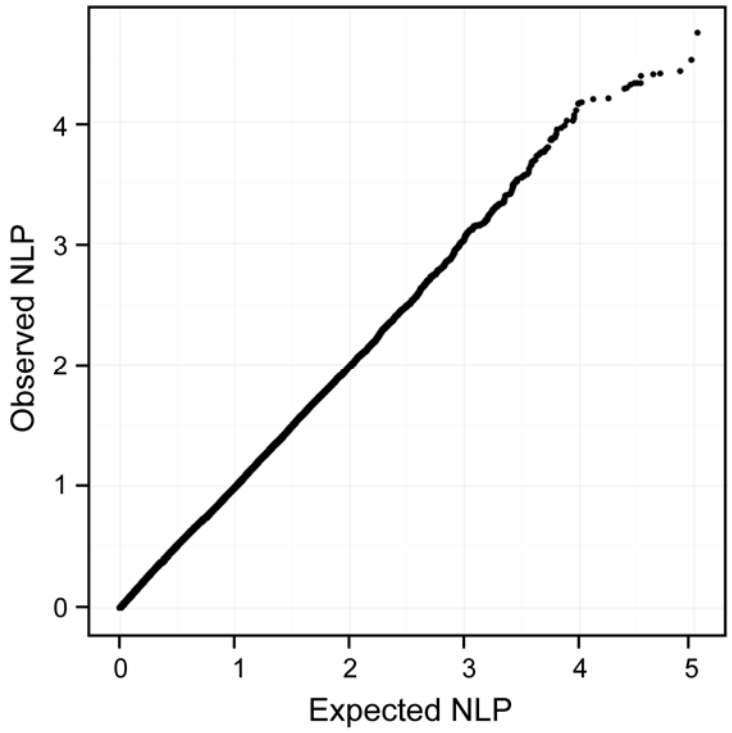

Figure S1 Absence of platform effect on allele frequency estimation. The Q-Q plot shows the observed versus expected NLP value for all pairwise comparisons of allele frequencies measured between the first and second replicates for each diet, which were sequenced on Illumina GAiix and Hi-Seq2000 platforms respectively. Significance values were assessed by simple binomial contrasts.

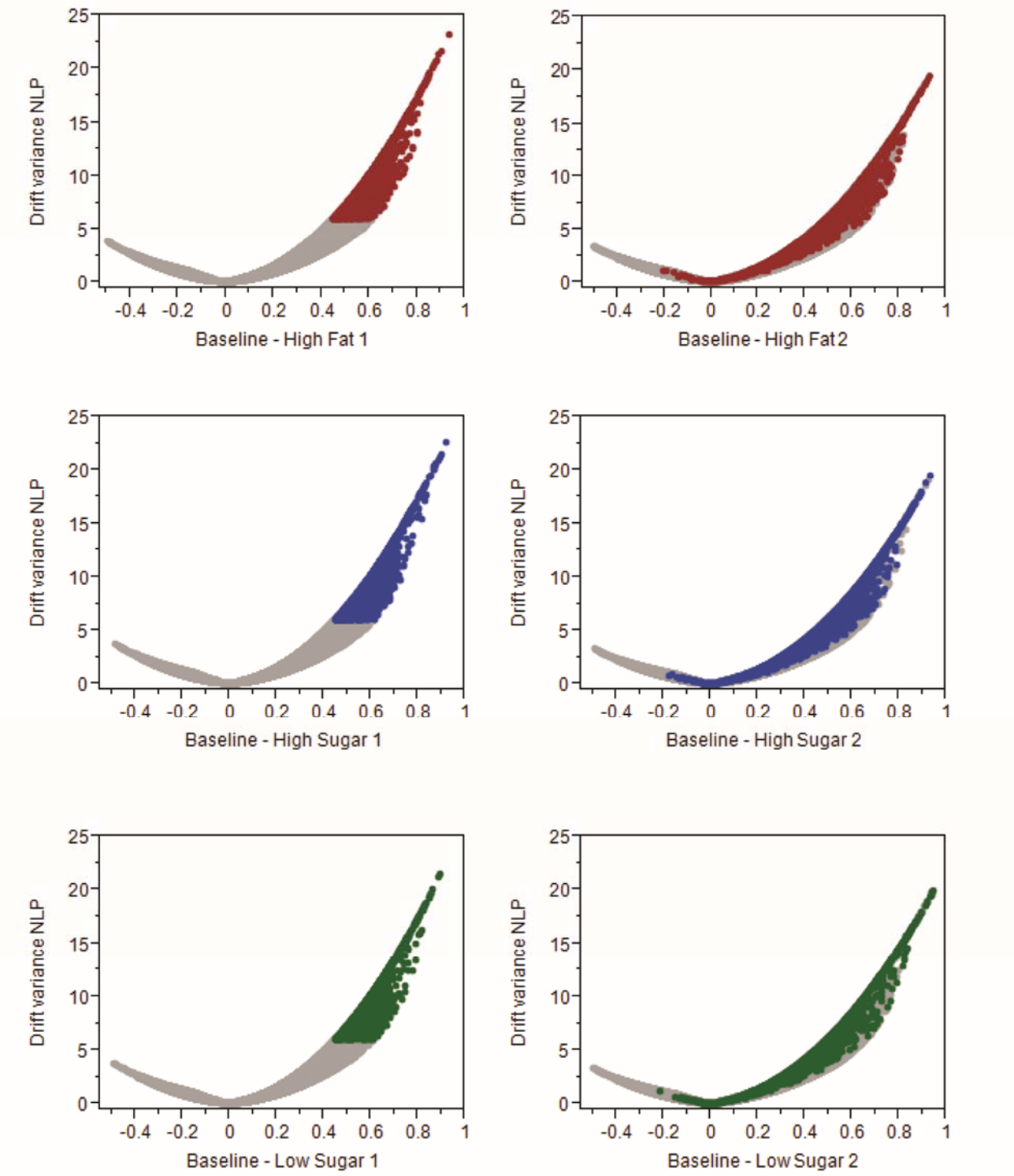

Figure S2 Volcano plots for each replicate. Significance computed as NLP from the Drift-Variance approximation is plotted against the allele frequency change for the major allele with higher frequency in Baseline to the right. Since the significance is calculated with respect to the initial major allele frequency, the maximum frequency change is bounded, with the consequence that very highly significant changes in frequency are more likely to occur when the allele frequency is reduced under selection. For each figure above, the significant SNPs (NLP>6) for the first replicate of each diet are colored, and the same SNPs are colored in the second replicate: the vast majority change in the same direction and many are highly significant.

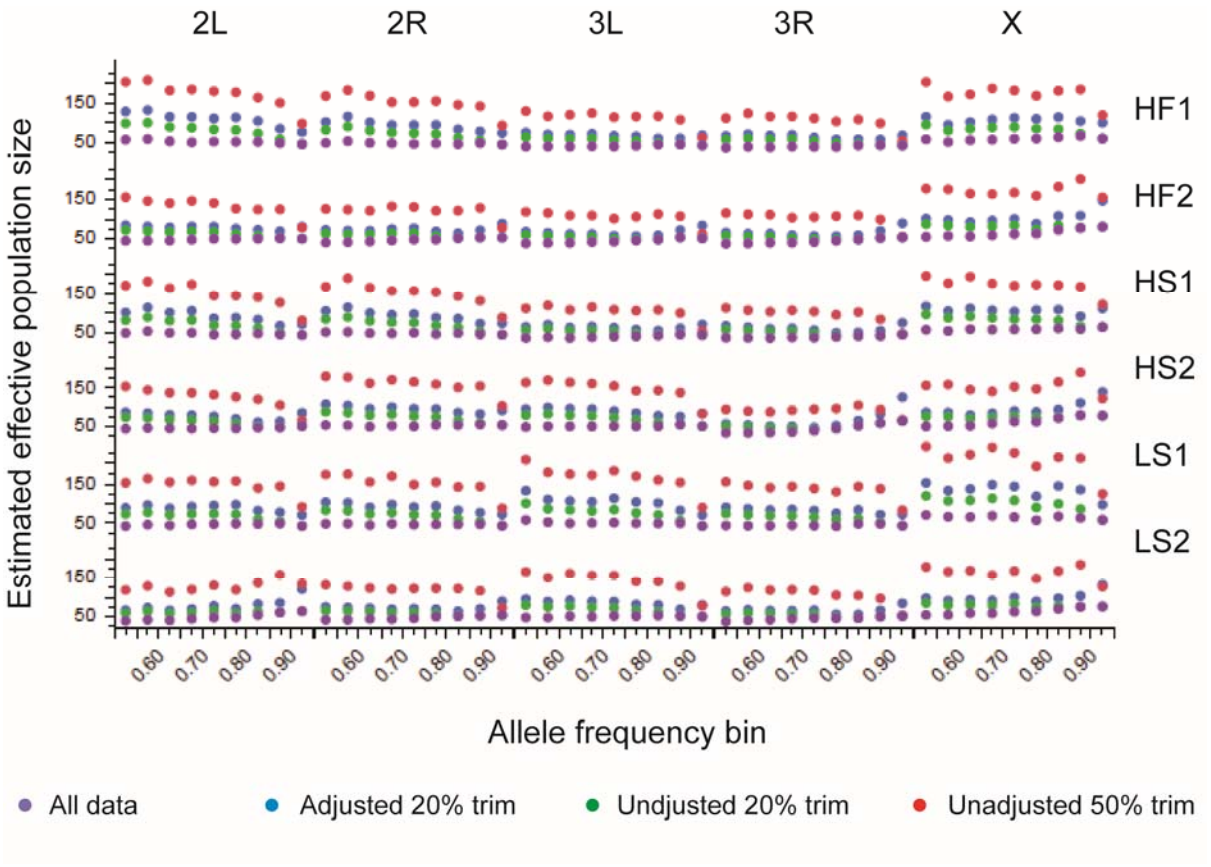

Figure S3 Estimation of effective population size. N_e is estimated for each replicate and each chromosome arm, using 4 different models. The lowest estimate (purple) uses all SNPs, but is downwardly biased because it does not account for SNPs that are under positive selection during lab adaptation. Trimming 20% of the SNPs that diverge the most in allele frequency (green, corresponding to the upper limit of the estimated fraction in LD with sites under selection) increases the estimate on average from N_e ~50 to N_e ~75. Further adjustment for sampling error in frequency estimation from the sequence data increases the estimate of N_e up to a further 25%. Trimming 50% of the SNPs results in N_e ~150, but is almost certainly an over-estimate since it reduces the variance estimate. Note that the estimates on 3R are lower, likely reflecting the increased selection on that chromosome arm possibly due to the presence of a common inversion - IN(3R)Payne, while those for the X are elevated and need to be down-weighted to account for the smaller number of X chromosomes in the population. We conclude that the effective population size is between 75 and 100 in all replicates, consistent with the experimental design in which 12 bottles with up to 15 flies of each sex were selected each generation.

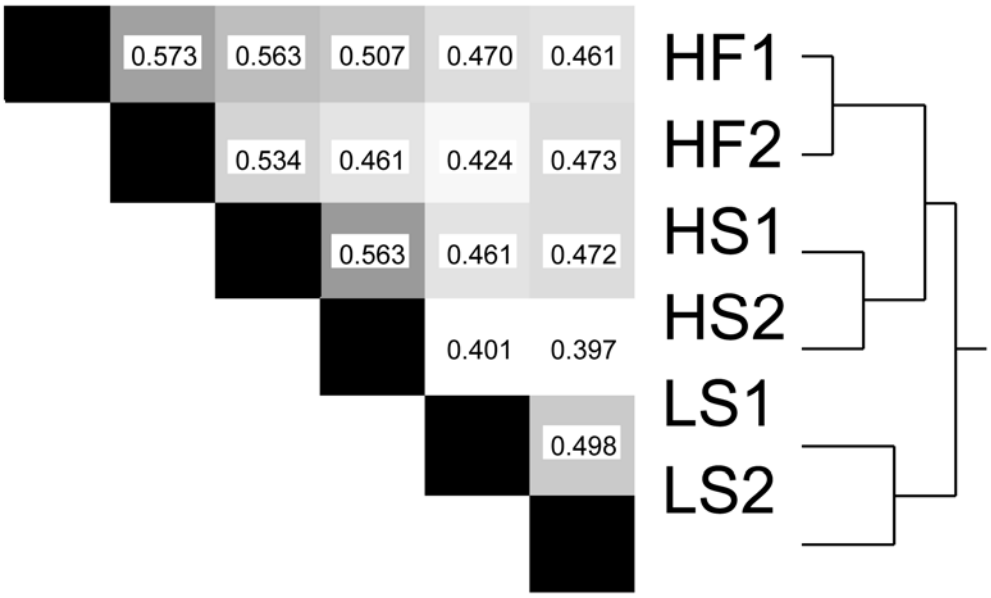

Figure S4 Correlation of allele frequencies between replicates. The heat map shows the Pearson correlation coefficient for each pairwise contrast of the difference between baseline and evolved frequencies, for the six replicates, from zero (white) to 1 (black). All alleles that passed the QC cut-offs (minimum depth 150, Q30) were included. Clustering of these correlations confirms that the two pairs of diets for the same replicate are closer to one another than to the other diets, and that the two high calorie diets (high fat and high sugar) are more similar than the low sugar diet.

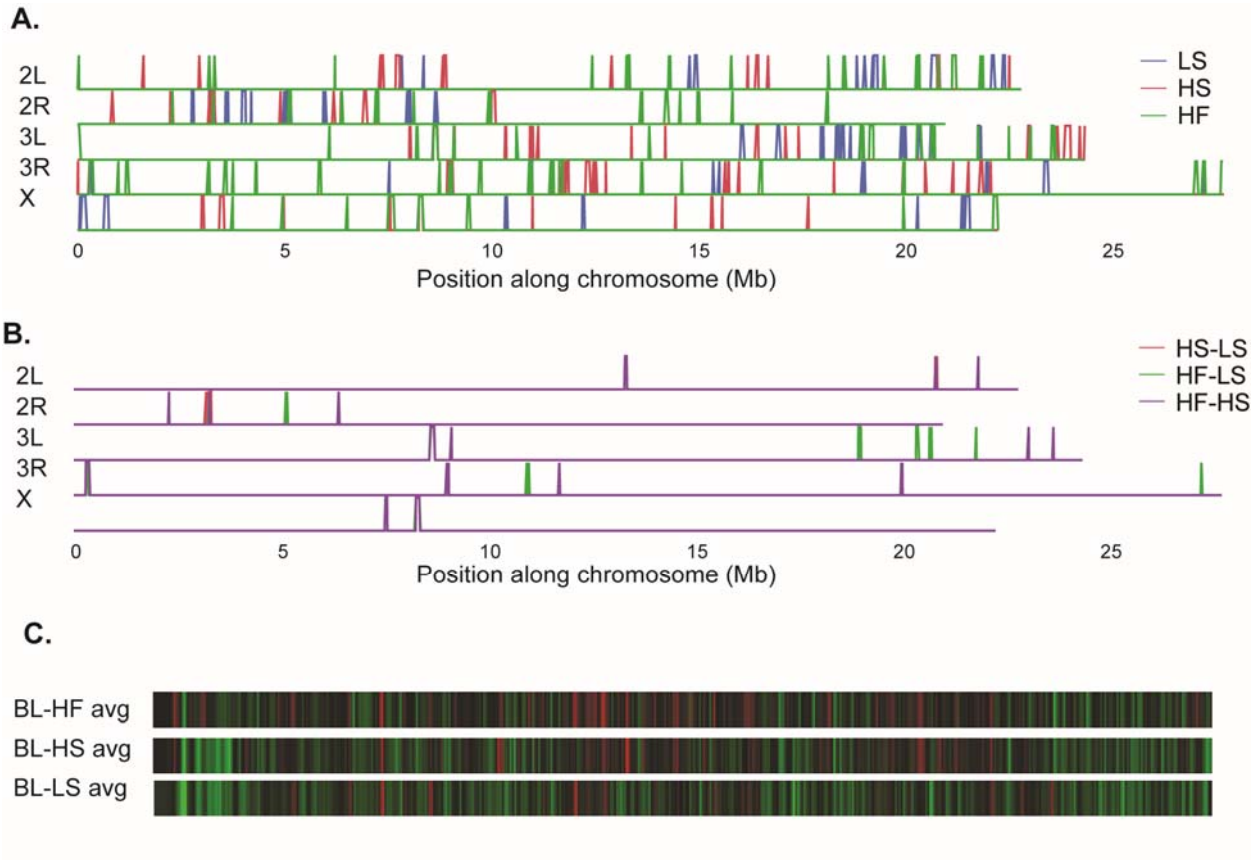

Figure S5 Heat maps of most significant selective events by chromosome. (A) Locations of the sliding windows in the top 10% of mean change in allele frequency across all 6 replicates, color coded by diet (HF green, HS red, LS blue). (B) Similar plot, but showing locations where comparisons between two diets were in the top 10%. (C) Heat map of sliding window average change in frequency on 3R for two diet replicates showing parallel evolution to the lab environment across all diet.

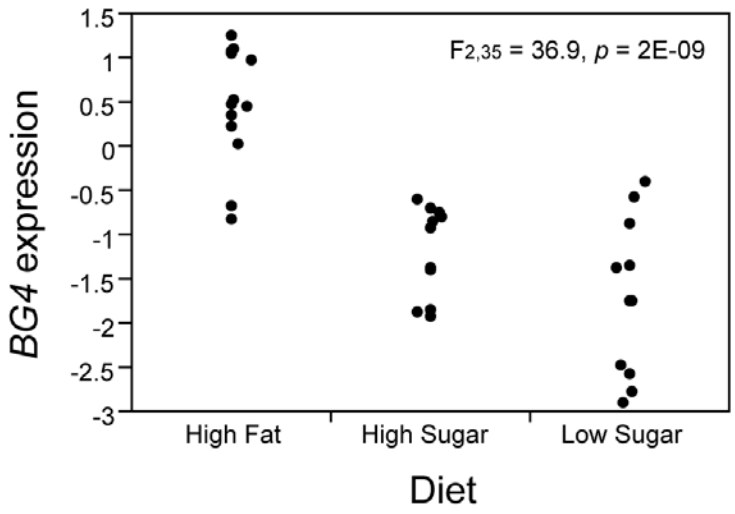

Figure S6 Example evolution of gene expression. Quantitative RT-PCR was used to monitor the expression of 41 transcripts located within regions showing laboratory adaptation in at least one diet. 23 of these showed a significant difference in abundance between the 12 flies sampled from one replicate of each diet. *BG4* expression is approximately 4-fold down-regulated on the high fat diet relative to the low sugar diet, as it shows Ct counts elevated by 2 cycles.

Files S1-S4

External Databases available for download as Excel files at authors' website, http://www.gibsongroup.biology.gatech.edu/supplemental-data-reed-et-al and at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.163857/-/DC1

File S1 Gene expression Line and Diet means

File S2 Metabolite Line and Diet means and identities

File S3 Phenotype Line and Diet means

File S4 Baseline and Evolved allele frequencies and significance estimates

Table S1 Proportion of Variance Explained (PVE) by Genetic, Dietary, and G×D contributions to gross phenotypes

Trait	Source	PVE	p Value
Weight	Genetic	0.256	$<10^{-324}$
Weight	Diet	0.006	5.1×10^{-15}
Weight	Genetic x Diet	0.047	2.6×10^{-73}
Weight	Replicate	0.112	6.6×10^{-151}
Triglyceride	Genetic	0.148	2.4×10^{-34}
Triglycerides	Diet	0.034	1.2×10^{-12}
Triglycerides	Genetic x Diet	0.140	1.5×10^{-19}
Triglycerides	Replicate	0.472	6.8×10^{-48}
Sugar	Genetic	0.123	3.9×10^{-18}
Sugar	Diet	0.015	5.3×10^{-4}
Sugar	Genetic x Diet	0.155	4.8×10^{-12}
Sugar	Replicate	0.357	2.2×10^{-19}
Larval Survival	Genetic	0.156	2.2×10^{-16}
Larval Survival	Diet	0.198	1.4×10^{-29}
Larval Survival	Genetic x Diet	0.076	1.1×10^{-1}
Larval Survival	Replicate	0.309	5.5×10^{-6}
Pupal Survival	Genetic	0.065	1.3×10^{-16}
Pupal Survival	Diet	0.487	2.3×10^{-86}
Pupal Survival	Genetic x Diet	0.116	2.4×10^{-17}
Pupal Survival	Replicate	0.186	1.4×10^{-12}
Development Time	Genetic	0.228	3.4×10^{-19}
Development Time	Diet	0.091	1.7×10^{-13}
Development Time	Genetic x Diet	0.087	4.2×10^{-2}
Development Time	Replicate	0.348	3.4×10^{-7}

Table S2 Correlations between the first 5 principal components of the gene expression (geppc) and metabolite (metpc) profiles based on line and diet means.

Expression PC	Metabolite PC	Correlation	p-value
geppc1 (20.8%)	metpc2 (7.8%)	-0.270	0.015
geppc1 (20.8%)	metpc5 (3.8%)	-0.205	ns
geppc1 (20.8%)	metpc3 (4.9%)	-0.072	ns
geppc1 (20.8%)	metpc1 (9.1%)	0.068	ns
geppc1 (20.8%)	metpc4 (4.4%)	0.056	ns
geppc2 (12.5%)	metpc2 (7.8%)	0.303	0.006
geppc2 (12.5%)	metpc5 (3.8%)	-0.145	ns
geppc2 (12.5%)	metpc3 (4.9%)	-0.034	ns
geppc2 (12.5%)	metpc4 (4.4%)	-0.032	ns
geppc2 (12.5%)	metpc1 (9.1%)	-0.004	ns
geppc3 (9.8%)	metpc2 (7.8%)	-0.231	0.039
geppc3 (9.8%)	metpc5 (3.8%)	-0.085	ns
geppc3 (9.8%)	metpc3 (4.9%)	0.059	ns
geppc3 (9.8%)	metpc4 (4.4%)	0.056	ns
geppc3 (9.8%)	metpc1 (9.1%)	-0.033	ns
geppc4 (7.5%)	metpc2 (7.8%)	0.330	0.003
geppc4 (7.5%)	metpc1 (9.1%)	0.292	0.009
geppc4 (7.5%)	metpc5 (3.8%)	0.206	ns
geppc4 (7.5%)	metpc3 (4.9%)	-0.101	ns
geppc4 (7.5%)	metpc4 (4.4%)	0.026	ns
geppc5 (5.4%)	metpc5 (3.8%)	0.230	0.041
geppc5 (5.4%)	metpc1 (9.1%)	-0.197	ns
geppc5 (5.4%)	metpc3 (4.9%)	0.061	ns
geppc5 (5.4%)	metpc4 (4.4%)	0.056	ns
geppc5 (5.4%)	metpc2 (7.8%)	0.010	ns

Table S3 Identified metabolites strongly correlated with Metabolite PC 2

Metabolite ID	Correlation	p-value
glycine	0.65	1.9×10^{-9}
arachidonoyl dopamine	0.54	1.4×10^{-5}
glucose	0.44	4.0×10^{-3}
fructose	0.39	1.0×10^{-2}
valine	-0.47	1.3×10^{-3}
leucine	-0.48	9.1×10^{-4}
l-dopa	-0.50	1.2×10^{-4}
methionine	-0.52	5.9×10^{-4}
isoleucine	-0.53	2.8×10^{-5}
phenylalanine	-0.55	1.8×10^{-7}

Table S4 Variance in metabolic traits explained by expression and metabolite profiles.

Trait	Gene Expression[a]	Metabolites[a]
Weight	0.320	0.522
Triglycerides	0.167	0.279
Sugar	0.295	0.180
Larval Survival	0.273	0.480
Pupal Survival	0.131	0.582
Development Time	0.353	0.427
Arrhythmia Index	0.452	0.382

[a] Cells show weighted sum of R^2 values fitting the trait as a function of the first 10 principal components of either gene expression or metabolite profiles.

Table S5 Dietary Change in Gene Expression measured by qRT-PCR

GENE	p_{diet}	RSq_{diet}	Sig_{diet}	Low in
amos	0.31	0.01		
BBS8	0.17	0.05		
beg	0.02	0.15	*	
BG4	2E-09	0.66	***	HF
CG10336	3E-05	0.42	***	HF
CG10505	0.31	0.01		
CG11035	0.39	0.00		
CG11251	0.02	0.16	*	
CG1138	5E-06	0.47	***	Sugar
CG11865	1E-05	0.44	***	HF, LS
CG12030	0.01	0.20	**	HS
CG13800	0.59	0.00		
CG14823	7E-06	0.46	***	HS
CG14826	0.07	0.09		
CG15506	7E-08	0.59	***	Sugar
CG31099	7E-06	0.46	***	Sugar
CG3124	7E-06	0.47	***	Sugar
CG3199	2E-06	0.50	***	Sugar
CG32982	3E-05	0.42	***	Sugar
CG34275	0.95	0.00		
CG3748	0.88	0.00		
CG4650	0.02	0.17	*	High Cal
CG8525	0.39	0.00		
cv-c	0.18	0.05		
daw	0.03	0.14	*	HF
Den1	0.003	0.25	**	High Cal
Dg	3E-04	0.34	**	High Cal
DmsR-1	5E-08	0.60	***	Sugar
dro3	0.64	0.00		
Gli	0.75	0.00		
Gr64e	0.07	0.09		
heph	0.0001	0.37	**	HF
IM2	0.05	0.11	*	High Cal
Lip4	0.07	0.09		
PH4αSG1	0.21	0.03		
PpD6	0.02	0.15	*	LS
Psf2	0.0001	0.37	**	HF
raw	0.33	0.01		
scramb1	0.16	0.05		
scrt	0.25	0.02		
Src64B	4E-08	0.60	***	HF

p_{diet} is the p-value associated with the R-squared measure by ANOVA for differential expression between the three diets (High Fat, HF; High Sugar, HS; and Low Sugar, LS), where gene expression was measured in a pool of 10 whole flies for each of 8 inbred lines for one replicate of each diet. The "Low In" column shows which diet(s) show the lower expression (higher Ct values) where High Calorie is both High Fat and High Sugar, and Sugar implies lower expression on both high and low sugar diets. Significance is summarized as * $0.05 > p > 0.01$ ** $0.01 > p > 0.0001$ *** $p < 0.0001$

Table S6 Lack of overlap between types of genomic response.

	Total	HS SNP	LS SNP	G×D Txt	TG RNAi	eQTL
HF SNP Genes[a]	571	287	208	34	22	25
HS SNP Genes[a]	661		222	28	21	24
LS SNP Genes[a]	484			28	15	14
G×D Transcripts[b]	697				38	22
TG RNAi Genes[c]	505					19
eQTL Genes[d]	486					

Cells show number of genes out of the Total listed for each of 6 genomic responses that are found in the indicated pair of responses. With 13,394 CG entries in the genome, and an average of 495 genes for each type of genomic response (3.7% of all genes), expected values are ~18 overlaps per pair. There may be an enrichment for the transcripts that show a significant G×D interaction and genes that affect Triglyceride content after RNAi-knockdown.

[a] Gene nearest to a SNP that shows significant change in frequency of both replicates of the High Fat (HF), High Sugar (HS) or Low Sugar (LS) diets at $p<10^{-5}$.

[b] Transcripts that show a significant Genotype×Diet interaction term in the microarray analyses.

[c] Genes that influence total adult triglyceride content when knocked down by RNAi (Pospisilik et al, 2010)

[d] regulatory eQTL detected in adults of both sexes (Massouras et al, 2012)