitmeyer CM *et al.* (1999) Elemental stoichiometry of *Drosophila* and their hosts. *Functional Ecology*, **13**, 78–84.

Marron MT, Markow TA, Kain KJ, Gibbs AG (2003) Effects of starvation and desiccation on energy metabolism in desert and mesic *Drosophila*. *Journal of Insect Physiology*, **49**, 261–270.

Montooth KL, Marden JH, Clark AG (2003) Mapping determinants of variation in energy metabolism, respiration, and flight in *Drosophila*. *Genetics*, **165**, 623–635.

Reinke V, White KP (2002) Developmental genomic approaches in model organisms. *Annual Review of Genomics and Human Genetics*, **3**, 153–178.

Soliman MH, van Herrewege J (1988) Nutrition. In: *Drosophila as a Model Organism for Ageing Studies* (eds Lints FA, Soliman MH). Blackie and Sons Ltd, Glasgow, Scotland.

Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences, USA*, **100**, 9440–9445.

Teixeira L, Rabouille C, Rorth P, Ephrussi A, Vanzo NF (2003) *Drosophila* perilipin/ADRP homologue Lsd2 regulates lipid metabolism. *Mechanisms of Development*, **120**, 1071–1081.

Wisotzkey RG, Johnson AN, Takaesu NT, Newfield SJ (2003) Alpha/beta hydrolase2, a predicted gene adjacent to Mad in *Drosophila melanogaster*, belongs to a new global multigene family and is associated with obesity. *Journal of Molecular Evolution*, **56**, 351–361.

Wolfinger RD, Gibson G, Wolfinger ED *et al.* (2001) Assessing gene significance from cDNA microarray expression data via mixed models. *Journal of Computational Biology*, **8**, 625–637.

Zinke I, Kirchner C, Chao L, Tetzlaff M, Pankratz M (1999) Suppression of food intake and growth by amino acids in *Drosophila*: the role of plumpless, a fat body expressed gene with homology to vertebrate glycine cleavage system. *Development*, **126**, 5275–5284.

Zinke I, Schutz CS, Katzenberger JD, Bauer M, Pankratz MJ (2002) Nutrient control of gene expression in *Drosophila*: microarray analysis of starvation and sugar-dependent response. *EMBO Journal*, **21**, 6162–6173.

All authors are in the department of Ecology and Evolutionary Biology at the University of Arizona. Laura Carsten is a PhD student with interests in life history and sexual selection. Tom Watts is Research Specialist Senior in the comparative biology and genetics of *Drosophila*. Therese Markow is Regents Professor of Ecology and Evolutionary Biology and Studies speciation, evolution of mating systems, and adaptation in *Drosophila*.