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Quantitative Trait Loci for Lipid Content in *Drosophila melanogaster*

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Abstract

Recombinant inbred lines derived from a natural population were used to investigate natural genetic variation for lipid abundance, protein abundance, and weight of *Drosophila melanogaster*. Females were heavier and contained more lipid and soluble protein than males. Lipid and protein abundance were genetically correlated with female weight, but male weight was not correlated with lipid or protein. Lipid and protein abundance were genetically correlated in males but not in females. Quantitative trait loci (QTLs) for weight and protein abundance were predominantly on the X chromosome, whereas QTLs for lipid abundance were found on the second and third chromosomes. QTLs for lipid proportion (lipid abundance normalized by weight or protein abundance) were present on all chromosomes; a lipid proportion QTL on the third chromosome correlated with a QTL for starvation resistance observed in a previous study using the same set of recombinant inbred lines, suggesting that it might underlie both traits. Candidate genes are discussed in relationship to lipid abundance, lipid proportion, and starvation resistance.

Keywords: lipid content, starvation, life span, quantitative trait loci mapping, *Drosophila melanogaster*

Nonstandard abbreviations: QTL, quantitative trait locus; **RIL**, recombinant inbred line

The characterization of natural genetic variation for the control of obesity in humans is an important goal in human health research. However, it is difficult to acquire this information because there are limited methods available for the study of the genetic architecture of complex traits in humans. Conversely, quantitative trait locus (QTL) studies of lipid

abundance in model organisms like *Drosophila* are especially suited for this purpose. The study of obesity in *Drosophila* is relevant to humans because pathways of lipid metabolism are generally conserved between vertebrates and invertebrates (1). In flies, nutrigenomics has so far relied on traditional developmental-genetic approaches such as genetic screens and expression analyses (2). This research has focused only on genes with qualitative, all-or-nothing phenotypes. It is possible, however, that an entirely different class of genes is required specifically to fine-balance resource partitioning without having major stand-alone effects. The goal of our research here is to describe genetic architecture of natural variation in a widely used human model—Drosophila. We have previously constructed a set of 144 recombinant inbred lines (RILs) that segregate for up to four natural alleles per locus (3). Here, we weigh males and females from these lines and assay for lipid and protein abundance and for lipid proportion, which is analogous to obesity. The goal was to map QTLs for genetic variation in these characters.

Protein and lipid content and body weight were all significantly higher in females (0.793 mg/mL, 0.552 μg/mL, and 0.315 mg, respectively) than in males (0.424 mg/mL, 0.369 μg/mL, and 0.217 mg). The genetics of body size differences in females and males is not known, but it may be relevant to note that body size is controlled by a nutrient sensor mechanism during *Drosophila* growth (4). Scaled by body weight, lipid content was no longer different between sexes (1.78 vs. 1.80 μg/mL in males and females, respectively), but protein content remained sexually differentiated (1.99 vs. 2.55 μg/mL). In two-way ANOVAs, effects of sex, line, and sex-by-line interactions were significant at the $p < 0.0001$ level for every trait (data not shown). Male-female line means were correlated within traits (table 1). In males, neither protein nor lipid abundance was correlated with body weight, but in females, these correlations were positive and highly significant. Protein and lipid abundance were significantly correlated in males but not in females. Genetic correlations between some of these characteristics have been previously studied by selection in the laboratory, but the results were not consistent between experiments (5). Partially due to this reason, selection experiment results are marginally suitable for comparison with the present study.

Table 1. Correlation matrix of line means between lipid and protein abundance and body weight of males and females

		Protein abundance		Lipid abundance		Body weight	
Trait	Sex	Male	Female	Male	Female	Male	Female
Protein content	Male		**0.376**[a]	*0.215*	0.091	0.034	0.168
	Female	**<0.0001**		0.138	0.053	*0.233*	**0.285**
Lipid content	Male	0.013	NS		**0.640**	–0.114	0.152
	Female	NS[b]	NS	**<0.0001**		0.037	**0.307**
Body weight	Male	NS	0.011	NS	NS		**0.613**
	Female	NS	0.002	NS	**0.001**	**<0.0001**	

a. Correlation coefficients above the diagonal (italicized if significant and bold if $p = 0.001$ or less) with the level of statistical significance indicated below the diagonal. p values remaining significant after correction for multiple testing are bold.

b. NS, not significant

The QTL likelihood profiles for three X chromosome haplotypes (linkage groups), four second chromosome haplotypes, and three third chromosome haplotypes are represented on the left panels of figures 1 to 3. Two haplotypes of the third chromosome (2 and 3) are indistinguishable in this set of RILs and have been merged together into a 2–3 haplotype. Altogether, we have detected 12 QTLs significant at the $p = 0.05$ level, whereas three are expected by chance alone. The false discovery rate might then be approximated as 0.25. The QTL effects are summarized in table 2. A majority of the protein abundance and weight QTLs were located on the X chromosome. QTLs affecting lipid abundance were observed on the second and third chromosomes. Contiguous haplotype QTLs might, in fact, represent a single large effect QTL, for instance, protein abundance QTLs 5C–12C on the X chromosome. As another consideration, estimates of QTL effects on different haplotypes of the same chromosome might not be independent. For instance, protein content QTLs at 5C on haplotype 1 and 5D on haplotype 3 of the X chromosome and body weight QTLs at 22A on haplotype 2 and 22B on haplotype 3 of the second chromosome (underlined in the table 2) might, in fact, represent the effect of a single allele (for more explanations, see "Research Methods and Procedures"). Despite these caveats, the effect of QTLs was similar to the pattern of line means.

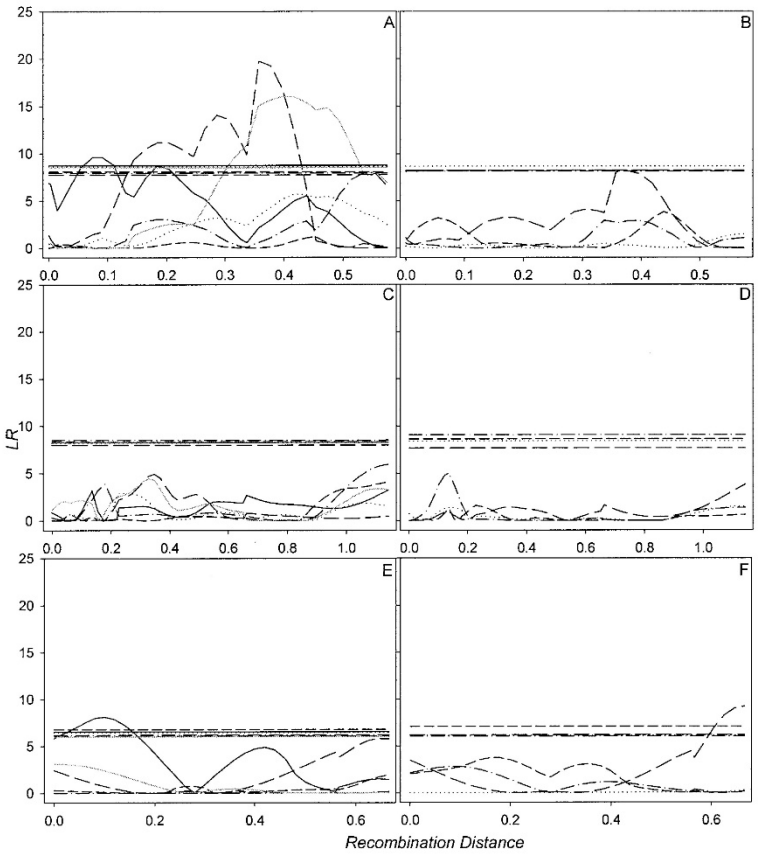

Figure 1. Left panels (A, C, and E) represent log likelihood ratio profiles for QTLs affecting male (medium dashes) and female (dots) protein content, male (short dashes) and female (dot-dashes) lipid content, and male (black) and female (gray) body weight on haplotypes 1 to 3 of the X chromosome. QTLs for lipid content weighed by protein content (medium dashes and dots) and for lipid content weighed by body weight (short dashes and dot-dashes) are in the three right panels. Horizontal lines correspond to permutation-based significance thresholds for same-colored traits.

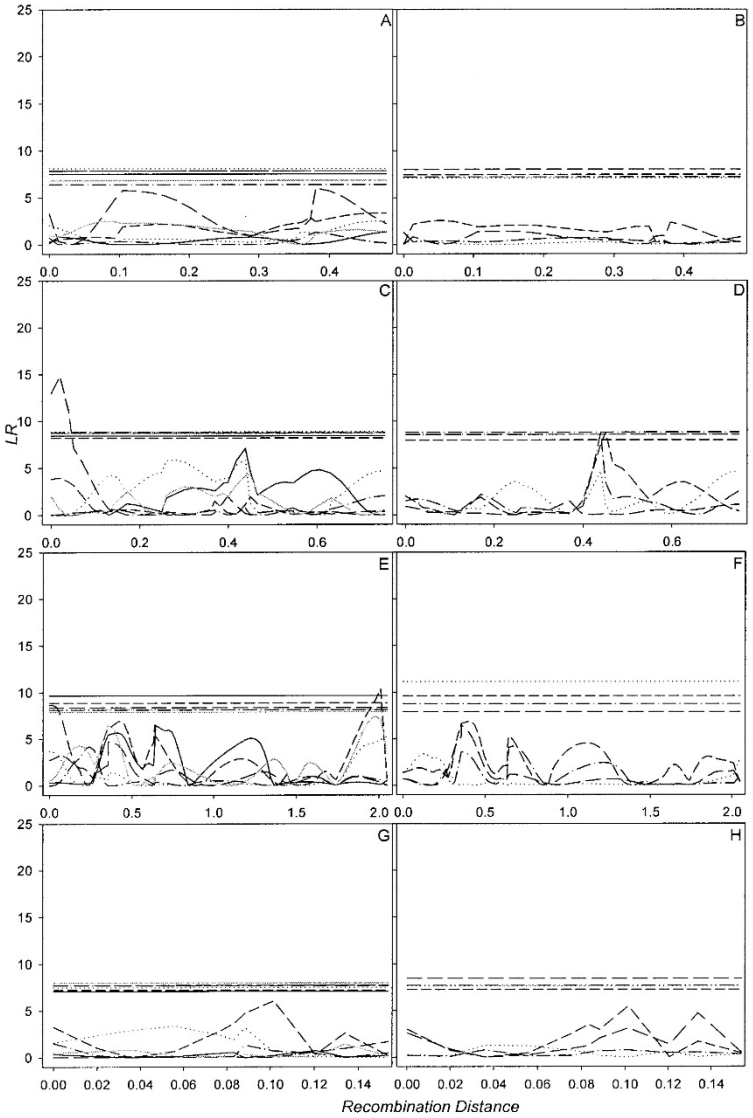

Figure 2. QTLs on haplotypes 1 to 4 of the second chromosome. The traits analyzed are: male (medium dashes) and female (dots) protein content; male (short dashes) and female (dot-dashes) lipid content; and male (black) and female (gray) body weight.

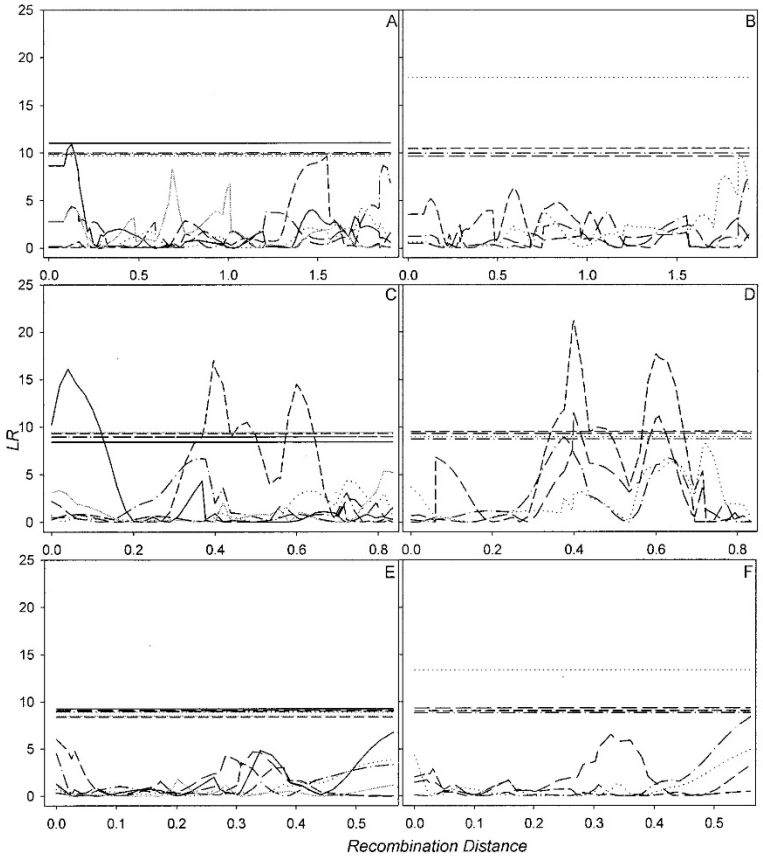

Figure 3. QTLs on haplotypes 1 to 4 of the third chromosome. The traits analyzed are: male (medium dashes) and female (dots) protein content; male (short dashes) and female (dot-dashes) lipid content; and male (black) and female (gray) body weight.

Table 2. Quantitative trait loci affecting lipid and protein abundance and body weight

		Protein content (mg/mL)		Lipid content (μg/mL)		Body weight (mg)	
Chromosome	Marker	Male	Female	Male	Female	Male	Female
X-1	4C	0.012	–0.014	–0.002	0.001	**0.01**[a]	–0.002
X-1	5C	**0.037**	0.018	0.007	0.027	0.014	0.009
X-1	9C	**0.038**	0.027	0.006	0.016	0.007	0.013
X-1	12C	**0.039**	0.033	–0.007	0.018	0.007	**0.018**
X-3	5D	–0.016	–0.005	0.007	–0.000	**–0.016**	–0.013
2–2	22A	**0.045**	0.015	0.024	–0.004	–0.000	0.005
2–3	22B	**–0.026**	–0.025	–0.015	0.002	0.002	0.004
2–3	47E	0.005	0.024	**0.037**	0.008	–0.001	0.010
3–23	63E	0.008	0.005	0.009	.008	**0.011**	0.008
3–23	73C	–0.001	0.004	**–0.041**	–0.026	0.003	–0.001
3–23	82C	0.009	0.023	**–0.043**	–0.009	0.002	0.005

a. Significant effects are in bold.

Because we were interested primarily in obesity QTLs, we standardized lipid abundance by protein content and body weight, which also has the effect of improving the power of detection by reducing unaccounted variation. Lipid proportion QTLs scaled by body weight and by protein content are presented in the right panels of figures 1 to 3. We detected an additional lipid proportion QTL at cytological position 11CD on the X chromosome (haplotype 3) and two additional lipid proportion QTLs at cytological position 51A on the second chromosome (haplotype 2). Notably, a third chromosome (cytological position 73C) female lipid proportion QTL (scaled by body weight) was also significant for lipid proportion in males, indicating a region of the genome responsible for a general impact on lipid proportion irrespective of sex. We conclude that the genetic architecture of lipid and protein abundance and lipid proportion variation in flies is likely to be multifactorial, comprised of major and smaller effects to varying degrees depending on the characteristic.

Because lipid content is associated with starvation resistance, it is tempting to compare our mapping results with whole-genome microarray data on gene expression during starvation and sugar feeding (6). In particular, the overlap of QTL and microarray data sets has been used to produce a list of candidate genes (7). However, it is not clear that phenotypic manipulations are expected to produce a similar pattern of gene expression to that observed for related evolved responses (8), including extant genetic variation maintained by selection.

Genetic variation in lipid composition might be due to allelic variation at the loci previously implicated in control of this trait (2). One can speculate about candidate genes corresponding to QTLs because numerous genes are positioned in the QTL proximity. For instance, the *Lsd2* gene (at cytological position 13A) belongs to the perilipin/ADRP/ TIP47 family of proteins. *Lsd2* mutants have reduced the level of neutral lipid, indicating that the gene is responsible for normal lipid storage (9). Another example is *pumpless* located at 78C (10), which is a gene involved in amino acid-dependent signaling arising from fat body

that induces cessation of feeding in the larva. Perhaps the most interesting example of a gene colocated with one of our QTLs is that of microRNA miR14 located at 45F (11). miR14 suppresses cell death and is required for normal fat metabolism. MicroRNAs seem to be involved in systemic regulation of multiple genes, including responding to stress conditions (12). *C. elegans* miR234 is probably induced as a consequence of nutrient stress. *Drosophila melanogaster* miR14 appears to synchronize multiple physiological responses related to stress, as supported by the fact that miR14 loss-of-function flies display increased sensitivity to a variety of stress conditions. Interestingly, lipid content was frequently increased in the lines selected for increased stress resistance (13). Intriguingly, QTL mapping in the same set of RILs as used in the present study identified a chromosomal region in proximity of 45F that was associated with *D. melanogaster* survival under starvation conditions (14). We, therefore, hypothesize that miR14 is a candidate locus potentially accounting for differential lipid abundance and starvation resistance. We will employ quantitative complementation tests (7) to test this prediction.

Research Methods and Procedures

RILs

A panel of 144 RILs was generated by crossing a single virgin female from the F_1 progeny of a fertilized female caught in the wild (Winters, CA) to a single male from the F_1 progeny of a different fertilized female caught at the same location. The genetic crosses employed and methods for genotyping the lines are described in detail in ref. 3. Briefly, recombinant F_2 genomes were isogenized by 25 generations of full-sibling inbreeding. We assayed positions of *roo* transposable elements by in situ hybridization to polytene salivary gland chromosomes with a biotinylated DNA probe (15). Locations were determined at the level of cytological bands on the standard Bridges' map in five individuals per line. A marker was recorded as present if detected in all larvae, absent if not detected in any larva, and otherwise segregating. Markers (123) were retained for the analysis, and recombination distances between them were calculated as in ref. 16. Two of the founding third chromosomes appeared to be identical except for chromosome tips (3); thus, we assumed three parental haplotypes for the third chromosome. (For the list of marker positions, see ref. 17.)

Fly Rearing

Flies were reared at 22°C in vials with 7 mL of *Drosophila* medium. This medium was made as follows. To 1.5 mL of water, 11 grams of agar, 90 mL of molasses, 150 grams of cornmeal, and 125 grams of Torula yeast were added, and the mixture was cooked and mixed, followed by the addition of 30 mL of ethanol, 3 mL of propionic acid, and 3 grams of Tegosept as antifungal agents. All flies from all lines were reared from vials that were seeded with ~75 eggs to control rearing density. Adult flies (15 males and 15 females) were transferred to fresh vials when they were 2 to 6 days old. Subsequently, flies were transferred to fresh food every 3 days for three successive transfers. At the end of the third transfer, the flies were frozen at –80°C for the assays described below.

Lipid Assay

Total lipid was measured using a method described by van Handel (18) adopted by Zera et al. (19) and slightly modified for the present study. Three females or three males were homogenized in 100 μL of chloroform-to-methanol (2:1) using plastic pestles and 1.5-mL Eppendorf tubes. After low-speed centrifugation (5000 rpm), the solvent was transferred to a glass test tube. Triolein (0.08, 0.1, 0.3, 0.5, 0.7, 0.9, 1.0, and 1.2 mg/mL) in 2:1 chloroform-to-methanol was used for standards. Glass tubes with samples and standards were incubated in a 85°C to 90°C water bath for 10 minutes to evaporate the solvent before addition of sulfuric acid and additional incubation in the 85°C to 90°C water bath for 10 minutes. The tubes were removed and allowed to cool to room temperature before addition of vanillin-phosphoric acid reagent followed by gentle vortexing. Two hundred fifty microliters of each sample or standard was transferred to a well in a 96-well plate and read in a spectrophotometer at 525 nm.

Protein Assay

Soluble protein was measured using the BCA Protein Assay Kit (Pierce Co., Rockford, Illinois). Five males or females from each line were homogenized as described in the lipid assay section, but in 250 μL of Tris-HCL (pH 8.0). The homogenate was duplicated 1:3 in homogenizing buffer, and 10 μL was added to each well (96-well plate) for the microplate assay procedure. The following standard concentrations of protein were employed: 2, 1, 0.5, 0.25, and 0.125 mg/mL. The bicinchronic acid reagents were added, and after a 30-minute incubation at 37°C, the plate was read at 562 nm.

Weight

Total body weight was determined after previously frozen flies were placed in a vacuum drier for at least 24 hours. For each RIL, the weight of five females and five males was determined using a Satorius M2P Microbalance (1 μg to 1 grams). Samples were removed from the vacuum drier such that flies were weighed within 2 hours after removal.

Statistical Analysis

For the ANOVA, we first transformed the data to improve normality of residuals. Box-Cox transformation was found that maximized Shapiro-Wilkinson test statistics (20). (SAS MACRO using UNIVARIATE procedure, option normal, is available from S.V.N.) Two-way ANOVAs were then performed with effects due to sex (fixed), line (random), and line × sex interaction (random). Statistical significance of components of variance was assayed with SAS procedure GLM. Genetic correlations between traits and sexes were approximated as correlations between line means and estimated with the SAS procedure CORR.

Our study makes use of wild, outbred founder flies. We adapted standard QTL mapping software that was designed for homozygous founders to analyze our data (for a detailed explanation, see ref. 14). Briefly, we tested whether an allele from one haplotype encodes a trait value significantly different from the average trait due to the combined effects of other haplotypes. We analyzed the following traits: lipid and protein contents and body weight (per animal) and lipid and protein abundance (lipid and protein contents

divided by the body weight). The number of different allelic effects that we can independently estimate is equal to the number of segregating haplotypes minus one. Using haplotype-specific marker alleles, we generated a separate log likelihood ratio (which is different from a conventional logarithm of the odds ratio score by a multiplier) profile for every haplotype with the CIM procedure in QTL Cartographer (21). Options in the CIM module were set to two background parameters, window size of 30 centimorgans and Kosambi mapping function. Significance thresholds were determined by 1000 permutations for each trait and chromosome.

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