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A System Genetics Analysis Of Energy Metabolism Traits In Drosophila Melanogaster

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A SYSTEM GENETICS ANALYSIS OF ENERGY METABOLISM TRAITS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Obesity is emerging as a global public health problem and it has shown to precede and predict the development of type 2 diabetes, a complex disease that has also reached epidemic proportions in the US and worldwide. Despite that obesity-related traits are highly heritable, the genetic basis underlying their natural variation and the loci playing pleiotropic roles among organismal traits have not been fully elucidated. The overall goals of these present studies were: to shed light on the architecture of the genetic co-expression networks regulating variations in obesity-related traits, elucidate the extent to which they are regulated by pleiotropic loci, and identify pleiotropic alleles between metabolism and life-history traits to provide key insights into why different alleles are allowed to persist in natural populations, despite the fact that some of them confer susceptibility to metabolic disorders. Using a wild-derived population of *Drosophila melanogaster* as model system, our results highlighted the relevance of non-metabolic pathways such as immune response, neurogenesis and neuronal function, cell growth, food processing and water balance as key regulators of organismal body weight, metabolic rate and body composition traits. Differential expression of cycling/photoperiodic genes among young adult flies underlies the genetic forces shaping

phenotypic variation in mitochondrial bioenergetic traits. Furthermore, the elucidation of pleiotropic transcriptional modules provided a key insight into the molecular basis of the well established trade-offs between body weight, reproduction, and survival of food deprivation. Our data further indicate that molecular regulation of mitochondrial respiration plays a critical role in mediating life history trade-offs in natural populations. In conclusion, our results confirm that the genetic basis of natural variations in obesity-related traits involves highly interactive co-regulated transcriptional networks, and identify various pleiotropic loci underlying evolutionarily conserved trade-offs among organismal survival and reproduction which account for the perpetuation of alleles that confer susceptibility to metabolic disorders among individuals from a natural population.

Keywords: obesity-related traits, *Drosophila melanogaster*, genetic networks, life-history traits, trade-off, *syndecan*.

DEDICATIONS

To my parents, my husband and my son.

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CHAPTER I:

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex disease which is characterized by a chronic hyperglycemic status as a consequence of insulin resistance in muscle (causing decreased glucose uptake) and liver (causing increased gluconeogenesis), together with impaired insulin secretion from pancreatic β-cells. The complexity of T2DM results from the interaction of environmental factors with genetic susceptibility factors. Linkage, candidate-gene, and genome-wide association studies have provided important insights into the genetic architecture of T2DM, and have identified potential genetic variants in several genes that confer T2DM risk [4]. Yet, numerous studies have demonstrated that increased fat mass, preferentially in the visceral compartment, is also an important risk factor for insulin resistance [5], and the development of T2DM [6]. According to Ford *et al.* [6], for every kilogram of weight gain, the risk of diabetes increases between 4.5 and 9%. The transition from obesity to diabetes is produced by a progressive defect in insulin secretion coupled with a progressive rise in insulin resistance. The reverse observation after weight loss of 10 kg [7] confirms the role of obesity in the impairment of insulin secretion and in the pathogenesis of insulin resistance.

Overweight and obesity continue to be leading public health concerns in the US. The latest estimates have shown that 17.1% of US children and adolescents are overweight and 32.2% of adults are obese [8]. Obesity has shown to precede and predict the development of T2DM [9] [10]. T2DM comprises 90% of people with diabetes around

the world. The World Health Organization (WHO) estimated that more than 180 million people worldwide had diabetes in 2006; and it has been anticipated that this number will double by 2030 [11].

Mitochondrial dysfunction and metabolism.

As mentioned above, skeletal muscle, adipose tissue and liver are the insulin-responsive organs responsible for maintaining normal glucose homeostasis. Dysregulation in any of the processes involved in the modulation of insulin action produces elevation in fasting and postprandial glucose levels. For several decades, it has been proposed that free fatty acids (FFA) play a critical role in the modulation of responsiveness of liver and muscle to insulin. This has been an area of intense research since Randle *et al.*'s [12] 1963 publication on the competition between glucose and fatty acids as oxidative fuel sources in isolated rat heart and diaphragmatic muscle preparations. According to Randle's model, when concentrations of plasma FFA increase, muscle fatty acid uptake is favored and fatty acids compete with glucose for oxidation. The enhanced fatty acid oxidation produces an increase in the acetyl-CoA/CoA ratio that suppresses glucose oxidation even further through activation of pyruvate dehydrogenase kinase, which phosphorylates and inhibits pyruvate dehydrogenase. The inhibition of this enzyme leads to increased cytoplasmic citrate concentrations that consequently inhibit phosphofructokinase resulting in increased concentrations of

glucose-6 phosphate; as a consequence, hexokinase is inhibited and, finally, glucose uptake is impaired.

Yet recent studies have postulated that this model provides only a partial explanation of the inhibition by FFA of insulin-stimulated glucose-uptake and oxidation in the muscle. Studies by Shulman and co-workers [13], using nuclear magnetic resonance spectroscopy, have shown a significant decrease in the intracellular glucose concentration of healthy subjects after a five-hour infusion of lipid/heparin through direct inhibition of glucose transport by fatty acids. In light of these findings, Lowell and Shulman [14] speculate that "any metabolic perturbation that promotes the accumulation of fatty acids in liver and/or muscle and/or any defects in the ability of these organs to metabolize fatty acids might result in insulin resistance". Indeed, recent biochemical evidence supports the idea that insulin resistance in humans arises from defects in mitochondrial fatty acid oxidation in skeletal muscle [14]. Furthermore, several cDNA microarray studies comparing transcriptional differences in skeletal muscle from non-diabetic controls and T2DM patients [15-17] have shown extensive down-regulation of genes involved in mitochondrial oxidative phosphorylation (OXPHOS). These findings in humans are also supported by gene-expression analysis in the skeletal muscle of other mammalian models [18]. Interestingly, in the studies conducted by Patti *et al*. [15] and Mootha *et al*. [17] a subset of down-regulated OXPHOS genes is known to be coordinately regulated by *peroxisome proliferator-activated receptor gamma co-activator* (*PGC*)-1α and *PGC-*1β. Expression of these genes is decreased in muscle tissue from diabetic and pre-diabetic subjects. *PGC-*1α and *PGC-*1β have been shown to regulate glucose and fat oxidation in

muscle and fat tissue [15] as well as gluconeogenesis in liver [17] and glucose-regulated insulin secretion in pancreatic β cells [15]. Overall, these findings strongly suggest that the biochemical network that regulates energy homeostasis is coordinated by genetic elements with regulatory control of pathway genes.

Mitochondrial Physiology and Genetics.

Mitochondria are subcellular organelles composed of "a smooth outer membrane surrounding an inner membrane of significantly larger surface area that, in turn, surrounds a protein-rich core, the matrix" [19]. Mitochondria are best known for their role in the production of adenosine triphosphate (ATP) through a process called OXPHOS [19,20]. Carbohydrate oxidation via glycolysis and the tri-carboxylic acid cycle (TCA) and β-oxidation of fats as well as protein metabolism supply reducing equivalents in the form of electrons that travel along the mitochondrial electron transport chain (ETC). The ETC is composed of 5 respiratory complexes initiating with electrons being transferred to either complex I (NADH dehydrogenase or NADH-ubiquinone oxidoreductase) or complex II (succinate dehydrogenase or succinate:ubiquinone oxidoreductase). Complex I is a very large enzyme composed of 43 subunits. It is the point of entry for the majority of reducing equivalents that travel along the ETC. Complex I transfers 2 electrons (e^-) from NADH to ubiquinone (UQ) and such transferring is associated to the translocation of 4 protons (H^+) across the membrane [21]. Complex II operates in both the ETC and the TCA cycle oxidizing succinate to fumarate and transferring electrons from succinate to UQ. It is composed of 4 subunits. Unlike

complex I, complex II does not behave as a redox-driven proton pump. Complex III (cytochrome (cyt) bc_1 complex or ubiquinol-cytochrome *c* oxidoreductase) delivers electrons from ubiquinol to cyt *c*. It is composed of 11 subunits, one of which has the capacity to pump protons [20,21]. The overall reaction involves the net oxidation of 1 UQH_2 to UQ, the reduction of two cyt c_1 (i.e. a complex III subunit), the release of 4 H^+s at one side of the membrane and the uptake of 2 H^+s from the other. Cyt *c* is a water soluble hemoprotein [20] which shuttles electrons from cyt c_1 in complex III to complex IV (cytochrome *c* oxidase; see Figure 1).

Complex IV is composed of 13 subunits and it is hypothesized to be the rate-limiting step of mitochondria bioenergetics [22]. At the active site, transfer of 4 e^-s is used to reduce O_2 into two molecules of H_2O given that no reactive oxygen species (ROS) is released. The complete cycle removes 4 H^+s from the matrix side involved in the reduction of O_2 to H_2O and additionally 4 more H^+s are pumped across the membrane producing an overall charge movement of 8q+/4 e^- [21]. A reduction in complex IV activity may increase the residence times of electrons upstream in the chain, thereby increasing the probability of electrons leaking to form superoxide anions [23,24]. Finally, complex V (i.e. mitochondrial ATP synthase) is a transmembrane protein composed of 11 subunits. Complex V is functionally reversible with the ability to synthesize ATP from adenosine diphosphate (ADP) and inorganic phosphate (P_i) powered by the OXPHOS-mediated proton motive force, coupling respiration to ATP synthesis. This form of respiration is known as ‘state 3’ respiration and is responsible for producing the ATP that powers multiple cellular reactions in eukaryotes [19]. However, mitochondria can also

use oxygen even in the absence of ADP, which occurs when protons leak back into the matrix via a mechanism that does not involve ATP synthase. This uncouples respiration from OXPHOS. Oxygen utilization in the absence of ADP is referred to as ‘state 4’ respiration [25]. Nuclear-encoded uncoupling proteins (UCPs) may play a role in augmenting this basal proton leak further rendering the mitochondrial inner membrane leaky for protons, “uncoupling” the ETC from ATP synthesis, and reducing ROS production [26,27].

Mitochondria OXPHOS is under dual genetic control in all eukaryotic cells. A single mitochondrion contains its own genome and protein-synthesizing machinery. Human mtDNA contains 37 genes: 22 encode transfer RNAs (tRNAs), 2 encode ribosomal RNAs (rRNAs), and the remaining 13 genes encode mitochondrial subunits of the ETC [21]. MtDNA consists mostly of coding sequences, has a very high mutation rate [28], and its repair mechanisms are poor [29]. Thus mtDNA replication during normal cellular and organismal aging could propagate mutant mtDNA within cell lineages [30] due to heteroplasmy, leading to bioenergetic defects of different magnitude between organs and individuals. Tissues heavily dependent on OXPHOS, as heart, muscle as well as the renal and endocrine systems are particularly affected [31] such [28]. MtDNA is maternally inherited [32,33]. Thus mitochondrial diseases have shown to be more severe in males than in females [34]. However, later reports have demonstrated the occurrence of paternal inheritance in interspecific crosses among mammalian models [35,36] as well as in *Drosophila* [37]. This biparental inheritance of mtDNA provides further chance for generating heteroplasmy within an individual. However, despite the important

contribution of the mitochondrial genome, nuclear genes code for most of the mitochondrial OXPHOS proteins and for the mitochondrial metabolic enzymes [38]. In addition numerous nuclear-encoded factors are required for either the expression of mitochondrial genes (i.e. DNA and RNA polymerases, ribosomal proteins and the mtDNA regulatory factors such as mitochondrial transcription factor A, Tfam) or the assembly of the respiratory complexes [28,38,39].

Resting metabolic rate, mitochondrial proton leak, and obesity.

Changes in body weight are known to result from an imbalance between the energy derived from food and the energy expended in maintaining life and performing physical work. This imbalance is reflected as a change in the amount of energy stored, mainly, as fat. Total daily energy expenditure comprises the resting metabolic rate (RMR), the thermic effect of food, and the caloric cost of physical activity [40]. RMR is the resting energy expenditure at thermoneutrality in the unfed state. It can be further subdivided into the sleeping metabolic rate and the additional energy necessary for wakefulness without physical activity. The thermic effect of food accounts for ~10% of the daily energy expenditure and the caloric cost of physical activity is variable depending on the individual's daily activity level. RMR is the component that explains the largest proportion of total daily energy needs in individuals. Assessment of RMR among siblings and monozygotic and dizygotic twins suggests that a good proportion of the inter-individual variability in RMR is inherited [41]. This is observed also after adjusting for individual differences in age, sex, and fat-free mass, which represents the major

determinants of RMR [42]. The heritability of RMR is also confirmed by studies in rodents [43] and *Drosophila* [44], which report that ~20-40% of the variability in RMR is explained by gene variants.

Numerous studies suggest that a low resting metabolic rate (RMR), adjusted for fat-free mass, fat mass, age, and sex, is a major risk factor for weight gain in humans [40,45-49]. However, there is still controversy about the influence of RMR on the development of obesity, with studies reporting that the resting component of organismal energy expenditure does not account for differences in weight gain patterns among individuals [50,51]. Because it is well-known that the influence of particular alleles on quantitative traits is context dependent, often influenced by the genetic background and the history of environmental conditions experienced [52], this discrepancy in findings may be the result of inadequate statistical power due to small sample sizes. Therefore, the use of model systems, such as the fruit fly *D. melanogaster*, which allow one to conduct studies in controlled environments and defined genetic backgrounds, is extremely valuable to address these issues.

Studies in rodents indicate that mitochondrial proton leak in skeletal muscle is a major contributor to RMR [27]. This holds true even when the data is extrapolated from isolated tissues to whole-organism RMR [25]. Up to 20%-25% of whole-body RMR is attributable to the mitochondrial proton leak process [26,53,54]. Coupling efficiency is the proportion of oxygen consumption used to produce ATP and do useful work. Mitochondrial proton leak (i.e. state 4) causes mild uncoupling, lowers mitochondrial efficiency and possibly plays a role in the regulation of ROS production [55]. By

contrast, under ADP-dependent respiration (i.e. state 3) coupling efficiency approaches 90%. Conditions of low coupling efficiency (i.e. increased proton leak) may thus lead to increased substrate oxidation and to reduced energy storage [56]. This is corroborated by the fact that patients with Luft's syndrome (i.e. a rare metabolic syndrome of unknown etiology) have inefficient mitochondria and display high caloric intake, high metabolic rate with profuse sweating, and a low body weight [56]. Therefore, increased RMR resulting from cellular inefficiency to generate ATP may have a potential antiobesity effect by increasing fat and glucose catabolism to meet organismal ATP requirements. In view of these observations, the goal of this project was to show that there is segregating genetic variation for skeletal muscle mitochondrial coupling efficiency among individuals from natural populations and that genetically determined differences in mitochondrial coupling efficiency may underlie some of inter-individual variability in organismal RMR, which in turn affects variation in whole-body energy storage.

Drosophila melanogaster as a model system for studying the genetic basis of metabolic traits.

Thoraces of *D. melanogaster* consist primarily of flight muscle, which is an important source of mitochondria, also known as sarcosomes [57]. Each muscle fiber contains relatively enormous, cross-striated, longitudinal columns or fibrils. Adjacent fibrils are separated by a zone of transparent sarcoplasm in which a longitudinal row of sarcosomes is embedded. In the apparent absence of connective tissue sheaths and sarcolemma, muscle fiber readily separates into fibrils liberating a turbid suspension of sarcosomes.

Watanabe and Williams [57] examined the sarcosomes from flies and showed a high titer of cytochrome *a*, *b* and *c*, an active cytochrome *c* oxidase and catalase, and significant titers of mitochondrial enzymes similar to vertebrate mitochondria. Furthermore, the phosphorylating respiratory chain of insect mitochondria strongly resembles that of mammalian mitochondria. Insect mitochondria have an exceptionally high respiratory and phosphorylative activity with α-glycerophosphate and pyruvate as physiological substrates [58]. However, there is a low rate of oxidation with Krebs-cycle intermediates and glutamate due to the very limited permeability of the mitochondria towards these substrates. Neither FFAs nor their carnitine esters can be oxidized by *Drosophila*. Moreover, one of the most characteristic features of insect tissues is the high concentration of free proline. Although pyruvate or proline alone is not oxidized at significant rates, pyruvate plus proline support high rates of coupled state 3 respiration [59]. This augmented respiratory rate obtained is due to an increase in the rate of pyruvate utilization mediated by proline. Fly mitochondrial respiration is also affected by the same inhibitors and uncouplers that affect mammalian system [58-60]. ATPase activity is inhibited by oligomycin and stimulated with the uncoupling agents 2,4-dinitrophenol and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) with dissipation of the electrochemical gradient across the inner membrane. In addition, similar to human nuclear-encoded UCPs, four UCP proteins have been identified in *D. melanogaster*: UCP4a, UCP4b, UCP4c and UCP5/BMCP1. Hanak and Jezek [61] identified the first three *D. melanogaster* UCPs as the closest human UCP4 analogs. *D. melanogaster* UCP5/BMCP1 shows a much higher similarity with the vertebrate UCP5

than any other UCP isoform [62]. A previous report showed an increase in respiration rate and a decrease in mitochondrial membrane potential as the result of *D. melanogaster* UCP5 expression in the heterologous yeast system [63].

It has long been established that metabolic pathways have changed little during the course of evolution. Insects capable of only short flights, such as fruit flies and bees, use carbohydrates as the main source of flight energy. High enzymatic activities for carbohydrate oxidation (i.e. trehalase, hexokinase, GPDH) and mitochondrial oxidative enzymes (i.e. cytochrome oxidase) along with high mitochondrial densities support the high rates of aerobic metabolism required to maintain flight [64]. Trehalose is a disaccharide that constitutes the major circulating sugar in these insects [65,66]. It can be stored in relatively high concentration in body fluids. Trehalose concentrations in insect hemoplymph are usually between 1-2% whereas blood glucose in humans is kept around 0.1% [65]. Fat is the other major energy reservoir in an organism. In *D. melanogaster*, fat is stored as triacylglycerol (TAG) in the fat body, which is distributed throughout the organism [67]. Similar to mammals, the fat body constitutes the flies' major energy reserve and it is the functional equivalent of both mammalian liver and white adipose tissue. Comparative sequence analysis shows that *Drosophila* is a good model system for studying energy metabolism traits because it possesses homologues for all of the known mammalian enzymes responsible for the activation, trafficking, and processing of fatty acids through the β-oxidation pathway [68]. Furthermore, the metabolic pathways for carbohydrate oxidation in vertebrate muscle have their counterpart in the insect flight muscle [69]. The insulin signaling pathway is highly conserved in insects particularly in

Drosophila [70]. In *Drosophila, a* group of insulin producing cells located in the brain is the equivalent of the human pancreas. Seven insulin–like peptides (Dilps) are recognized (Dilp1-7). The fly insulin-like peptides have been shown to control energy metabolism, growth, reproduction, and longevity similarly to the vertebrate insulin/IGF signaling pathway. Adipokinetic hormone (AKH) is another important metabolic hormone in insects with structural and functional similarities to vertebrate glucagon [71,72]. Insulin- and AKH-producing cells comprise a specialized network that controls organismal energy metabolism and growth sharing a common evolutionary ancestry with the ∝- and β-cells of the human pancreas [70].

The measurement of CO_2 production is the most sensitive and accurate method used to investigate the rate of metabolism in small organisms, such as fruit-flies [73]. CO_2 is produced as a direct by-product of oxidative metabolism, but the ratio of CO_2 produced to oxygen consumed varies with the metabolic substrate utilized. In *Drosophila*, carbohydrate is the sole fuel metabolized to provide substrate for the aerobic production of energy during flight [64,69]. Therefore, the CO_2 production of fed flies is correlated with oxygen consumption and the respiratory quotient is near 1 [64], unless the flies are starved over a 4-hour period and fat starts being consumed concurrently [74]. Genetic differences affecting variation in RMR have been reported in natural populations of *D. melanogaster* [44]. Previous publications have recognized body size as an important variable affecting metabolic rate [75,76]; however, later reports have shown no correlation between these two traits under a wide range of environmental conditions [73]. This is because “the more energy spent on activity, the higher the mass-independent

resting metabolic rate". Thus, insects that fly, an energetically demanding behavior, have higher RMRs than species that use energetically less demanding types of locomotion. There is evidence that these higher RMRs among the more active organisms are caused by either larger sizes of those organs that have high metabolic activity or by higher metabolic rates in some or all organs [77]. The small size of *D. melanogaster* makes it impractical to assess the size or metabolic rates of individual organs.

Life history theory and energy allocation.

Life-history theory predicts that the amount of energy available is finite, and energy devoted to one function cannot be devoted to other functions [55]. The way in which organisms allocate their available nutrients to growth and reproduction at different ages has been shown to affect their survival [55]. Both early fecundity and egg-laying rate appear to have an antagonistic relationship with lifespan [78-82]. Early reproduction may result in early cessation of repair, producing fast growth, fast aging, and a short lifespan. Selection for late reproduction may favor investment in repair, delay maturity and aging, and lengthen lifespan [81,83].

Over the past 50 years, ROS, mainly produced by mitochondrial OXPHOS, have also been investigated as putative mediators of the process of aging [84]. This has been confirmed by several reports across different species [85-88]. ROS are endogenously and continuously produced throughout life under normal physiological conditions, leading to deleterious effects on biological macromolecules and irreversible damage, especially in

post-mitotic tissues. Respiratory complex I and III are important ROS generators in the ETC [60].

Under normal conditions, mitochondria are incompletely coupled allowing a natural leak of protons back into the matrix (i.e. state 4). This appears to be a general pathway of ecologically significant energy loss in all aerobes and not an artifact of mitochondrial isolation [30]. So during OXPHOS some of the redox energy is lost as heat. Considering that up to 20%-25% of the RMR may be used to drive this proton leak [25,26,53], its function must be so important that organisms are prepared to pay a very high energetic price to maintain it. It is clearly a thermogenic mechanism because it stimulates respiration without energy conservation [30]. However, it also occurs in ectothermic organisms such as *Drosophila*, so thermogenesis cannot be its primary function. Brand and colleagues [30] have hypothesized that the potential function of the mitochondrial basal proton leak (i.e. state 4) is to decrease the production of superoxide and other ROS to protect against cellular degeneration and aging. These functions seem to be important enough to warrant such high energetic investment. Similarly, other reports have shown that mitochondrial superoxide production has a strong dependence on the magnitude of the proton-motive force across the inner membrane, so it can be strongly decreased by mild uncoupling [60,84]. The proton-motive force would affect ROS generation by altering the redox state of ubiquinone. At high proton-motive force respiration slows, so electrons accumulate on ubiquinone leading to an increase in the steady-state concentration of ubisemiquinone, an important determinant of ROS production. Partial uncoupling by the basal proton leak will tend to lower the proton-motive force leading to

a more oxidized ubiquinone pool and a lower concentration of ubisemiquinone. This proton leak will also increase oxygen consumption rate so it will lower the oxygen tension around the mitochondrion. Therefore, both a reduced concentration of oxygen and ubisemiquinone in mitochondria may lead to a lower rate of mitochondrial ROS production. In this context, one of the objectives of this research project was to elucidate the genetic basis of natural variation in mitochondrial state 4 respiration as a surrogate measure of basal proton leak. These findings may provide a key insight into the identification of candidate genes regulating mitochondrial ROS generation as well as potential therapeutic targets to prevent ROS-mediated cellular degeneration and aging.

Genetics of quantitative traits and gene co-expression network in natural populations.

Mitochondrial bioenergetic and energy metabolism traits are continuous traits. Continuous traits are referred to as quantitative traits and the area of genetics that studies their mode of inheritance is called **quantitative genetics** [89]. The value observed when the character is measured is the phenotypic value of the trait and it may vary among individuals in a population. The amount of variation is measured and expressed as the variance. The phenotypic variance (V_P) is composed of a genotypic component (V_G) and an environmental component (V_E):

$$V_p = V_G + V_E$$

The fraction of the phenotypic variance due to genetic effects is called the "heritability" of the character [89]. In this project, we determined the broad-sense heritability, denoted as H^2, or the degree of genetic determination of the trait of interest, which is expressed as **Vg/Vp**.

Genome-wide measurements have shown that there is extensive variation in gene expression levels in humans and other organisms and that variation in transcript-specific abundance of many genes has a heritable component [90-97]. The so called "genetical genomics" approach has been used to map loci controlling gene-expression differences that may underlie functional trait variation [98]. This approach integrates trait, expression, and genetic marker data to infer causal associations between gene expression and trait variation [99]. This is accomplished by several levels of analysis. The first step consists of performing a genome-wide scan to map the chromosomal regions controlling the level of expression for each transcript abundance phenotype. The second step consists of reconstructing co-expression network pathways underlying multiple transcript abundance phenotypes to identify transcriptional "modules" of highly interconnected genes [100]. One characteristic of many transcriptional networks in the biological system is a "scale-free" topology, where a small subset of highly linked genes, or "hubs", links the rest of less connected genes to the system [101,102]. The third step concerns the mapping of the transcripts responsible for expression regulation of the gene modules underlying the trait of interest [103].

Recent "genetical genomics" studies in human and animal models have provided strong evidence of the existence of co-expression genetic networks that control energy

homeostasis [43,104,105]. This indicates that identifying single genes controlling variation in a quantitative trait or conferring susceptibility to a disease has limited utility in uncovering the genetic mechanisms underlying complex diseases, unless the genetic networks associated with the trait or disease are unraveled [99]. In addition, this type of network analysis provides hypotheses about functional relationships among transcripts and provides insight into how variation in the network of co-expressed genes can give rise to variation in the associated traits.

Significant Studies Included

In this dissertation, we used a group of 40 *Drosophila* inbred lines derived from a single natural population in Raleigh, NC, to investigate whether there is considerable segregating variation in energy metabolism and mitochondrial bioenergetic traits among individuals from a single natural population. The overall goals of the present studies were:

1. to shed light on the genetic architecture of the co-expression networks regulating variation in organismal obesity-related traits in *D. melanogaster*,
2. to elucidate the extent to which body composition and energy metabolism traits are regulated by pleiotropic loci, and
3. to identify evolutionary conserved genes with pleiotropic effects on body composition, energy metabolism, and life-history traits to provide key insights into why variation in body composition traits, such as fat mass, and in

mitochondrial traits, such as state 4 respiration rate, are allowed to persist in natural populations.

The following questions and topics were addressed:

-Chapter II: SYSTEMS GENETICS ANALYSES OF BODY WEIGHT AND ENERGY METABOLISM TRAITS IN *DROSOPHILA MELANOGASTER*. The present study aimed to elucidate the genetic architecture of co-regulated transcriptional networks underlying natural variations in *Drosophila* body weight, metabolic rate, and whole-body metabolite pools. The study also assessed whether there were genetic correlations between energy metabolism and life-history traits (such as fitness, starvation resistance, and lifespan) and identified pleiotropic gene-expression modules underlying these correlations.

-Chapter III: IDENTIFICATION OF NUCLEAR-ENCODED GENES REGULATING *DROSOPHILA* MITOCHONDRIAL RESPIRATION TRAITS THROUGH TRANSCRIPTOME ANALYSIS. The present study aimed (i) to determine the genetic basis underlying variation in mitochondrial respiration trait in a natural population of *D. melanogaster* and (ii) to investigate genetic correlations between mitochondrial and life-history traits.

-Chapter IV: A ROLE FOR *DROSOPHILA SYNDECAN* IN THE REGULATION OF WHOLE-BODY ENERGY METABOLISM AND HOMEOSTASIS. The present study aimed to corroborate the role of *Drosophila syndecan (dSdc)* in mitochondrial function as *dSdc* was identified as one of the candidate genes regulating mitochondrial

state 3 respiration rate among a highly interactive network of cycling genes. For this purpose, we used a viable mutant allele of the gene, *Sdc*^{BG02774}.

CHAPTER II:

SYSTEMS GENETICS ANALYSIS OF BODY WEIGHT AND ENERGY METABOLISM TRAITS IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

Obesity has reached epidemic proportions in the US and worldwide. Quantitative genetic analyses in humans and animal models have provided compelling evidence that several genes underlie the genetic architecture of obesity-related traits. However, there has been little focus on documenting the extent to which variation in energy metabolism traits contribute to life history variation and trade-offs in natural populations. Here, we quantified phenotypic variation in body weight, body composition, and energy metabolism traits among 40 wild-derived inbred lines of *Drosophila melanogaster* established from a single natural population in the Raleigh area (NC). These lines were chosen because they were previously screened for whole-genome transcript abundance and life-history traits. Our data confirmed that there is segregating variation in all the traits, with broad-sense heritabilities ranging from 25% to 68%. Regression models identified several hundred candidate genes significantly ($P<0.01$) associated with variation in metabolic phenotypes in both sexes forming modules of biologically meaningful correlated transcripts. Gene ontology analyses revealed that body weight co-expression sub-networks were overall enriched for genes involved in immune response and neuronal development and function. Genes involved in cell growth and metabolism were identified as regulators of body composition traits, while genes associated with food processing and water balance were shown to underlie the basis of natural variation in metabolic rate. Our study also showed that there was a tendency for lines with higher

body weight and glycogen reserves to survive starvation better at the expense of reproductive fitness and mating. Several pleiotropic gene-expression modules were identified that provide key insights into the molecular mechanisms underlying the trade-off between body weight, glycogen storage, and fitness.

INTRODUCTION

Obesity is a condition characterized by an excess of adipose tissue that adversely affects human health [1]. The clinical problem of excessive adipose tissue resides in its strong association with a number of chronic diseases, such as insulin resistance [2,3], type 2 diabetes (T2DM) [4], coronary artery disease [4,5] and stroke [6]. In 2003-2004, 32.2% of the adults in the United States were obese [7]. This estimate represents a significant increase in obesity prevalence over the past 20 years. Similar trends are being observed worldwide [8]. As the rise in the incidence of obesity and related health problems continues, considerable efforts have been made towards a better understanding of the etiology of obesity.

Large twin, adoption, and family studies have firmly established that obesity-related traits, such as body mass index (BMI) and measures of body composition (e.g., fat mass, lean mass, and percentage fat mass), are highly heritable in humans [9-16]. Segregating variation in obesity-related traits has also been observed in natural populations of most other organisms, including invertebrates [17,18]. Yet the genetic architecture of these traits is very complex [19]. This is reflected by the numerous gene-by-gene interactions (e.g. epistasis) reported in a diverse group of organisms [20-27] as well as the extensive genotype-by-environment interactions [24,28-30].

Furthermore, a growing body of research in humans and animal models has provided strong evidence of the existence of co-expression genetic networks that control body

composition and energy metabolism traits [31-33]. For example, Ghazalpour *et al*. [34] used a “genetical genomics” approach in a segregating population of mice to map gene sets that are differentially perturbed in lean and obese mice. They identified 13 metabolic pathways whose genes are coordinately regulated in association with subcutaneous fat-pad mass traits [34]. Interestingly, follow-up studies that compared the human and mouse adipose gene co-expression networks [31] identified a single common module enriched for genes associated to immune response and macrophage activation that co-localize or are in causal relationship to obesity-related traits in both humans [31] and rodent models [33]. These findings are reinforced by a recent study that investigated tissue-to-tissue co-expression (TTC) networks between genes in the hypothalamus, liver, and adipose tissue of obese mice [32]. The study demonstrated a strong cross-tissue communication and identified genes involved in circadian rhythm, energy balance, cellular response to starvation, and immune response as specific to the TTC networks in this obesity model [32].

The link between adiposity and immune function is of particular relevance as previous studies in mammals have shown that molecules, such as leptin and peroxisome-proliferator activated receptors, known to play a pivotal role in metabolism, also play a role in the regulation of the immune response [35]. These molecules have also been shown to play a role in reproduction [36-38], which proposes them as mediators of the physiological trade-off between reproduction and immune function [39]. Organisms partition dietary resources acquired from the environment among growth and development, reproduction, and survivorship [40]. Since these resources are limited, the

way in which they are acquired and partitioned may be critical to the fitness of the individual and often result in trade-offs between demanding physiological functions [41]. Furthermore, the environment poses constant challenges to an organism's survival and fitness. For example, fighting a microbe infection, finding a mate, or surviving a stress require significant energy, and trade-offs are expected between investments in these energetically costly functions and other critical life-history functions involving growth and reproduction [39,42-47].

A number of studies have identified physiological correlates of life-history variation and trade-offs within species [48-51]. Selection studies in *D. melanogaster* have demonstrated that energetic investment in early reproduction, possibly involving lipids [52], may result in early cessation or reduction of somatic maintenance and repair, allowing increased fecundity, but faster rates of aging [44,53]. On the other hand, selection for late reproduction may favor investment in repair, delayed maturity and aging, resistance to environmental stresses, and lengthen lifespan [44,50,53]. Based on these observations and the findings of the systems genetics studies cited above, we hypothesized that the architecture of the genetic networks underlying natural variation in body composition traits may have been shaped by adaptive relationships between fitness-related life-history traits and energy metabolic traits, as these ultimately control the amount of available energy that can be invested in these competing demands [50].

To test our hypothesis, we used *D. melanogaster* as a model system. Much of what has been learnt about the evolutionary biology of life-history variation, in particular about the genetic and molecular aspects, comes from studies in *Drosophila*. In addition, in

recent years, we have learned that several genetic mechanisms controlling lipid metabolism and energy homeostasis are shared between invertebrates and mammals (reviewed in [54,55]). Thus, insights about the genetics of body weight and energy metabolism traits gained from *Drosophila* may apply also to mammals. In the present study, we quantified genetic variation in wet body weight (BW), triacylglycerol storage (TAG), total protein content (PRO), total glycogen level (GLY), total glycerol level (GLYC), and metabolic rate (MR) in 40 wild-derived lines of *D. melanogaster*. These lines were chosen because they were previously evaluated for several life-history traits, including longevity, resistance to starvation stress, mating behavior, and competitive fitness [56], which allowed us to determine genetic correlations between traits. Such correlations (either positive or negative) would indicate the extent to which these traits are influenced by the same polymorphisms [57] and could reveal genetically based trade-offs among traits (if negatively correlated). Furthermore, these lines were previously quantified for transcript abundance [56].

Thus, we used this data to establish gene co-expression networks and to identify "modules" of highly-interconnected genes associated with variation in each trait [56]. Several algorithms have been developed to predict coherent transcriptional modules from gene expression profiles, the key difference between these methods and the method used in this study is that the module building algorithm is objective and the number of modules/clusters is self-determined to maximize the genetic correlation within a module [56]. Our network analysis requires that we first regress the transcript levels of each gene that was significantly associated with the trait against the trait value for each line. The

residuals from each regression are then saved. Finally, the procedure calculates the degree to which the residuals from each regression are significantly correlated (or co-expressed) across the 40 lines. Use of residuals is necessary because all genes used in the network analysis are chosen based on their significant association with the trait (and so by default all transcript expression levels should be correlated with each other). Each of the resultant modules is then used to form an interaction network that can be represented as a graphical model in which nodes represent transcripts and edges identify genetic correlations between transcripts exceeding a threshold value [58]. This approach has been highly effective for identifying gene co-expression networks that contribute to variation in *D. melanogaster* life span, starvation resistance, male mating behavior, chill-coma recovery, fitness, and sleep among the 40 wild-type lines of *D. melanogaster* used in this study [56,59]. This provided us with the opportunity to gain invaluable insights into the molecular causes of the adaptive relationships evolved in a natural population between energy metabolism and life-history traits.

MATERIALS AND METHODS

Drosophila stocks.

The 40 unrelated wild-type inbred lines of *D. melanogaster* were established from a sample of isofemale lines collected in Raleigh, NC and inbred to near-homozygosity by 20 generations of full-sib inbreeding [60]. Insertional mutations and their co-isogenic control lines were obtained from the Berkeley *Drosophila* Gene Disruption Project (http://flypush.imgen.bcm.tmc.edu/pscreen/). Exelixis *PiggyBac* transposon insertional mutations and their co-isogenic w^{1118} control line were obtained from the Bloomington *Drosophila* Stock Center (http://www.flybase.org).

We maintained each stock at constant parental density for at least two generations to minimize environmental effects. To control for larval density, we allowed the parents of the experimental flies to mate for 3 hours to generate egg collections on apple juice/agar medium in laying plates. After 24 hours, we picked groups of 100 first-instar larvae from the surface of the medium and put into replicate vials. To minimize the influence of genetic variation in reproduction on energy metabolism, we performed all the phenotypic assays on virgin flies that were randomly collected from the replicate vials for each line on days 10 to 16 under brief CO_2 exposure.

For all assays, we used ten replicate vials per line, with each vial containing a group of 10 single-sexed individuals aged 3-5 days. Due to the size of this experiment, we conducted the phenotypic assays in 5 overlapping blocks, with each block including 2-4 of the 10 replicate vials per line. We reared flies in vials containing 10 ml of standard

cornmeal, agar, sugar, and yeast medium at a constant temperature of 25°C, 60–75% relative humidity, and a 12-hr light-dark cycle.

MR measurements

We measured MR as CO_2 production using a flow-through respirometry system (Qubit System Research, Kingston, Ontario, Canada) and a modification of the method described in Van Voorhies *et al*. [61]. Briefly, a pump is used to push air through a CO_2 scrubber therefore providing CO_2-free air to the system. The airstream is saturated with H_2O by passing through a series of gas syringes filled with sterile H_2O and cotton wool. Pressure in the line is controlled by a precision pressure regulator that sets the input pressure to the 4-channel mass flow meter/controller where the flow is divided into 4 gas streams and provided to the sample chambers. The flow rate entering the chamber was 50ml/min. After leaving the sample chambers, air enriched in CO_2 enters into the 4-channel gas switcher that directs the flow to either the analysis system or to waste (vented). For the determination of CO_2, sample air was pulled through a drying column to remove H_2O, a mass flow meter, and then the CO_2 analyzer that has a range of 0-2000 ppm CO_2 with a resolution of better than 1 ppm. We measured CO_2 for 10 minutes/chamber with a 30 second flush period between measurements. The amount of CO_2 produced by each group of flies was calculated using C950 Data Acquisition software (Qubit System Research, Kingston, Ontario, Canada).

BW and metabolite measurements

We first starved the flies for one hour under non-dehydrating conditions to reduce the food-derived TAG and GLY present in the gut. We then weighed each group of flies to

0.1 mg accuracy with an analytical balance and stored them at -70°C. Finally, we homogenized each group and measured TAG, GLYC, and PRO using the protocol described in De Luca *et al*. [62]. GLY was measured from the same homogenates using a modification of the protocol described in Clark *et al*. [17]. Briefly, aliquots of 1.67 µl of homogenate were added to 250 µl of a reagent containing 0.1 U/ml of amyloglucosidase, 5 U/ml of glucose oxidase, 1 U/ml of peroxidase, 0.04 mg/ml of O-dianisidine dihydrochloride. After 30-minute incubation period at 37°C, OD_{540} was measured. Concentration of glycogen was determined from glucose and glycogen standards run with each replicate. Each sample was assayed twice and the mean used in the analysis. Previous studies showed that this protocol accurately reflects glycogen concentration and that endogenous glucose present in the flies contributes only negligibly to the results [17].

Quantitative genetic analyses

All statistical analyses were performed using SAS version 9.1. We used two-way ANOVA to partition variation in each trait among the inbred lines according to the model, $Y = \mu + L + S + LxS + E$, where μ is the overall mean; L and S are the main effects of Line (Random) and Sex (Fixed); LxS is the random effect of sex-by-line interaction; and E is the within-vial error variance. Reduced models by sex were also run. Broad-sense heritabilities (H^2) were computed as $H^2 = (\sigma_L^2 + \sigma_{SL}^2)/(\sigma_L^2 + \sigma_{SL}^2 + \sigma_E^2)$ for the analyses pooled across sexes, where σ_L^2, σ_{SL}^2 , and σ_E^2 are the among line, sex-by-line and within line variance components, respectively. H^2 values by sex were also computed as $H^2=(\sigma_L^2)/(\sigma_L^2 + \sigma_E^2)$ [57]. Cross-sex genetic correlations (r_{MF}) were also estimated as $r_G = cov_{♀♂}/\sigma_{♀}\sigma_{♂}$, where $cov_{♀♂}$ is the covariance of lines means between females and

males, and $\sigma_{♀}$ and $\sigma_{♂}$ are the square roots of the among line variance components for males and females. Genetic correlations between phenotypic traits were calculated as $r_{GT} = cov_{G12} /(\sigma_{G1}\sigma_{G2})$, where cov_{G12} is the covariance between traits among line means from the joint analysis, and σ_{G1} and σ_{G2} are the square roots of the variances among lines from the analyses of each trait separately. The coefficients of genetic (CV_G) and environmental (CV_E) variances were calculated as $CV_G = 100\sigma_G/\mu$ and $CV_E = 100\sigma_E/\mu$, respectively, where σ_G and σ_E are the square roots of the line and within line variance components, respectively. Differences in metabolic traits between *P{GT1}*, *PiggyBac* transposon insert lines and their co-isogenic controls were assessed using the same ANOVA models described above.

Transcript-phenotype associations

We used regression models ($Y = \mu + S + T + S \times T + \varepsilon$, where *S* denotes sex and *T* the trait covariate) to identify transcripts significantly ($P < 0.01$) associated with organismal phenotypic variation in both sexes. Modules of co-expressed transcripts associated with variation in each metabolic trait were constructed using the residuals from regression models ($Y= \mu+ S+ E+ SxE+\varepsilon$, where E is the covariate median $\log_2$ expression level) to compute the genetic correlations between transcripts significantly associated with each phenotype.

Transcriptional modules

Transcripts significantly associated with metabolic phenotypes across the 40 wild-derived inbred lines were organized into statistically correlated transcriptional modules as described previously [56]. The correlation between all pairs of significant transcripts *i*

and *j* was computed and the absolute correlation values $|r_{ij}|$ were transformed to define edge weights $e^{\frac{|r_{ij}|-1}{\sigma^2}}$ in a graph of genes indexed by the free parameter σ. The clustering $P = \{V_1, \ldots, V_k\}$ and the value of σ that jointly maximize the modularity function:

$$Q(P,\sigma) = \sum_{c=1}^{k} \left[\frac{A_\sigma(V_c, V_c)}{A_\sigma(V,V)} - \left(\frac{A_\sigma(V_c, V)}{A_\sigma(V,V)} \right)^2 \right]$$

were determined, where $A_\sigma(X,Y)$ denotes the total edge weight in the graph indexed by σ that connects any vertex in set X to a vertex in set Y. The optimal partition $P = \{V_1,\ldots, V_k\}$ defines k transcriptional modules $V_1,\ldots,V_k$ at which the genetic correlation within a module is maximal.

Pleiotropic modules

Transcriptional modules common to more than one metabolic trait as well as to the other phenotypes were identified by considering pairs of traits and comparing the lists of significant transcripts for each module from the first phenotype to each module from the second across the 40 inbred lines. Fischer's Exact Test was used to quantify the extent that the overlap between two modules exceeded chance expectation.

RESULTS AND DISCUSSION

Natural variation in body weight and energy metabolism traits

We observed significant genetic variation among the lines for all the organismal traits analyzed (P <0.01), with H^2 ranging from 25% to 68% in the combined sex analyses (Table 1 and Figure 1A-F). These estimates are in general agreement with those determined by several clinical studies in humans [9-15,63,64] as well as various reports on mammalian [65-67] and non-mammalian models [67,68]. We also found that all traits exhibited significant sex-by-line interactions (Table 1). These results, however, are most likely caused by differences in one sex in one line for most of the traits. Indeed, the genetic correlation coefficients across sexes among lines, r_{MF} (±SEM), were very high for BW (0.94±0.05; P <0.0001), TAG (0.72±0.11; P <0.0001), PRO (0.72 ±0.11; P <0.0001) and GLYC (0.97±0.04; P <0.0001) indicating that the same loci affect these traits in the two sexes. Moderate values were observed for GLY (0.44±0.14; P=0.0032) and MR (0.39±0.15; P =0.0116).

Next, we tested whether there were significant genetic correlations between the traits. We did not find any significant correlation using all data pooled across sexes (Table 2A). On the other hand, when we analyzed the data stratified by sex we observed positive correlations significant at P < 0.05 between PRO and BW, GLYC, and TAG only in females (Table 2B). These results are in perfect agreement with previous findings by Wang and co-workers [69] who reported that protein and lipid abundance were highly

correlated with body weight in females from a set of *Drosophila* recombinant inbred lines derived from a natural population, but not in males.

Positive correlations were also found between MR and TAG in females (Table 2B) and between BW, GLY, and MR in males (Table 2B). However, it is possible that the sex-specificity of some of the correlations detected by our analysis may be the result of inadequate statistical power due to small sample size. Indeed, a marginally significant correlation between BW and GLY was also detected in females (P = 0.06; Table 2B). Laboratory selection studies in *D. melanogaster* have shown that both female and male adult flies selected for resistance to desiccation and starvation stresses are significantly heavier than unselected controls [48]. Desiccation-selected flies also have increased whole-body water content and elevated glycogen content [48]. Glycogen binds 3-5 times its own mass in water [70], and therefore the increase in glycogen storage has been suggested as a mechanism of increasing intracellular water [48]. Insects and other terrestrial arthropods are susceptible to water loss because of their small size and variation in desiccation resistance has been documented for populations of *D. melanogaster* [71,72]. In addition, a positive correlation between body size and desiccation tolerance in natural populations of *D. melanogaster* has been previously reported [72,73]. We therefore speculate that the genetic correlation between BW and GLY identified in our study may reflect the influence of body mass and water balance on desiccation resistance in the wild.

One interesting finding of our study is that there is a tendency for male flies from lines that have higher BW to have higher MR (Table 2B). The association between body

mass and metabolic rate in *Drosophila melanogaster* has been extensively studied by Van Voorhies *et al*. [61]. They compared wet or dry body mass and metabolic rate in male flies from different laboratory lines at different ages and over different metabolic sampling periods and found no correlation between the traits [61]. A possible explanation for these conflicting findings lies in the origin of the flies used in the two studies. While Van Voorhies *et al*. used primarily flies from laboratory lines [61], the *Drosophila* lines tested in our study were established from a natural population. The patterns of correlations that have evolved under laboratory conditions, in which abiotic factors such as temperature and humidity are usually constant, are not always identical to those that have differentiated in wild populations. In support of this idea, studies of desiccation resistance and water balance in natural populations of *Drosophila* have shown a positive correlation between metabolic rates and water-loss rates, both of which were found to be positively correlated with body mass [73]. The relationship between body mass, metabolic rate, and water loss is explained by the fact that a reduction in metabolic rates can help the fly to conserve water by reducing the need for gas exchange. Hence, the genetic correlation between BW and MR observed in our study could be the result of the phenotypic integration between these traits and water-loss rate. Consistent with this hypothesis, as indicated below, we found that several genes regulating variation in MR are expressed in the Malpighian tubules (Figure 3E). Insect Malpighian tubules represent the functional equivalent of the mammalian kidney [74]. They play a crucial role in organismal survival due to the fact that, as indicated above, insects are highly susceptible to water loss particularly under dehydrating environmental conditions [75].

Quantitative trait transcripts (QTTs) regulating natural variation in body weight and energy metabolism traits

In order to identify candidate genes responsible for variation in BW and energy metabolism traits among the 40 *Drosophila* inbred lines, we searched for significant correlations between transcript abundance (QTTs) and variation in each trait. At a *P*-value of 0.01, we found 275, 125, 130, 298, 389, and 93 transcripts associated with natural variation in BW, TAG, GLY, PRO, GLYC, and MR, respectively (see **Supplementary Table 1**: Analysis of modules of correlated transcripts associated with each of the body composition and energy metabolism traits).

To independently validate the finding that the genes identified by our analysis affect the trait, we tested for phenotypic differences between homozygous mutants of candidate genes and their controls. We chose to focus on candidate genes associated with variation in TAG, GLY, and GLYC for which homozygous *P-element* and *PiggyBac* mutations have been generated in an isogenic background (see **Supplementary Table 2**: Effects of *P[GT1]* and *PiggyBac* transposon insertional mutations in candidate genes affecting TAG, GLY, and GLYC).

We selected six genes affecting TAG, *dead-box-1* (*Ddx1*); *RhoGAP71E*, *rutabaga* (*rut*), *sugarless* (*sgl*), *SIRT7*, and *GXIVsPLA2*. We found that all the mutations affected the trait at least in one sex (Figure 4; see **Supplementary Table 2**: Effects of *P[GT1]* and *PiggyBac* transposon insertional mutations in candidate genes affecting TAG, GLY, and GLYC). *Ddx1* codes for an evolutionarily conserved protein with a pivotal role in the control of cell growth and division [76]. *rut* encodes a Ca^{2+}/calmodulin-responsive

adenylyl cyclase involved in learning and memory. Interestingly, screening of *P*-element insert lines of *Drosophila* identified *Ddx1* as a potential candidate gene regulating variations in odor-guided behavior, while a single mutation in *rut* has been reported to influence food choice behavior [77]. In most organisms, olfactory behavior is an essential trait required for food localization [78]; and feeding behavior is an important component of organismal energy balance. *Sgl* codes for a homologue of mammalian *UDP-glucose dehydrogenase* and is essential for proteoglycan synthesis. *P*-element insertions within the *sgl* coding region have previously shown to significantly impact fly energy stores [79]. *SIRT7* is a member of the Sirtuins histone deacetylase enzyme family. Its mammalian ortholog has been previously suggested as an important regulator of organismal cell growth in accord with energy status [80]. *SIRT7* NAD^+-dependency establishes the link to fat accumulation as fatty acid esterification to triglyceride is determined by the cellular $NADH/NAD^+$ ratio [81]. *RhoGAP71E* and *GXIVsPLA2* represent novel candidate genes affecting variation in fat storage in *Drosophila*. *RhoGAP71E* encodes a protein that belongs to the group of RhoGAPs proteins. They are evolutionarily conserved regulators of RhoGTPases [82], which have been shown to modulate several cellular processes (i.e. cell growth and division, cell dynamics, membrane trafficking, gene transcription and apoptosis) in response to environmental cues [83]. *GXIVsPLA2* encodes an enzyme involved in phospholipid metabolism [84].

We selected five genes affecting GLY, *β amyloid protein precursor-like* (*Appl*), *Calbindin 53E (Cbp53E)*, *transferrin 1* (*Tsf1*), *sevenless* (*sev*), and *junctophilin* (*jp*). We found that, except for *transferrin 1,* all the mutations affected the trait at least in one sex

(Figure 4; see **Supplementary Table 2**: Effects of *P[GT1]* and *PiggyBac* transposon insertional mutations in candidate genes affecting TAG, GLY, and GLYC). *Drosophila Appl* is a pan-neural protein involved in axonal growth and synapse formation [85] with a pivotal role in the pathogenesis of neurodegenerative diseases [86] and behavioral defects in flies [87]. *Cbp53E* is a calcium-binding protein that modulates the activation of many intracellular effector proteins. Neuronal development and function underlie the basis of food-related behaviors in *Drosophila* [88] and hence differential expression of neuronal-related genes (such as *Appl* and *Cbp53E)* may impact feeding behaviour and subsequently, organismal energy balance. *Sev* encodes a tyrosine kinase receptor required for photoreceptor fate specification in the developing eye [89]. Interestingly, components of the *sev* signaling pathway have been linked to the regulation of glucose and lipid homeostasis via insulin signaling [90]. *jp encode*s a protein that belongs to a novel group of highly conserved transmembrane proteins mediating optimal ionic signaling among excitable cells [91]. These proteins have not been previously linked to glycogen storage.

We selected seven genes affecting GLYC, *b4GalNAcTA, CG10133, CG5946, CG8920, Gliotactin* (gli), *Glutamate dehydrogenase* (*Gdh*), and *tweety (tty*). Glycerol is an important intermediate in carbohydrate and lipid metabolism. We found that, except for *CG5946,* all the mutations affected the trait at least in one sex (Figure 4; see **Supplementary Table 2**: Effects of *P[GT1]* and *PiggyBac* transposon insertional mutations in candidate genes affecting TAG, GLY, and GLYC). *Drosophila b4GalNAcTA* is implicated in glucosamine metabolism. Previous quantitative genetic analysis reported *b4GalNAcTA* as a candidate gene regulating variations in 24-hr sleep

time [79]. Interestingly, sleep disorders in *Drosophila* have been linked to increased adiposity accompanied by coordinated transcriptional changes in genes involved in lipid metabolism [92]. *CG10133* and *CG8920* are involved in phospholipase A2 activity, mitochondrial electron transport function and nucleic acid binding, respectively [93]. Mitochondrial function has been shown to regulate glucose and lipid homeostasis in mammalian [94] and non-mammalian models [95-97]. *Drosophila gli* is a transmembrane protein transiently expressed in peripheral glia in which loss of function has been implicated in defects in axonal guidance and synaptogenesis [98]. *Gdh* codes for a nuclear-encoded mitochondrial enzyme with a pivotal role in metabolism as it has been linked to differential utilization of metabolite pools for energy production [99,100]. *Tty* encodes a highly conserved calcium-activated chloride channel associated to flight behavioral abnormalities [101].

Transcriptional networks associated to body weight and energy metabolism traits

Previously, Ayroles *et al.* showed that the *Drosophila* transcriptome is characterized by high rates of correlation between transcripts [56], we therefore sought to use a weighted gene co-expression network procedure [56] to identify “modules” of highly-interconnected genes associated with variation in each trait. This type of network analysis provides hypotheses about functional relationships among transcripts and provides insight into how variation in the network of co-expressed genes can give rise to variation in the associated traits. We identified 13 modules of correlated transcripts associated with BW, 5 with TAG, 9 with GLY, 18 with PRO, 13 with GLYC, and 6 with MR (Figure 2 and **Supplementary Table 1**: Analysis of modules of correlated transcripts associated

with each of the body composition and energy metabolism traits). To determine the biological significance of the genes in these network modules, we used gene ontology categories [102], tissue-specific expression [103], and published protein-protein interactions or shared domains.

Body Weight. We found that several of the QTTs associated with variation in BW are enriched for genes encoding antimicrobial peptides, infection-induced proteins, as well as proteins involved in microbial recognition, phagocytosis, melanization and signaling (modules 1, 2, 4, 5, and 13) (Fig. 3A; **Supplementary Table 3**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits). These genes are predominantly expressed in the fat body, gut, and carcass of the adult fly (Fig. 3C). While the fat body is recognized as the major immune-responsive tissue responsible for the synthesis and secretion of antimicrobial peptides in response to a pathogenic challenge, the gut and carcass possess the ability to fight infection via local production of reactive oxygen species and antimicrobial peptides as the main barrier epithelia in constant contact with exogenous microorganisms [104].

The remaining BW modules are enriched for genes involved in chemical stimulus and behavior (modules 7 and 11); organismal development, sensory perception and transduction (module 10). Reports on the genetic basis of rare monogenic forms of obesity in humans [105] and single-gene approaches in mammalian models [106-108] have long suggested a strong involvement of the central nervous system in body weight regulation [109]. Recently, tissue-to-tissue co-expression networks have highlighted the role of the hypothalamus as the controlling tissue in organismal energy balance [32].

Furthermore, recent genome-wide association studies in humans have mapped body weight-associated loci near genes that are highly expressed in the brain, particularly in the hypothalamus, and are involved in neuronal development and activity [110,111]. Consistent with the data in mammals [32], BW module 10 is enriched for genes involved in neuronal development, such as *nightblind (cac)*, *inebriated (ine)*, *soxneuro (SoxN)*, *erect wing (ewg)* and *reverse polarity (repo)*, and genes that are expressed in the adult brain and thoracicoabdominal ganglion (Fig. 3B-C), the equivalent of mammalian central and peripheral nervous systems.

BW module 10 is also enriched for genes associated with sensory perception and transduction (**Supplementary Table 3**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits). In mammals, the central nervous system (CNS) integrates information regarding nutrient status and organismal energy stores with cognitive, visual, olfactory and taste stimuli to elicit appropriate behavioral responses in relation to feeding [109]. Anatomically, various parts of the CNS in insects have shown to be connected to neuroendocrine organs and the enteric nervous system innervating their feeding apparatus [112]. Interestingly, the neuropeptide gene *hug*, one of the hub genes in module 10 (Figure 3B), is highly expressed in the subesophageal ganglion, a region involved in feeding and taste response [113]. These neurons have been shown to project axons to the ring gland, the central neuroendocrine organ in *Drosophila*, which produces adipokinetic hormone (AKH) and receive inputs from *Drosophila* insulin-like peptides-secreting cells. Both insulin- and AKH-producing cells comprise a specialized network that controls

organismal energy metabolism and growth and hence body weight, sharing a common evolutionary ancestry with the α- and β-cells of the human pancreas [114]. Further experiments have suggested that *hug* neurons integrate chemosensory and nutrient signals to determine feeding behavior in *D. melanogaster* [112].

Triacylglycerol storage. QTTs associated with variation in TAG storage are enriched for genes that mediate response to stress (modules 1 and 4) and cell growth (module 4; **Supplementary Table 3**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits). Remarkably, five of the hub genes in module 4, *debcl*, *Sce*, *viaf*, *Sirt7*, and *Srp54*, (Fig 3D), have human orthologs, *BOK*, *RING1*, *Pdcl3*, *SIRT7*, and *SFRS12*, respectively, whose transcript abundance has been shown to regulate obesity in mice [32].

Total glycogen level. QTTs associated with variation in GLY are enriched for genes implicated in multicellular organismal development (module 6 and 7), cell communication, signal transduction and synapsis (module 7), and mitochondrial genome maintenance and replication (module 9; **Supplementary Table 3**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits). Interestingly, *puckered*, the most highly connected gene in module 6, encodes a *Drosophila* mitogen-activated protein kinase (MAPK) phosphatase, an important negative regulator of one of the MAPK pathways, the Jun N-terminal kinase (JNK) signaling pathway (see **Supplementary Table 1**: Analysis of modules of correlated transcripts associated with each of the body composition and energy metabolism traits). MAPK signaling cascades induce coordinated transcriptional changes

in response to environmental cues. Studies in *Drosophila* [115], *Caenorhabditis elegans* [116] and mammalian models [117] suggest antagonistic relationships between JNK and the insulin signaling pathway. Mutations in the JNK signaling cascade induce significant reduction in glycogen stores with rapid depletion of metabolite reservoirs upon starvation [118]. Thus, ***puckered***-mediated differential activation of the JNK pathway would be expected to have a regulatory effect on whole-body glycogen pools.

The inclusion of signal transduction genes as hub genes in module 7 (Figure 5A), particularly those associated with acetylcholine receptor signaling and metabolism (*AchR protein of Drosophila, muscarinic receptor, acetylcholinesterase, choline acetyltransferase*), highlights the role of the central nervous system in the regulation of organismal energy homeostasis. Acetylcholine has been shown to play a pivotal role in olfactory learning in *Drosophila* [119]. Olfactory learning provides the basis of feeding motivation in insects as it derives from previous dietary experience [120].

Total protein content. QTTs associated with variation in PRO are enriched for genes involved in gene expression and RNA metabolism (module 3 and 4), cellular metabolism and tissue development (module 7) and immune response (module 13) (**Supplementary Table 3**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits). Coordinated transcriptional changes via three main signaling cascades, the insulin/insulin growth factor 1, the target of rapamycin (TOR) and the MAPK pathways, play a pivotal role in the body-wide control of protein synthesis [121]. Interestingly, module 7 includes *Tif-IA* as a hub gene (Figure 5B). *Tif-IA* is a nutrient sensitive molecule previously identified as a downstream

target of TOR in the yeast [122], *Drosophila* [123] and mammalian systems [124], which is involved in the control of ribosomal biogenesis, translational machinery, and cell growth. We also identified *nemo* in module 7 as a QTT associated with variation in whole-body protein. Gene ontology analysis describes a mitogen-activated protein kinase (MAPK) activity for *nemo*. Furthermore, there is evidence associating *mitochondrial transcription factor A*, a QTT in module 4, to mitochondrial biogenesis/function [125]. Similarly, *scribble*, a QTT in module 13, has been linked to olfactory behavior [126]. These findings highlight the biological relevance of the modular components comprising our protein co-expression network as mitochondrial bioenergetics and olfaction have also shown to play a pivotal role in organismal energy homeostasis and feeding behavior [120,127,128].

Total glycerol level. QTTs associated with variation in GLYC are enriched for genes involved in cellular redox homeostasis (module 5), gene expression and RNA metabolism (module 6), cellular organization and biogenesis (module 7), protein metabolism (module 8) and neuronal development and behavior (module 12) (**Supplementary Table** 3: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits). The maintenance of cellular redox homeostasis (module 5) in *Drosophila* when facing an immune challenge has shown to trigger acute coordinated proteomic changes in Gpdh levels as a result of increasing energy demand that shuts down normal biosynthetic processes, such as those involved in glycerol metabolism [129]. Interestingly, *Gpdh*, the ortholog of mammalian *glycerol-3-phosphate dehydrogenase*, is included in module 12, and has been previously shown as

an evolutionarily conserved enzymatic component involved in *Drosophila* glycerol metabolism pathways [130].

Metabolic rate. QTTs associated with variation in MR are enriched for genes mediating proteolysis (modules 1 and 2) and carbohydrate metabolism (module 5 and 6; **Supplementary Table 3**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits). It is interesting to note that modules of correlated transcripts associated with MR are enriched for genes that are mainly expressed in the midgut and Malpighian tubule (Figure 3E). The midgut is the site for carbohydrate and protein digestion [131]. Food processing entails a metabolic cost that can increase metabolic rates between two and fourfold with a subsequent decline that can be either rapid or prolonged depending on the species [132,133]. Data on lepidopteran caterpillars suggest that the feeding-induced increase in oxygen consumption rates is long-lasting [134]. Similar to insects, mammalian [135] and non-mammalian vertebrates [136] display a post-prandial increase in metabolic rate mainly attributable to the energy requirements for protein and lipid synthesis, along with those of temporary nutrient storage (i.e. fat and glycogen) derived from food ingestion. Overall, these observations and our findings suggest that meal size and composition are important regulators of the duration of the effect of food processing on metabolic rate.

Genetic correlations between energy metabolism and life-history traits

Since the Raleigh lines were previously evaluated for several life-history traits [56], we next asked whether there were genetic correlations between energy metabolism and life-history traits. The results of the analysis are shown in Table 3A-C. We observed a

clear trade-off between BW and reproductive fitness (Table 3A). A similar trade-off was observed between GLY and reproductive fitness and between PRO and time to initiate copulation (Table 2A). Such trade-offs evolve as a consequence of the limited nature of internal energy reserves and their differential allocation [50]. As previously proposed, "an increment of resources allocated to one trait necessitates a decrement of resources to another trait" [137]. Indeed, diversion of energy flow, particularly of fatty acids and amino acids, towards reproductive processes has been shown to determine high-fecundity phenotypes at the expense of somatic reserves as shown by artificial selection studies in insects [138,139]. Similarly, carbohydrates play a pivotal role in reproductive success [140]. As periodic episodes of food shortage are ubiquitous in nature, genetically-determined physiological adaptations are expected to evolve towards greater resistance to starvation [49] at the expense of reproductive fitness. Indeed, previous data published on these 40 lines indicated that those lines that are resistant to food deprivation tend to have reduced competitive fitness [56]. Several selection experiments in *Drosophila* have shown that an increase in energy reserves, in particular lipid stores, seems to be a mechanism underlying evolution of greater starvation resistance. Yet the relationship between fat reserves and starvation appears to be a consequence of laboratory selection since no correlation was found among isofemale strains derived from wild populations [141]. Consistent with these findings, we found a tendency for lines in which flies are heavier and have higher levels of GLY to display higher resistance to starvation (Table 2). Yet no correlation was observed between TAG and starvation resistance.

We then analyzed the data stratified by gender as sexual dimorphism among body weight and energy metabolism traits is well recognized across different species [18,142,143]. BW is positively correlated to copulation latency in females but not in males (Table 3B-C), which is in good agreement with previous reports suggesting that reproductive traits are strongly dependent on female body size but not or much less on male body size [144-146]. Similar to the combined sex analysis, whole-body protein was positively correlated to copulation latency and glycogen storage was positively correlated (at a significant p-value<0.05) to starvation survival in both sexes (Table 3B-C).

In males, there was also a tendency for lines with lower MR to live longer (Table 2C). Several reports have highlighted the existence of a negative correlation between longevity and metabolic rate in mammalian [147,148] and non-mammalian models [149,150]. These findings underlie the basis of the oxidative-stress theory of aging [151,152] by which increased by-products derived from higher rates of aerobic metabolism are hypothesized to induce organismal cumulative damage and hence early mortality. Recent studies have challenged the validity of this theory [153,154]. This discrepancy in findings may stem from undisclosed gender-specific allelic effects on quantitative traits as shown by our data.

To gain insight into the molecular basis of the observed genetic correlations, we tested whether there was overlap of common genes between modules for the energy metabolism traits and life-history traits. We found substantial modular pleiotropy between BW and other life-history traits (Table 4). Pleiotropic modules between BW, starvation resistance, and fitness traits were enriched for genes involved in immune

response (BW module 2 and 4; see **Supplementary Table 1**: Analysis of modules of correlated transcripts associated with each of the body composition and energy metabolism traits), as well as in lipid and protein metabolism (BW module 8; see **Supplementary Table 1**: Analysis of modules of correlated transcripts associated with each of the body composition and energy metabolism traits). *molting defective* is one of the genes with pleiotropic effects on BW, reproductive fitness, and starvation resistance. Interestingly, *molting defective* is involved in the biosynthesis of ecdysone [155], previously suggested as an important hormonal modulator of organismal post-embryonic development, oogenesis, and reproduction [50,156] and linked to a positive regulation of cellular and humoral innate immunity in *D. melanogaster* [157-159]. A recent report has shown that insect cell culture display increased expression of *diptericin, cecropin* and *attacin* (three other genes with pleiotropic effects on energy metabolism and life-history traits) under pre-treatment with 20-hydroxy-ecdysone and upon immune stimulation [160]. Differential expression of *molting defective* may impact organismal body size (and hence BW) as *molting defective* mutants have shown to display developmental arrest [155]. Furthermore, its transcriptional defect may affect the biosynthesis of ecdysone with subsequent changes in the *Drosophila* immune response.

Among the genes with pleiotropic effects on GLY and reproductive fitness, we identified two photosensory opsins, *Rh4* and *Rh6*. Feeding behavior displays a 24hr circadian rhythm under the control of peripheral clocks in metabolic tissues but is also influenced by light [161]. Indeed, disruption of this circadian organization of feeding rhythms has been reported to alter glycogen storage in *Drosophila* [161]. In agreement

with our findings, a wide range of reproductive behaviors in *Drosophila* (i.e. sexual receptivity, oviposition, mating, courtship, and locomotion) are also under circadian regulation [162,163].

CONCLUSION

The orchestrated expression of highly complex co-expression networks underlies the basis of phenotypic variation in metabolism traits among young adult flies with significant impact on reproduction and starvation resistance. Our gene ontology enrichment profiles highlight the relevance of signaling pathways non-directly related to energy metabolism, as regulators of natural variations in obesity-related traits. Indeed, genes involved in immune response, neuronal development and function, cell growth and cell metabolism were identified as regulators of organismal energy balance. Several of these genes have also been identified as exerting pleiotropic roles among energy metabolism and fitness traits setting the basis of existent life-history trade-offs.

Table 1. Quantitative genetics analyses of body composition and energy metabolism traits for 40 wild-derived inbred lines of *D. melanogaster*. Estimates of genetic variance for the combined sex analyses

Trait[a]	Mean (±SE)	σ_L^{2}[b]	σ_{SL}^{2}[c]	σ_G^{2}[d]	σ_E^{2}[e]	σ_P^{2}[f]	H^2[g]	CV_G[h]	CV_E[i]
BW (mg/fly)	0.75 (± 0.01)	0.02**	0.01****	0.03	0.09	0.12	0.25	23.09	40.00
TAG (ug/fly)	6.27 (± 0.04)	0.42****	0.18****	0.60	0.64	1.24	0.48	12.35	12.76
GLY (ug/fly)	6.02 (± 0.14)	30.37**	31.23****	61.60	43.42	105.02	0.59	130.37	109.46
GLYC (ug/fly)	4.11 (± 0.11)	0.23****	0.01**	0.24	0.13	0.37	0.65	11.92	8.77
PRO (ug/fly)	64.35 (± 1.14)	219.21*** *	88.39****	307.6	144.39	451.99	0.68	27.25	18.67
MR ($mlCO_2$/fly)	3.80 (± 0.03)	0.12**	0.14****	0.26	0.36	0.62	0.42	13.42	15.79

[a] BW: body weight; TAG: triacylglycerol storage; PRO: total proteins; MR: metabolic rate; GLYC: glycerol levels. [b] Among line variance component. [c] Sex by line interaction variance component. [d] Total genetic variance ($\sigma_L^2 + \sigma_{SL}^2$). [e] Variance within replicates or lines. [f] Total phenotypic variance ($\sigma_G^2 + \sigma_E^2$). [g] Broad-sense heritability (σ_G^2 / σ_P^2). [h] Coefficient of genetic variation ($100\sigma_G$/Mean). [i] Coefficient of environmental variation ($100\sigma_E$/Mean). $^{*}P \leq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Table 2 - Genetic correlations between body weight and energy metabolism traits. (A) Genetics correlations averaged across sexes. (B) Genetic correlations for females (above the diagonal) and males (below the diagonal).

A

	TAG	**GLY**	**PRO**	**GLYC**	**MR**
BW	0.11±0.16	0.22±0.16	0.27±0.16	-0.14±0.16	0.21±0.16
	TAG	0.04±0.16	0.20±0.16	0.16±0.16	0.27±0.16
		GLY	-0.15±0.16	0.05±0.16	-0.15±0.16
			PRO	0.25±0.16	-0.03±0.16
				GLYC	-0.07±0.16

B

	BW	**TAG**	**GLY**	**PRO**	**GLYC**	**MR**
BW		0.19±0.16	0.30±0.16	**0.34±0.16***	-0.17±0.16	0.18±0.16
TAG	0.17±0.16		0.16±0.16	**0.35±0.15***	0.25±0.16	**0.45±0.15****
GLY	**0.32±0.15***	0.05±0.16		-0.19±0.16	0.01±0.16	-0.03±0.16
PRO	0.27±0.16	0.23±0.16	-0.19±0.16		**0.37±0.16***	0.05±0.16
GLYC	-0.16±0.16	0.07±0.16	0.06±0.16	0.10±0.16		-0.03±0.16
MR	**0.38±0.15****	0.03±0.16	-0.16±0.16	-0.09±0.16	-0.11±0.16	

BW: body weight; TAG: triacylglycerol; GLY: glycogen levels; PRO: total proteins; GLYC: glycerol levels; MR: metabolic rate. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$.

Table 3 - Genetic correlations between energy metabolism and life-history traits averaged across sexes (A) for females (B), and for males (C).

A

	FT	CL	SR	LS
BW	**-0.48±0.14****	0.21±0.16	**0.52±0.14*****	0.01±0.16
TAG	-0.14±0.16	0.08±0.16	0.12±0.16	-0.15±0.16
GLY	**-0.38±0.15****	0.08±0.16	0.29±0.15	0.17±0.16
PRO	-0.17±0.16	**0.43±0.15****	0.26±0.16	0.13±0.16
GLYC	0.05±0.16	0.22±0.16	0.06±0.16	-0.07±0.16
MR	0.14±0.16	-0.11±0.16	-0.04±0.16	-0.26±0.16

B

	FT	CL	SR	LS
BW	**-0.52±0.14*****	**0.38±0.15***	**0.53±0.14*****	-0.01±0.16
TAG	-0.10±0.16	0.20±0.16	0.14±0.16	0.01±0.16
GLY	-0.27±0.16	0.10±0.16	**0.36±0.15***	0.10±0.16
PRO	-0.15±0.16	**0.51±0.14*****	0.19±0.16	0.17±0.16
GLYC	0.04±0.16	0.22±0.16	0.00±0.16	-0.08±0.16
MR	0.06±0.16	-0.11±0.16	0.00±0.16	-0.16±0.16

C

	FT	CL	SR	LS
BW	**-0.42±0.15****	-0.06±0.16	**0.67±0.12******	0.07±0.16
TAG	-0.17±0.16	-0.04±0.16	0.14±0.16	-0.29±0.16
GLY	**-0.59±0.13******	0.04±0.16	**0.42±0.15****	0.24±0.16
PRO	-0.22±0.16	**0.32±0.15***	0.23±0.16	0.09±0.16
GLYC	0.06±0.16	0.22±0.16	0.13±0.16	-0.06±0.16
MR	0.25±0.16	-0.10±0.16	-0.04±0.16	**-0.41±0.15****

BW: body weight; TAG: triacylglycerol; GLY: glycogen levels; PRO: total proteins; GLYC: glycerol levels; MR: metabolic rate; FT: competitive fitness; CL: copulation latency; SR: starvation resistance; LS: lifespan. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; $^{****}P < 0.0001$.

Table 4. Modular pleiotropy between energy metabolism and life-history traits

Energy Metabolism Trait	**Module**	**Life-history trait**	**Module**	***p*-value**
BW	5	FT	2	2.80E-04
BW	2	FT	6	3.05E-08
BW	4	FT	11	1.09E-06
BW	8	FT	15	6.92E-13
BW	10	FT	17	7.18E-11
BW	3	FT	18	8.50E-04
BW	13	FT	19	1.03E-04
BW	7	FT	20	3.46E-05
BW	2	SR	2	3.81E-05
BW	4	SR	4	7.22E-05
BW	7	SR	8	4.04E-03
BW	8	SR	9	3.21E-04
BW	8	CL	8	4.76E-03
BW	10	GLY	7	1.40E-03
GLY	2	FT	14	4.33E-03
GLY	7	FT	17	2.03E-05
PRO	13	FT	15	5.00E-04
PRO	7	CL	3	2.07E-04
PRO	15	CL	6	1.84E-09
PRO	17	CL	8	1.90E-03
PRO	15	SR	7	4.08E-06
PRO	13	SR	9	3.95E-03

BW: body weight; GLY: glycogen levels; PRO: total proteins; FT: competitive fitness; CL: copulation latency; SR: starvation resistance.

Figure 1. **Variation in body weight and energy metabolism traits in *D. melanogaster*.** Distribution of BW (panel A), TAG (panel B), GLY (panel C), PRO (panel D), GLYC (panel E), and MR (panel F) among the 40 Raleigh wild-type inbred lines. Data represent means ± SEM for n = 10 independent replicates. The blue and pink bars in panels A-F depict females and males, respectively.

Figure 1

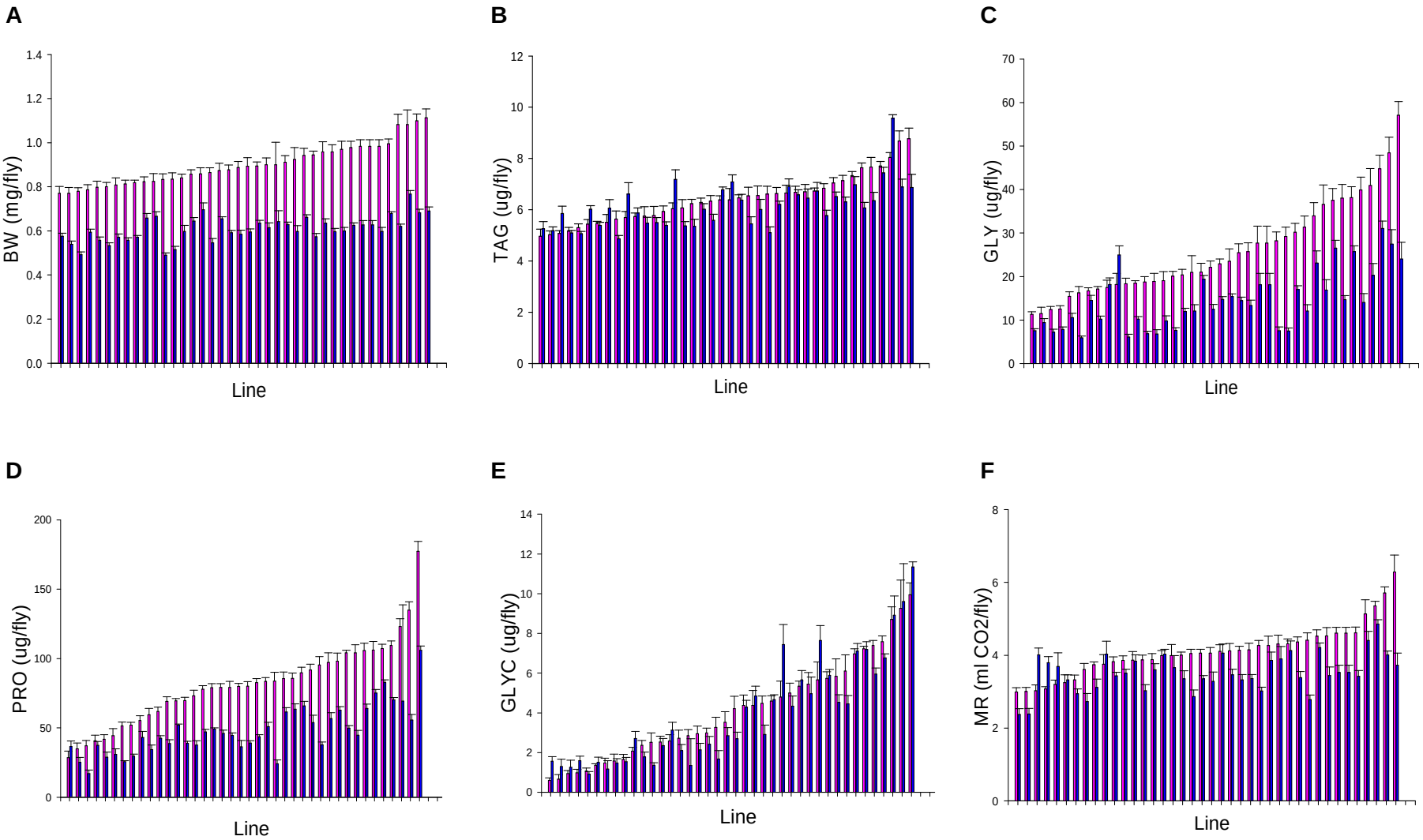

A
BW (mg/fly)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Line
B
TAG (ug/fly)
12
10
8
6
4
2
0
Line
C
GLY (ug/fly)
70
60
50
40
30
20
10
0
Line
D
PRO (ug/fly)
200
150
100
50
0
Line
E
GLYC (ug/fly)
14
12
10
8
6
4
2
0
Line
F
MR (ml CO2/fly)
8
6
4
2
0
Line

Figure 2. QTTs and transcriptional networks associated to variation in body weight and energy metabolism traits. (A) Clustering of the 275 transcripts significantly associated with variation in BW into 13 modules. The color scale bar indicates the value of the correlation. (B) Clustering of the 125 transcripts significantly associated with variation in TAG into 5 modules. (C) Clustering of the 130 transcripts significantly associated with variation in GLY into 9 modules. (D) Clustering of the 298 transcripts significantly associated with variation in PRO into 18 modules. (E) Clustering of the 389 transcripts significantly associated with variation in GLYC into 13 modules. (F) Clustering of the 93 transcripts significantly associated with variation in MR into 6 modules.

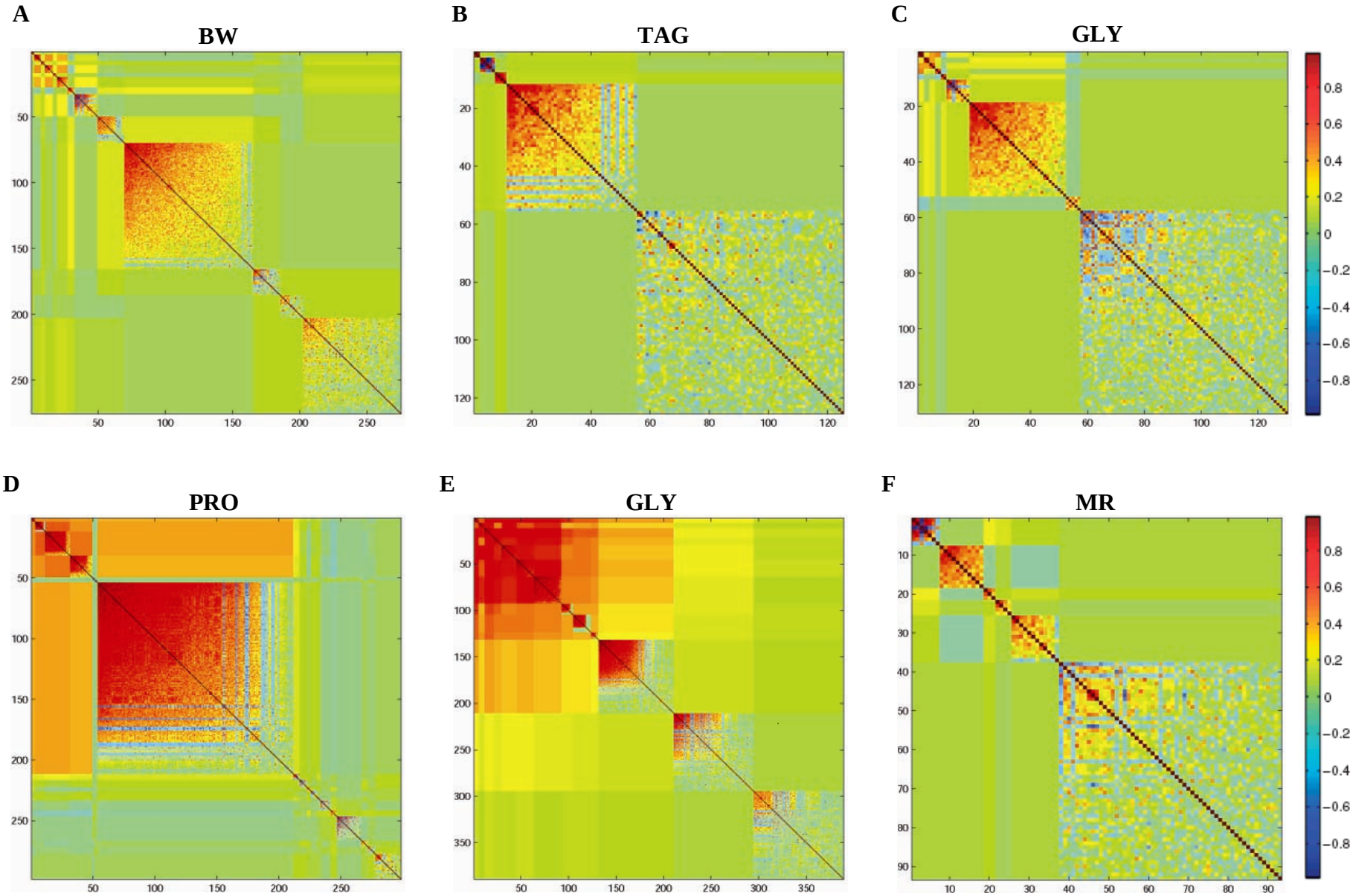
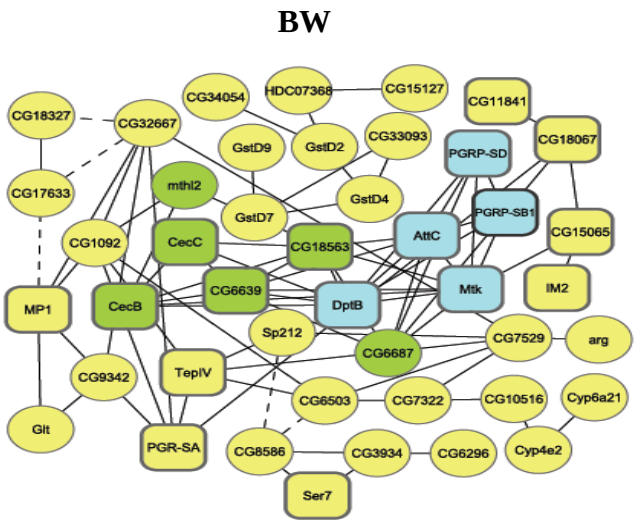
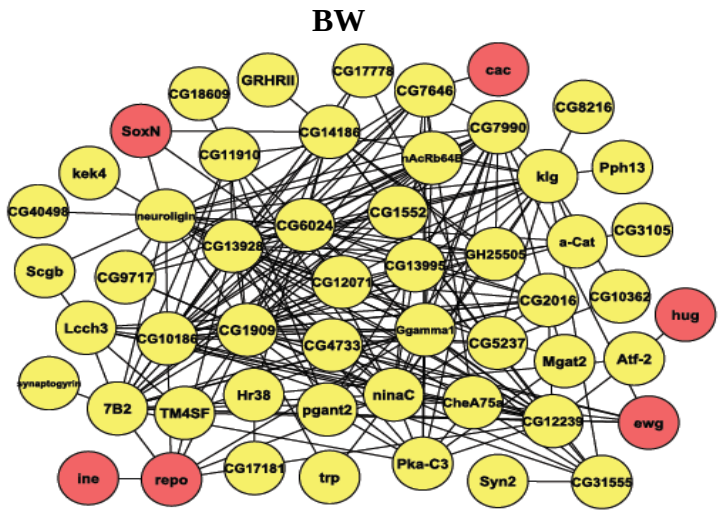

A
BW
B
TAG
C
GLY
D
PRO
E
GLY
F
MR

Figure 3. Genetic networks underlying variation in BW, TAG and MR. (A) Network of correlated (|r|≥0.5) transcripts for BW module 2 (light blue nodes), 4 (green nodes) and 13 (yellow nodes). Each node represents a gene and each edge a significant correlation between a pair of genes. Nodes shown as round rectangles represent hub genes involved in responses to microbial infection in *Drosophila*. Dashed lines between nodes represent correlation of |r| = 0.4-<0.5. (B) Network of correlated (|r|≥0.6) transcripts for body weight module 10. Each node represents a gene and each edge a significant correlation between a pair of genes. Nodes shown as pink represent hub genes associated with neurogenesis in *Drosophila*. (C) Distribution of tissue-specific expression of QTTs in module 2, 4, 13 and 10. Module 2, 4 and 13 are enriched for QTTs involved in immune response and predominately expressed in immune-responsive tissues: midgut, fat body and carcass. Module 10 is enriched for transcripts mainly expressed in brain and thoracicoabdominal ganglion. (D) Network of correlated (|r|≥0.5) transcripts for TAG module 4. Each node represents a gene and each edge a significant correlation between a pair of genes. Nodes shown as pink represent those genes previously identified as "hub" genes in multi-tissue co-expression networks [32]. (D) Distribution of tissue-specific expression of all QTTs comprising the MR co-expression network. QTTs are overall predominately expressed in the midgut.

A

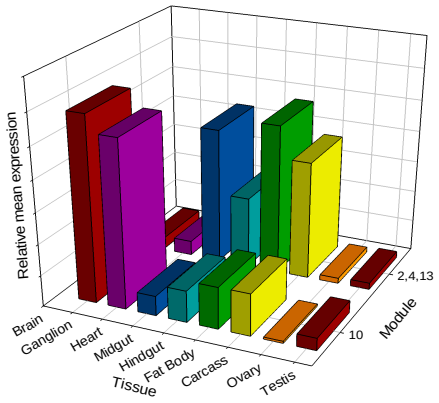


Modules 2, 4 and 13



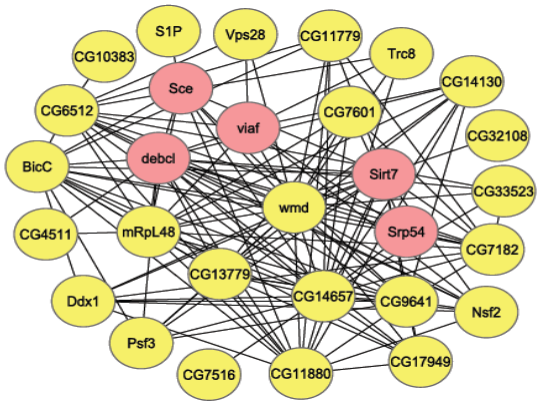
Module 10

C



D

TAG



Module 4

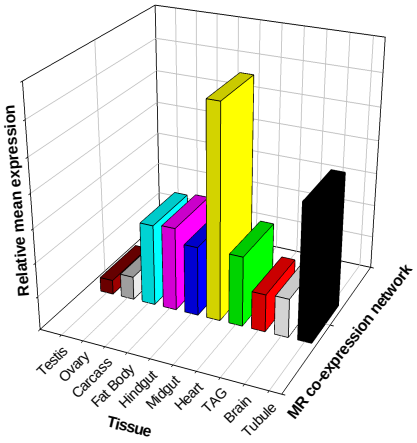


Figure 4. **Effects of *P[GT1]* and *PiggyBac* transposon insertional mutations in candidate genes affecting variation in TAG, GLY, and GLYC**. Mutational effects are given as deviations from the co-isogenic control line. Pink and blue bars represent females and males, respectively. Mutations in all genes shown have significant effects in one or both sexes (see **Supplementary Table 3**: Effects of *P[GT1]* and *PiggyBac* transposon insertional mutations in candidate genes affecting TAG, GLY, and GLYC). Error bars, S.E.M. (A) TAG. (B) GLYC. (C) GLY.

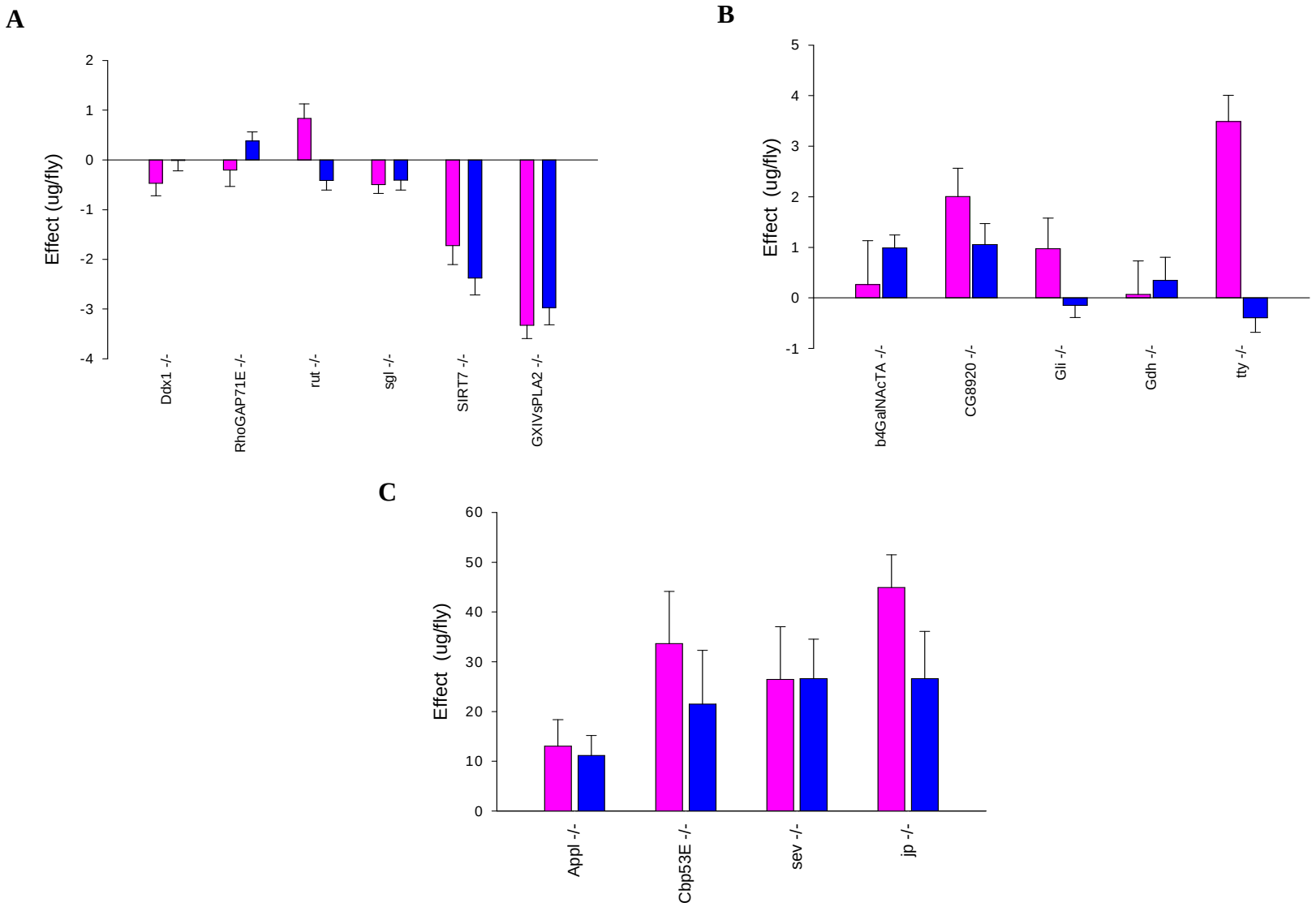
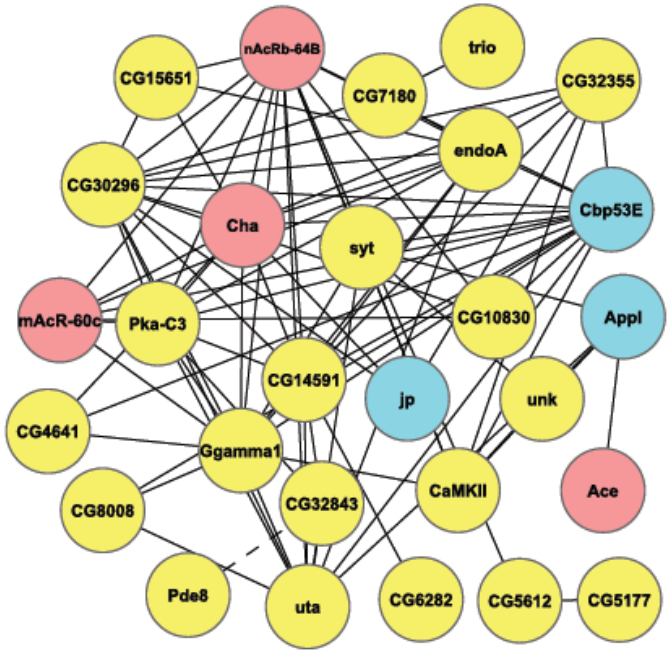

A
Effect (ug/fly)
2
1
0
-1
-2
-3
-4
Ddx1 -/-
RhoGAP71E -/-
rut -/-
sgl -/-
SIRT7 -/-
GXIVsPLA2 -/-
B
Effect (ug/fly)
5
4
3
2
1
0
-1
b4GalNAcTA -/-
CG8920 -/-
Gli -/-
Gdh -/-
tty -/-
C
Effect (ug/fly)
60
50
40
30
20
10
0
Appl -/-
Cbp53E -/-
sev -/-
jp -/-

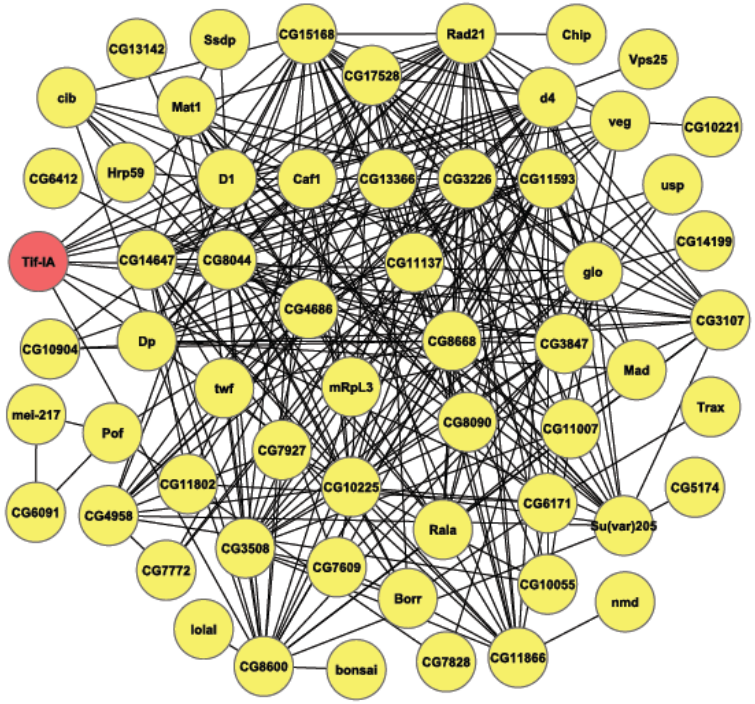
Figure 5. Genetic networks underlying variation in GLY and PRO. (A) Network of correlated ($|r| \geq 0.5$) transcripts for GLY module 7. Each node represents a gene and each edge a significant correlation between a pair of genes. Nodes shown as pink are involved in acetylcholine receptor signaling and metabolism. Nodes shown as light-blue represent those candidate genes for which homozygous mutants were tested. Dashed lines between nodes represent correlation of $|r| = 0.4-<0.5$. (B) Network of correlated ($|r| \geq 0.8$) transcripts for total proteins module 7. Each node represents a gene and each edge a significant correlation between a pair of genes. Node shown as pink (*TifIA*) represents a TOR-regulated gene.

GLY



Module 7

PRO



Module 7

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CHAPTER III:

IDENTIFICATION OF NUCLEAR-ENCODED GENES REGULATING *DROSOPHILA* MITOCHONDRIAL RESPIRATION TRAITS THROUGH TRANSCRIPTOME ANALYSIS

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ABSTRACT

Obesity is the major contributor to the increasing prevalence of chronic diseases such as insulin resistance and type 2 diabetes mellitus (T2DM). T2DM is a complex disease that adversely affects human morbidity and mortality. Recent genetic evidence provides strong support that T2DM in humans may develop from downregulation of mitochondrial oxidative phosphorylation (OXPHOS). Furthermore, alteration of mitochondrial function has emerged as a key factor in the development of diabetic complications. Despite the extensive research into mitochondrial pathology, we have no understanding of the genetic basis of mitochondrial respiration traits in natural populations. Here, we quantified variation in mitochondrial respiration traits [ADP-stimulated state 3 respiration, non-ADP-stimulated state 4 respiration (a surrogate of basal proton leak), and mitochondrial ATP synthesis efficiency (ADP/O ratio)] among 40 wild-type inbred lines of *Drosophila melanogaster*. We chose these lines because they were previously evaluated for variation in nuclear genome-wide transcription abundance and several energy metabolism and life-history traits. This allowed us to identify nuclear-encoded genes regulating *Drosophila* mitochondrial respiration traits and to investigate genetic correlations between these traits. We showed a genetic component of variance for all mitochondrial respiration traits, with broad-sense heritabilities ranging from 14% to 23%. Notably, we did not observe significant genetic correlations between mitochondrial state 3 and state 4 respiration traits, suggesting that these traits have different genetic properties. On the other hand, a

negative correlation was found between ADP/O and state 4 in males, which is consistent with the contribution of proton leak to mitochondrial efficiency. We used a gene co-expression network analysis to identify nuclear-encoded genes and pathways affecting natural variation in mitochondrial respiration rates. We found that 293, 105, and 19 quantitative trait transcripts (QTTs) underlie differences in mitochondrial state 3, state 4, and ADP/O ratio, respectively. Regression analysis showed that genes regulating mitochondrial state 3 respiration rate are involved in sensory perception and signal transduction, and mitochondrial state 4 respiration network comprises genes engaged in reproduction and cell replication. The analysis of genetic correlations revealed positive correlations between state 3 respiration rate and total glycogen levels and between ADP/O ratio and total protein levels. A positive correlation was also observed between state 4 respiration rate and starvation resistance. That is, higher mitochondrial proton leak improves the ability of the organism to survive starvation stress. Finally, we found a negative correlation between state 4 respiration and copulation latency, suggesting a trade-off between proton leak and reproduction. These results strongly indicate that molecular regulation of mitochondrial respiration play a critical role in mediating life history trade-offs in natural populations.

INTRODUCTION

Obesity and overweight both pose a major risk for the development of chronic diseases such as insulin resistance and type 2 diabetes mellitus (T2DM). T2DM and its complications have imposed a substantial economic burden on individuals, families, health systems and countries as it has reached epidemic proportions in the US and worldwide [1] [2]. T2DM is a complex disease characterized by chronic hyperglycemia as a consequence of insulin resistance in skeletal muscle (causing decreased glucose uptake) and liver (causing increased gluconeogenesis), together with defects in insulin secretion from pancreatic β-cells. The complexity of T2DM results from the interaction of environmental factors with genetic susceptibility factors [3]. Recent genetic evidence strongly supports the idea that insulin resistance in humans develops from downregulation of mitochondrial oxidative phosphorylation (OXPHOS) [4-9]. In addition, mitochondrial cytopathy has been linked to defects in lipid metabolism [10] and abnormal fat accumulation, preferentially in the visceral compartment, which is an important risk factor for insulin resistance [11] and the development of T2DM [12].

The oxidation of metabolic fuels is an essential process regulating energy balance in aerobic organisms. Oxidation takes place in mitochondria, where the electrochemical proton gradient is used to convert ADP into ATP via OXPHOS for use in cellular processes [13]. In normal mitochondria, electrons from reducing equivalents (electron donors) derived from substrate oxidation

n fuel into complexes I and II of the mitochondrial electron transport chain and travel through complexes III and IV to reduce oxygen (electron acceptor) to water. Coupled to electron transport, protons are pumped across the mitochondrial inner membrane (Figure 1). The proton-motive force established by proton pumping drives protons back through complex V, also known as the ATP synthase, forming ATP from ADP and inorganic phosphate.

In a perfectly coupled system, protons only re-enter the mitochondrial matrix through ATP synthase in the presence of ADP. This form of respiration is classified as 'state 3' (i.e. O_2 is consumed only in the presence of substrate and ADP). However, mitochondria also use oxygen in the absence of ADP, which occurs when protons leak back into the matrix via a mechanism that does not involve ATP synthase. This proton leak *uncouples* respiration from OXPHOS. 'State 4' respiration rate represents a surrogate measure of mitochondrial basal proton leak. Electrons flowing through complex I and III can also escape redox transfer to produce reactive oxygen species (ROS). Strong evidence exists that mitochondrial ROS production plays a key role in the pathogenesis of T2DM and its complications through modification of various cellular events in many tissues, including kidney, pancreatic β cells, and liver [14].

Despite the extensive research into mitochondrial pathology, little is known about the genetic and molecular mechanisms underlying variation in mitochondrial respiration traits in natural populations. Several fundamental questions remain to be resolved: is there considerable segregating variation in mitochondrial bioenergetic traits in natural populations? Do genetically based differences in mitochondrial respiration in skeletal

muscle underlie some of the inter-individual variability in organismal energy metabolism? What role does molecular regulation of mitochondrial respiration play in mediating life history trade-offs? In this context, the goal of the present study was to investigate the genetic basis of mitochondrial state 3 respiration, state 4 respiration, and mitochondrial efficiency (ADP/O ratio) using 40 wild-type inbred lines of *D. melanogaster* recently established from a single natural population in Raleigh, NC [15]. *D. melanogaster* was used as a model because of the strong resemblance of the phosphorylating respiratory chain of insect mitochondria to that of mammalian mitochondria [16]. Exceptionally high respiratory and phosphorylative activities have been reported in insects using pyruvate plus proline (feeding into complex I) or α-glycerophosphate (feeding into complex III) as physiological substrates [16]. The mitochondrial OXPHOS in insects is affected by the same inhibitors and uncouplers affecting the mammalian system [16-18]. Furthermore, we chose these lines because they were previously evaluated for variation in nuclear genome-wide transcription abundance and several energy metabolism and life-history traits. This allowed us to identify nuclear-encoded genes regulating differences in mitochondrial respiration traits among *Drosophila* lines, and to investigate genetic correlations between all these traits.

MATERIALS AND METHODS

Drosophila stocks

The 40 unrelated wild-type inbred lines of *D. melanogaster* were established from a sample of isofemale lines collected in Raleigh, NC and inbred to near-homozygosity by 20 generations of full-sib mating [15]. Each stock was maintained at constant parental density for at least two generations to minimize environmental effects. To control for larval density, we allowed the parents of the experimental flies to mate for 3 hours to lay eggs on apple juice/agar medium in laying plates. After 24 hours, we picked groups of 100 first-instar larvae from the surface of the medium and placed them into replicate vials. To minimize the influence of genetic variation in reproduction on energy metabolism, we performed all the phenotypic assays on virgin flies that were randomly collected from the replicate vials for each line on days 10 to 16 under brief CO_2 exposure. For mitochondrial function assays, we used seven replicate vials per line, with each vial containing a group of 20 single-sexed individuals aged 3-5 days. Due to the size of this experiment, we conducted the phenotypic assays in 14 overlapping blocks. We reared flies in vials containing 10 ml of standard cornmeal, agar, molasses, and yeast medium at a constant temperature of 25°C, 60–75% relative humidity, and a 12-hr light-dark cycle.

Mitochondrial respiration rate assay

Live flies were anesthetized on ice and thoraces were severed. Briefly, thoraces were placed into 200 µl of ice-cold isolation buffer [250 mM sucrose, 5 mM Tris-HCl, 2 mM EGTA, 1% (w/v) bovine serum albumin (BSA), pH 7.4 at 4°C; [18]) supplemented with

protease inhibitors (leupeptin 1mg/ml, aprotinin 1mg/ml and pepstatin 1mg/ml) in a 1.5 ml Eppendorf tube. The samples were pounded gently 126 times over a 2 minute period, using a motorized micromortar. Mashed flies were filtered through a 5 micron nylon mesh, and the volume was raised to 400 µl by washing the nylon membrane with additional isolation buffer. After a cycle of low-speed centrifugation followed by centrifugation of the filtered solution for 10 min at 3000x*g*, at 4°C, the pellet was re-suspended in 100 µl of isolation buffer. Protein concentrations in the mitochondrial fractions were determined using a Lowry assay.

Mitochondrial respiration assays were performed using a polarographic oxygen sensor (Oroboros oxygraph, OROBOROS® INSTRUMENTS**,** Innsbruck, Austria) in 0.1 mg/ml of freshly isolated mitochondria incubated in respiration medium (120 mM KCl, 5 mM KH_2PO_4, 3 mM Hepes, 1 mM EGTA, 1 mM $MgCl_2$, and 0.2% BSA, pH 7.2; [18]). Oxygen consumption rates were measured at 25° C, previously shown as the optimum temperature for such experiments [19]. As implemented by Miwa *et al*., we measured state 3 and state 4 respiration rates [18] using NAD^+-linked substrates pyruvate 5 mM/proline 5 mM to feed electrons to mitochondrial complex I, along with ADP 400 µM to elicit ADP-dependent state 3 and ADP-independent state 4 respiration once ADP is exhausted. The concentrations of substrates were chosen to achieve maximal state 3 respiration rates. Respiratory control ratio was obtained as state 3/state 4. ADP/O ratio was calculated from the amount of oxygen consumed after a 400 µM load of ADP. All assays were performed within 3 hours of mitochondrial isolation. Data was analyzed using the software *DatLab* Version 4.1.0.8.

Quantitative genetic analyses

We used two-way ANOVA to partition variation in each trait among the inbred lines according to the model, $Y = \mu + L + S + LxS + E$, where μ is the overall mean; L and S are the main effects of Line (Random) and Sex (Fixed); LxS is the random effect of sex-by-line interaction; and E is the within-vial error variance. Reduced models by sex were also run. Broad-sense heritabilities (H^2) were computed as $H^2 = (\sigma_L^2 + \sigma_{SL}^2)/(\sigma_L^2 + \sigma_{SL}^2 + \sigma_E^2)$ for the analyses pooled across sexes, where σ_L^2, σ_{SL}^2, and σ_E^2 are the among line, sex-by-line and within line variance components, respectively. H^2 values by sex were also computed as $H^2=(\sigma_L^2)/(\sigma_L^2 + \sigma_E^2)$ [20]. Cross-sex genetic correlations (r_{MF}) were also estimated as $r_{MF} = cov_{♀♂}/\sigma_{♀}\sigma_{♂}$, where $cov_{♀♂}$ is the covariance of lines means between females and males, and $\sigma_{♀}$ and $\sigma_{♂}$ are the square roots of the among line variance components for males and females. Genetic correlations between phenotypic traits were calculated as $r_{GT} = cov_{G12}/(\sigma_{G1}\sigma_{G2})$, where cov_{G12} is the covariance between traits among line means from the joint analysis, and σ_{G1} and σ_{G2} are the square roots of the variances among lines from the analyses of each trait separately. The coefficients of genetic (CV_G) and environmental (CV_E) variances were calculated as $CV_G = 100\sigma_G/\mu$ and $CV_E = 100\sigma_E/\mu$, respectively, where σ_G and σ_E are the square roots of the line and within line variance components, respectively. All statistical analyses were performed using SAS version 9.1.

Transcript-phenotype associations

Regression models ($Y= \mu+ S+ T+ SxT+\varepsilon$, where T denotes the trait covariate) were used to identify transcripts significantly associated ($P<0.01$) with variation in each

mitochondrial respiratory trait in both sexes [21]. Modules of co-expressed transcripts associated with variation in each metabolic trait were constructed using the residuals from regression models ($Y = \mu + S + E + SxE + \varepsilon$, where E is the covariate median $\log_2$ expression level) to compute the genetic correlations between transcripts significantly associated with each phenotype.

Transcriptional modules

Transcripts significantly associated with metabolic phenotypes across the 40 wild-derived inbred lines were organized into statistically correlated transcriptional modules as described previously [21]. The correlation between all pairs of significant transcripts *i* and *j* was computed and the absolute correlation values $|r_{ij}|$ were transformed to define edge weights $e^{\frac{|r_{ij}|-1}{\sigma^2}}$ in a graph of genes indexed by the free parameter σ. The clustering $P = \{V_1, \ldots, V_k\}$ and the value of σ that jointly maximize the modularity function:

$$Q(P,\sigma) = \sum_{c=1}^{k} \left[\frac{A_\sigma(V_c, V_c)}{A_\sigma(V, V)} - \left(\frac{A_\sigma(V_c, V)}{A_\sigma(V, V)} \right)^2 \right]$$

were determined, where $A_\sigma(X,Y)$ denotes the total edge weight in the graph indexed by σ that connects any vertex in set *X* to a vertex in set *Y*. The optimal partition $P = \{V_1,\ldots, V_k\}$ defines *k* transcriptional modules $V_1,\ldots,V_k$ at which the genetic correlation within a module is maximal.

RESULTS AND DISCUSSION

Natural variation in mitochondrial respiration traits

Using ANOVA, we found a significant genetic component of variance for mitochondrial state 3 and state 4 respiration rates. No significant sex or interaction effects were observed for these traits. In contrast, there was significant variation between females and males for ADP/O ratio (P =0.0012), with females displaying higher mitochondrial efficiency than males (Figure 2C). However, this difference was not constant across lines; the sex-by-line interaction term was highly significant (P <0.0001; Table 1), suggesting that the loci that control ADP/O ratio have different effects in males and females. The heritability (H^2) estimates for the mitochondrial respiration traits ranged from 14% to 43% (Figure 2A-D and Table 1). The genetic correlation coefficients across sexes among lines, r_{MF} (±SEM), were very high for mitochondrial state 3 (0.91 ± 0.07, P <0.0001), mitochondrial state 4 (0.64 ± 0.12; P <0.0001) and RCR (0.67 ± 0.12; P <0.0001), but non-significant for ADP/O ratio (-0.12 ± 0.79, P =0.21), further supporting the existence of loci with sex-specific effects affecting variation in this last trait.

Despite evidence of the existence of a genetic component underlying inter-individual variations in mitochondrial bioenergetics [22-28], ours is the first study reporting heritability estimates for mitochondrial traits. Interestingly, compared to the estimates previously reported for other energy metabolism traits (*Jumbo-Lucioni et al*, manuscript in preparation), heritability for mitochondrial bioenergetic traits, particularly mitochondrial state 3 and state 4 respiration rates, are low (H^2= 0.14-0.20). It has been

previously reported that traits with low heritability tend to be closely correlated to fitness as fitness-related traits display greater environmental sensitivity and are thus less heritable [29,30]. Evolutionary changes in fitness-related traits are a consequence of fitness response to natural selection as "the 'character' that natural selection selects for is fitness" [20]. Paradoxically, phenotypic variation in these fitness-related traits simultaneously introduces further variation in fitness. Analogously, phenotypic differences in mitochondrial bioenergetic traits have shown to impact fitness (i.e. reproduction and survival) in populations of *Drosophila* [28,31,32].

We also identified sex-specific effects on mitochondrial efficiency (i.e. ADP/O ratio) (Table 1) under pyruvate plus proline-stimulated respiration. Sexual dimorphism in energy metabolism traits is well recognized across different species [33-35]. Consistent with our findings, a previous report in *Drosophila simulans* showed higher mitochondrial efficiency for female flies under pyruvate plus proline-induced respiration [31]. Our results are also in good agreement with findings in mammals showing that female rodents possess a higher mitochondrial capacity and efficiency for substrate oxidation across several tissues [36-40]. Furthermore, clinical studies [41,42] have reported gender-specific variations for mitochondrial traits, with female sex steroids proposed as the modulators of mitochondrial biogenesis and function. Attempts to provide an explanation for the mechanisms underlying the sexual dimorphism in mitochondrial bioenergetic traits focused on the way evolution selects and optimizes certain genes for each sex. Throughout evolution, genes from the mitochondrial genome and X chromosome spend relatively more time under selection in females due to their asymmetric inheritance [43-

45] and are therefore expected to be better optimized for function in females compared to males [45]. Since females usually engage in more energetically demanding behaviors than males to attain reproductive success, it has been proposed that sexual differences may have arisen as an evolutionary adaptation to such differences in energetic demands [46].

Genetic correlations between mitochondrial respiration traits

Next, we tested whether there were significant genetic correlations between traits. There was no significant correlation between state 3 and state 4 respiration rates for both males and females (data not shown), which indicate that different loci regulate inter-individual variability in these traits. In contrast, there was a tendency for males from those lines that have higher state 4 respiration rate to have lower mitochondrial efficiency (e.g. ADP/O ratio) (Table 2C). State 4 is the respiration rate in the presence of carbon substrates (i.e. pyruvate plus proline) but in the absence of ADP and it is usually attributed to mitochondrial basal proton leak [47]. These proton leak events decrease the energy available to drive ATP synthesis thus reducing mitochondrial efficiency [48]. Our findings of a negative correlation between state 4 and mitochondrial efficiency in males but not in females may arise from differences in genetic architecture between male and females as a result of the different energetic demands between sexes.

Quantitative trait transcripts (QTTs) regulating natural variation in mitochondrial respiration traits

Previously, Ayroles *et al.* identified 10,096 genetically variable transcripts (quantitative trait transcripts or QTTs) in these lines [21]. We therefore used regression of

the mitochondrial traits on transcript abundance to identify nuclear-encoded genes that might mediate mitochondrial respiration traits. At a significance level of 0.01, we detected 293, 105, and 19 QTTs associated with variations in mitochondrial state 3, state 4, and ADP/O ratio, respectively (**Supplementary Table 4**: Analysis of modules of correlated transcripts associated with mitochondrial bioenergetic traits).

The small number of nuclear-encoded genes associated with variations in mitochondrial ADP/O ratio highlights the potential role of mitochondrial-encoded and/or nucleo-mitochondrial interacting alleles in controlling inter-individual variability in this mitochondrial trait. Analyses of the relevance of mito-nuclear interactions and mtDNA variations in mitochondrial bioenergetics have been performed at the between-population level. A previous report has demonstrated differences in various surrogate markers of mitochondrial efficiency among sympatric *D. simulans* hosting distinct mtDNA haplogroups [26]. Western hemisphere populations of *D. melanogaster* have been reported to be the least diverse with a single dominant haplotype (i.e. haplotype # 7) [49] as opposed to *D. simulans*. However, non-neutral genetic variation in mtDNA is also expected at the within-population level in *Drosophila melanogaster* as the mutation rate of this genome is generally high and the influence of natural selection (to decrease genetic variation) is reduced compared to the nuclear genome [50,51]. Likewise, within-population differences in mito-nuclear interactions are expected to evolve as mtDNA variations have shown to trigger adaptive responses from the nuclear genome. These events set the basis of mito-nuclear co-evolution [51,55]. Various experiments in mammalian [52,53] and non-mammalian [54] models highlight the role of mito-nuclear

co-adaptation in maintaining optimal respiratory chain function. Therefore, one can hypothesize that the number of genes influencing variations not only in mitochondrial efficiency but in all the mitochondrial bioenergetic traits may actually be bigger.

Transcriptional networks associated to mitochondrial bioenergetic traits

As mitochondrial abundance, morphology and function have been shown to be coordinately regulated to meet cell-specific energetic, metabolic and signaling demands, [56,57] individual variations in mitochondrial bioenergetics are expected to involve a complex process of individual-specific changes in the coordinated transcription of several genes [57]. To address this idea, we used a gene network analysis to provide insight into how variation in these QTTs can give rise to variation in mitochondrial respiration traits in our population of flies.

We identified 20 modules of correlated transcripts associated with mitochondrial state 3 respiration rate, 8 with mitochondrial state 4 and 2 with ADP/O ratio(Figure 4A-B and **Supplementary Table 4**: Analysis of modules of correlated transcripts associated with mitochondrial bioenergetic traits). To determine the biological significance of the genes in these network modules, we used gene ontology categories [58], tissue-specific expression [59], and published protein-protein interactions or shared domains.

Mitochondrial State 3 Respiration Rate. Gene ontology analysis revealed that 30% of the QTTs associated with variations in mitochondrial state 3 respiration rate are mainly enriched for genes involved in sensory perception and signal transduction (Figure 2A). These alleles are significantly clustered in modules 10, 13, 16, 18 and 20 (**Supplementary Table 5**: Over-representation of Gene Ontology Categories, KEGG

Pathways and Keywords for transcripts associated with quantitative traits). Among them, module 10 displays the highest degree of transcriptional connectivity, which highlights its essentiality across the whole network as highly connected genes have been shown to play a pivotal role "in organizing the behavior of biological networks" [60]. Indeed, as shown in Figure 5A, the expression of "hub" genes in module 10 is highly correlated to the expression of other "hub" genes throughout the network (Figure 5A).

Gene ontology analysis showed that module 10 is particularly enriched for loci linked to photoreception (**Supplementary Table 5**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits, Figure 5A), which are mainly expressed in *Drosophila* eye and head (Figure 5B). This module includes two photosensory opsins, *rhodopsin 4 (Rh4)* and *6 (Rh6). Rh6* belongs to a structurally related group of extraocular photoreceptors [61] located close to *Drosophila* compound eye and optic lobe. Similar to *Rh4, Rh6* transcription is under circadian control [62,63] as its function has been implicated in the light-mediated signaling for the entrainment of circadian rhythms and timing of photoperiodic responses independent of visual imaging [64,65]. These findings are further strengthened by anatomical evidence of axonal projections from *Rh6*-containing photoreceptors to *Drosophila* circadian pacemaker center in the brain [65].

Furthermore, *Rh6* is the *Drosophila* ortholog of mammalian melanopsin, a photopigment found in the retina which has shown to substantially mediate circadian signaling [67]. While *Rh6* ectopic expression has shown to fully restore light response in mutant flies, heterologous expression of human melanopsin has shown to similarly rescue

photoreception in mammalian neuronal cells [66,67]. Besides melanopsin, other retinal photoreceptors or photopigments have been suggested to contribute to the transduction of the photic stimuli to the circadian system. Such redundancy in photoreception has also been demonstrated in plants [68], flies [69] and other vertebrates [70]. Previous studies have suggested that melanopsin and circadian rhythm are both mediators of light signaling effects on sleep and brain activity during wakefulness [72]. Both vertebrates and invertebrates display a circadian organization of sleep and activity with similar responses to exogenous modulators [71]. Analogous to melanopsin, *Rh6* is highly correlated with genes mostly involved in sensory perception and transduction (i.e. modules 13, 16, 18 and 20; Fig. 5A) which is in good agreement with the activation of sensory systems that characterizes wakefulness [73].

Thus, light-mediated circadian signaling via such photosensory opsins coordinates the daily rhythm of organismal physiology and behavior [72,74]. Out-of-phase light exposure has been shown to alter the temporal organization of behavior in *D. melanogaster* [75,76]. Mammalian models have shown a similar response [77-80]. Interestingly, module 17 in the mitochondrial state 3 co-expression network is enriched for genes involved in circadian rhythm and behavior (Figure 5C). The behavioral component specifically includes reproduction, olfaction, locomotion, learning and memory (**Supplementary Table 5**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits).

Indeed, there is extensive evidence of circadian modulation of a wide range of organismal behaviors in *Drosophila,* such as sexual receptivity, oviposition, mating,

courtship, locomotion [76,81], olfactory learning and memory [82,83]. Long-term memory formation has shown to be clock-controlled in many invertebrate [82,84,85] and vertebrate model systems [86-88]. Similarly, a recent report has highlighted a comparable role for the central clock in short-term memory formation in *Drosophila* via axonal projections from *Drosophila* central oscillator in the brain to the vicinity of the mushroom bodies, the brain centers for associative (i.e. olfactory) learning and memory in *Drosophila* [83]. Similar to the suprachiasmatic nuclei in mammals [67,89], a group of ventral lateral neurons in the brain have been identified as the central clock regulators of behavioral rhythmicity in *Drosophila melanogaster* [69,89,90]. Interestingly, QTTs in module 17 are predominately expressed in *Drosophila* brain (Figure 5B) which further strengthens the validity of our findings. Furthermore, a similar central oscillatory control has been described for locomotion [91,92] and reproductive behaviors [93] in *Drosophila*.

However, QTTs in module 17 are not exclusively brain-specific (Figure 4B). In *Drosophila*, besides a central clock, peripheral circadian oscillators have also been identified [94]. Although previous reports have suggested diffusible hormonal signals as mediators of the central control of peripheral oscillators [95,96], there is also strong evidence of the existence of independent photoreceptive clocks throughout the fly body [97-99]. Later reports have suggested that a wide range of sexual reproductive behaviors and locomotion are controlled by multiple oscillators [76,81]. Likewise, it has been shown that olfactory behavior in insects displays circadian-dependent rhythm under the control of self-contained peripheral clocks in the olfactory receptor neurons [99,100].

Such clocks are thought to share common pathways supporting a multi-oscillatory organization of the circadian system [76].

Therefore, the body-wide distribution of circadian clocks may account for the impact of circadian regulation on almost every aspect of organismal life. Circadian clocks have been shown to govern the rhythmic expression of hundreds of transcripts that are involved in diverse cellular functions including developmental timing, physiology, and biochemistry [62,101-107]. Previous studies have shown that mRNA translation is a critical event in the light entrainment process of circadian oscillators [111]. TOR is an evolutionarily conserved serine-threonine kinase, which coordinates cell growth in accordance with nutrient quantity and quality via the activation of the cellular mRNA translation machinery [108-110]. Interestingly, this nutrient sensitive pathway has shown to be under photic regulation [111]. Brief light exposure has been shown to trigger TOR activation in mammalian species dependent on the light-induced activation of the mitogen-activated protein kinase (MAPK) signaling pathway [111]. Similar to mammals, MAPK also plays a crucial role in circadian output in *Drosophila* [112] as well as in other vertebrate models [113-119]. Although there are no reports in flies about light-induced activation of TOR pathway via MAPK signaling, there is evidence of TOR dependency on MAPK for complete activation [120].

Increasing evidence highlights TOR signaling as crucial for a wide range of critical biological functions [121-123]. Indeed, TOR has been proposed as a sensor not only of cellular energy status, but also of mitochondrial activity [124]. Interestingly, a previous mammalian report has shown that down-regulation of the TOR/S6K signaling pathway is

linked to coordinated transcriptional changes of genes involved in mitochondrial electron transport and mitochondrial biogenesis via up-regulation of peroxisome proliferator-γ coactivator-1 (*PGC1α*) [125]. Physical interaction [126], modulation of TOR activity via mitochondrial ROS production [127], modulation of mitochondrial bioenergetics (i.e. oxygen consumption, oxidative capacity, membrane potential) dependent on TOR stimulation and TOR-mediated regulation of glycolytic versus oxidative (i.e. mitochondrial) metabolism [121,128] have been suggested as potential mechanisms underlying the molecular basis linking TOR and mitochondrial function. Consistent with these observations, we have identified *RPS6-p70-protein kinase* (*RPS6K*, **Supplementary Table 4**: Analysis of modules of correlated transcripts associated with mitochondrial bioenergetic traits), one of TOR downstream molecular targets, as one of the hub genes in mitochondrial state 3 module 18 (Figure 5C). Interestingly, *RPS6K* expression highly correlates with the expression of various hub genes within the network, such as *syndecan* in module 19 (Figure 5C). *Syndecan* is a type-I transmembrane protein involved in cell-matrix adhesion, migration, neuronal development, inflammation, and feeding behavior [129,130], which are physiological processes with known impact on energy homeostasis.

Analogous to the crucial role of MAPK signaling in the light-dependent TOR activation, MAPK has also been shown to play a pivotal role in modulating other circadian output pathways. We identified downstream targets of MAPK signaling pathway in our mitochondrial state 3 co-expression networks. *Neurofibromin 1 (Nf1)*, a "hub" gene in mitochondrial state 3 module 17 (Figure 4C), is highly conserved between

humans and flies, and has been linked to the circadian control of locomotor activity [112], learning and memory [131] and overall growth in *Drosophila* [132] via a MAPK-dependent signaling pathway [112]. Furthermore, *cAMP-dependent protein kinase 1 (PKa-C1),* has been suggested to interact or be a downstream target of *Nf1* [132]. In agreement with these findings, we identified *PKa-C1* and *Nf1* as two hub genes within module 17, and our genetic network data shows that the expression of *PKa-C1* is highly correlated with *Nf1* transcript levels (Figure 5C). S*yndecan* expression is also co-regulated by expression of hub genes in module 17.

Mitochondrial State 4 Respiration Rate. Gene ontology analysis revealed that 30% of the QTTs associated with variations in mitochondrial state 4 respiration rate are mainly enriched for genes involved in reproduction and cell replication (Figure 3B), significantly clustered in modules 1 and 4 (**Supplementary Table 5**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits). Module 1 may play a key role in organizing the behavior of this mitochondrial bioenergetic network as it displays the highest degree of transcriptional connectivity within the network. It is particularly enriched for genes involved in eggshell formation. *Drosophila* eggshell is an extracellular structure laid during late oogenesis between the oocyte and the overlying follicle cells targeted towards a wide variety of functions spanning from egg fertilization to larva hatching during late embryogenesis [133]. Interestingly, a well-defined temporal organization of transcriptional, anabolic, cleavage and transport events of various proteins and molecular targets is relevant for *Drosophila* eggshell assembly. Thus, a time-dependent transcription of most of the QTTs

in module 1: *chorion protein 16* and *19*, *chorion protein a at 7F (i.e. CG33962)*, *CG13083* and *CG13084* (the latter two are putative chorion genes), has been implicated in eggshell biogenesis. In agreement with our findings, previous reports have suggested that certain stages of oogenesis are under circadian control via self-contained autonomous ovarian clocks [98].

Furthermore, several QTTs regulating variation in mitochondrial state 4 outside module 1 (i.e. *CG32397*, *longitudinals lacking*, *Usf*, *CG7408* and *nop5* in module 4; *CG30427* and *odorant binding protein 99b* in module 5; *CG13067* and *CG31304* in module 6; *no receptor potential A*, *Mec2* and *CG17124* in module 7; *huntingtin*, *CG32556*, *CG11142*, *CG9953*, *CG11357* and *Cyp4d21* in module 8) represent clock- or sleep-regulated alleles [62,73,134]. Interestingly, among these genes, *nop5* previously shown to be differentially expressed in a fly model of human insomnia [73], is a highly conserved size-control gene implicated in ribosomal biogenesis [135]. Characterization of this gene in yeast has shown that it is a transcriptional target of *Sfp1*, a downstream effector of the TOR pathway [136]. Interestingly, *slimfast*, another QTT in module 4, has also been shown to be under TOR regulation in *Drosophila* [137]. Although an ortholog of *Sfp1* has not been described in flies, TOR pathway is evolutionarily conserved in all eukaryotes [138] and has been previously shown to be under photic regulation [111]. Thus, our findings highlight the non-visual effects of light as an exogenous signal coordinating the temporal rhythms of organismal physiology in *Drosophila* and mammals [72,139,140]. In addition, the identification of genes involved in ribosomal biogenesis

suggest a key role for nutrient-sensitive cell size- and/or cell growth-controlling genes in determining variations in mitochondrial bioenergetic traits.

Huntingtin expression, a hub gene in module 8, has also been shown to be under sleep regulation [73]. *Huntingtin* encodes a protein of unknown function involved in the pathogenesis of Huntington's disease. *Huntingtin* has been previously suggested to regulate the transcription of *PGC1α*, a transcriptional coactivator that regulates mitochondrial biogenesis and respiration [141]. Such findings propose *PGC1α* as a molecular mediator of Huntington's disease with a potential role in the pathogenesis of other neurodegenerative diseases. *PGC1α* has been previously shown to be under TOR-dependent transcriptional regulation [142], which supports the relevance of this nutrient sensitive pathway as mediator of the circadian output pathways regulating organismal metabolism and energy homeostasis.

Mitochondrial ADP/O Ratio. Only two modules comprise the mitochondrial ADP/O ratio co-expression network (**Supplementary Table 4**: Analysis of modules of correlated transcripts associated with mitochondrial bioenergetic traits). Gene ontology enrichment analysis suggests that ADP/O ratio network alleles are primarily involved in muscle development, display a predominant membrane location and are mainly devoted to oxidoreductase activities (Figure 3C, **Supplementary Table 5**: Over-representation of Gene Ontology Categories, KEGG Pathways and Keywords for transcripts associated with quantitative traits).

Genetic correlations between energy metabolism and life-history traits

All forty Raleigh inbred lines were previously screened for body weight, metabolic rate and total levels of protein, triacylglycerol, glycerol, and glycogen (*Jumbo-Lucioni et al,* manuscript in preparation), as well as for several life-history traits (i.e. copulation latency, competitive fitness, starvation resistance, and chill-coma recovery) [21]. Thus, we sought to determine whether there were significant genetic correlations between mitochondrial, energy metabolism, and life-history traits. Identification of significant correlation would give us information on why genetic variation in loci mediating mitochondrial respiration is preserved in natural populations.

In the combined sex analyses, there was a tendency for those lines that have a higher mitochondrial state 3 respiration rate and coupling (i.e. respiratory control ratio= state 3/state 4) to store more glycogen, and for those lines that have a greater mitochondrial efficiency (i.e. ADP/O ratio) to have higher total proteins (Table 2A). These findings highlight the central role of mitochondrial function in organismal energy homeostasis. Highly coupled mitochondrial respiration (i.e. high respiratory control ratio, RCR) allows for a higher level of metabolic efficiency and favors energy storage [143]. Factors that adversely impact mitochondrial coupling and efficiency in eukaryotes have been shown to have a deleterious impact on energy conservation [144]. Glycogen represents the major fuel source of flight energy, which can be quickly mobilized to meet skeletal muscle metabolic requirements [145].

Several of the QTTs identified in our study that have pleiotropic effects on both mitochondrial state 3 (particularly clustered in module 20) and glycogen storage are differentially expressed in a fly model of human insomnia [73]. Sleep displays a circadian

organization in both vertebrate and invertebrate models but sleep behavioral modifications also provide timing information to the central clock [71]. A recent report has suggested that the regulation of feeding behavior and metabolism in *Drosophila* requires a complex circadian network of transcriptional events involving input signaling from central and peripheral clocks that couple to maintain whole-body energy homeostasis [146]. Disruption of this multi-level circadian regulatory system has been shown to adversely impact food consumption and glycogen storage in *Drosophila* [146]. Based on these observations and our findings, we propose that differential expression of circadian-regulated alleles (as a result of differential clock signal transduction) induces variations in mitochondrial bioenergetic function leading to concomitant changes in organismal metabolite reserves.

We further observed that those inbred lines displaying higher mitochondrial state 4 respiration rate tended to be more resistant to starvation (Table 2A). We identified *Nop5* as one of the genes with pleiotropic effects on both mitochondrial state 4 respiration rate and starvation resistance. *Nop5* has been previously shown to be differentially expressed in a fly model of human insomnia [73] and has been identified as a downstream target of the TOR pathway [136]. As previously discussed, downregulation of this nutrient sensitive pathway triggers coordinated transcriptional changes of genes involved in mitochondrial electron transport and mitochondrial biogenesis via up-regulation of mammalian *PGC1α* [125]. Up-regulation of *PGC1α* has been associated to increased mitochondrial state 4 respiration rate in muscle cells [147,148]. A homolog of *PGC-1*, C*G9809*, has been described for *D. melanogaster* [142].

Most animals face periods of food shortage and are thus expected to evolutionarily develop genetic-based physiological adaptations towards greater resistance to starvation [149]. Translational regulation allows an organism to rapidly respond to environmental cues such as food scarcity, as it regulates protein expression from existing cellular mRNAs [150]. Protein translation is highly dependent on TOR signaling as TOR activation up-regulates translation via the release of eukaryotic translation initiation factor (eIF) 4E from eIF4E-binding protein (BP)-mediated repression. Genome-wide transcriptional analysis has shown that *eIF4EBP* is up-regulated under starvation conditions [151]. Later reports have highlighted *eIF4EBP* as a key nutrient-sensitive gatekeeper of translational events [142] and provide evidence that expression of *Drosophila eIF4EBP* and its binding to eIF4E are essential for survival to starvation stress [150]. Thus, down-regulation of TOR signaling may underlie the transcriptional events leading to enhanced mitochondrial state 4 respiration rate accompanied by concomitant improvement in resistance to starvation stress via *eIF4EBP*-mediated translational repression.

Additionally, those lines with higher mitochondrial state 4 respiration rate tended to initiate copulation faster. Genes with pleiotropic effects on both mitochondrial state 4 and copulation latency are mainly clustered in state 4 co-expression network module 6 (**Supplementary Table 4**: Analysis of modules of correlated transcripts associated with mitochondrial bioenergetic traits) and involve genes differentially expressed during *Drosophila* metamorphosis (i.e. *syntaxin interacting protein 2*, [152]), and tissue morphogenesis .(i.e. *brachyenteron*, [153]). Organismal body shape and size have been

shown to have a strong genetic component and nutrient sensitive pathways such as TOR play a pivotal role during organismal development since nutrition modifies developmental timing [110]. Interestingly, inhibition of TOR signaling in the prothoracic gland, a tissue responsible for the hormonal production required for larva-to-pupa transition, prolongs larva development and increases *Drosophila* adult weight by as much as 25% [110]. Along with TOR-associated changes in *PGC1* expression (as discussed above), TOR-induced changes in body size may confer fitness advantages. Consistent with this idea, previous studies in *D. simulans* provide evidence that females mate faster with larger males under different environmental conditions [154].

Since sexual dimorphism in mitochondrial bionergetic traits is well recognized across different species [31,36-38], we then analyzed the data stratified by sexes. In females, mitochondrial respiratory coupling (i.e. respiratory control ratio) and mitochondrial efficiency (i.e. ADP/O ratio) were correlated to both glycogen and total proteins, respectively, in a similar way as in the combined sex analysis (Table 2A-B). In addition, there was a tendency for females from lines that have a higher mitochondrial state 4 respiration rate to have lower total glycerol levels (Table 2B). Glycerol is a key precursor for the synthesis of phospholipids, one of the major classes of membrane lipids in all biological membranes [155]. A decrease or absence of cardiolipin, a phospholipid exclusively located in the inner mitochondrial membrane, has a deleterious impact on mitochondrial OXPHOS through a decrease in mitochondrial coupling [156,157]. Such uncoupling effect is attributed to an increase in mitochondrial state 4 respiration rate [157].

Likewise in males, we found that those lines that have higher mitochondrial efficiency, mitochondrial state 3 and 4 respiration rates tended to have a higher resistance to food deprivation, and those that have higher oxygen consumption rates at mitochondrial state 3 and 4 tended to take longer to recover from chill-coma (Table 2C). In agreement with our findings, studies using *D. simulans* have suggested that variations in mitochondrial bioenergetics confer differential resistance to starvation and chill-coma recovery time [28,158]. Energy metabolism genes are central to temperature tolerance and climatic adaptation [56,159] as well as to resistance to nutrient deprivation [160].

CONCLUSIONS

By constructing gene co-expression networks we provided evidence that nuclear-encoded genes involved in processes regulated by photoperiod and circadian clocks are key players of the molecular network underlying phenotypic variation in mitochondrial respiration traits in a natural population of *D. melanogaster*. Moreover, our data corroborate the pivotal role of the evolutionary conserved TOR signaling pathway in mediating the effect of cycling/photoperiodic genes on mitochondrial respiration. These results add to a growing body of evidences showing a link between molecular controls of circadian rhythm and energy metabolism traits [161-166] as disruption of biological rhythms has been repeatedly linked to obesity, insulin resistance, T2DM and cardiovascular disease. Thus, differential regulation of the temporal organization of organismal physiology and behavior via differential expression of circadian-regulated genes may underlie inter-individual variations in obesity-related traits that confer

susceptibility to metabolic disease. Our findings identify such alleles which become immediate candidates for targeted pharmacological treatment.

One limitation of this study is that we specifically focused on determining the extent to which nuclear genes influence mitochondrial respiration traits. Although we identified several nuclear-encoded genes regulating variation in state 3 and state 4 mitochondrial respiration rate, it is clear from our findings that variability in mitochondrial efficiency (e.g. ADP/O ratio) among the Raleigh inbred lines may be mostly explained by variation in the mitochondrial genome and/or genetic interaction between nuclear and mitochondrial alleles (i.e. intergenomic epistasis). This limitation will soon be overcome by the completion of the sequencing of the whole mtDNA genome of the 40 Raleigh lines.

ACKNOWLEDGEMENTS

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Table 1. Quantitative genetics of organismal mitochondrial traits for 40 wild-derived inbred lines. Estimates of genetic variance for all sexes combined.

Trait [a]	Mean (±SE)	σ_L^{2} [b]	σ_{SL}^{2} [c]	σ_G^{2} [d]	σ_E^{2} [e]	σ_P^{2} [f]	H^{2} [g]	CV_G [h]	CV_E [e]
ST3 (pmol/sec/mg protein)	1848.93 (± 26.06)	39960.7****	5122.4	45083.1	178195.6	223278.7	0.20	11.48	22.83
ST4 (pmol/sec/mg protein)	237.33 (± 3.01)	484.48***	52.29	536.77	3458.8	3995.57	0.14	9.76	24.78
P/O	2.67 (± 0.01)	-0.003	0.075****	0.072	0.097	0.169	0.43	10.05	11.67
RCR	8.41 (± 0.15)	1.78****	0.33	2.11	6.95	9.06	0.23	17.27	31.35

[a] ST3: mitochondrial state 3 respiration; ST4: mitochondrial state 4 respiration; PO: ADP/O ratio; RCR: respiratory control ratio (state 3/state 4); [b] Among line variance component. [c] Sex by line interaction variance component. [d] Total genetic variance ($\sigma_L^2 + \sigma_{SL}^2$); [e] Variance within replicates. [f] Total phenotypic variance ($\sigma_G^2 + \sigma_E^2$). [g] Broad-sense heritability (σ_G^2 / σ_P^2). [h] Coefficient of genetic

variation ($100\sigma_G$ /Mean). [i] Coefficient of environmental variation ($100\sigma_E$/Mean). Asterisks indicate *P*-values: $^{*}P \leq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$.

Table 2. Genetic correlations among organismal mitochondrial, metabolism, and life history traits for 40 wild-derived inbred lines.

(A) Genetic correlations averaged across sexes. (B) Genetic correlations for female flies. (C) Genetic correlations for male flies.

A

Trait[a]	GLY	PRO	SR[b]	CL[b]
ST3	**0.35±0.15***	-0.29±0.15	0.24±0.15	-0.08±0.16
ST4	-0.10±0.16	-0.12±0.16	**0.40±0.15***	**-0.33±0.15***
PO	-0.09±0.16	**0.70±0.11******	0.18±0.16	0.17±0.16
RCR	**0.35±0.15***	-0.16±0.16	-0.06±0.16	0.08±0.16

B

Trait	GLY	PRO	GLYC	FT[b]	CL[b]	CC[b]
ST3	**0.64±0.12******	**-0.46±0.14****	-0.04±0.16	-0.01±0.16	-0.11±0.16	0.02±0.16
ST4	-0.03±0.16	-0.30±0.15	**-0.46±0.14****	-0.05±0.16	**-0.43±0.14****	-0.10±0.16
PO	-0.13±0.16	**0.93±0.06******	0.13±0.16	**-0.32±0.15***	0.24±0.15	**0.33±0.15***
RCR	**0.45±0.14****	-0.13±0.16	0.24±0.15	0.07±0.16	0.07±0.16	-0.06±0.16

C

Trait	SR[b]	CC[b]	PO
ST3	**0.31±0.15***	**0.32±0.15***	-0.20±0.15
ST4	**0.63±0.12******	**0.34±0.15***	**-0.40±0.14***
PO	**0.37±0.15***	0.05±0.16	**-**

[a] ST3: mitochondrial state 3 respiration; ST4: mitochondrial state 4 respiration; PO: ADP/O ratio; RCR: respiratory control ratio (state 3/state 4); GLY: glycogen storage; GLYC: total glycerol; CL: copulation latency; FT: competitive fitness; SR: starvation stress resistance. [b] Data from *Ayroles et al*, Nature Genetics 2009. Asterisks indicate *P*-values: $^{*}P \leq 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$

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Figure 1. Mitochondrial electron transport chain showing the five respiratory complexes and the proton leak across the inner mitochondrial membrane (adapted from *Zeviani and Lamantea, Science and Medicine 2005*).

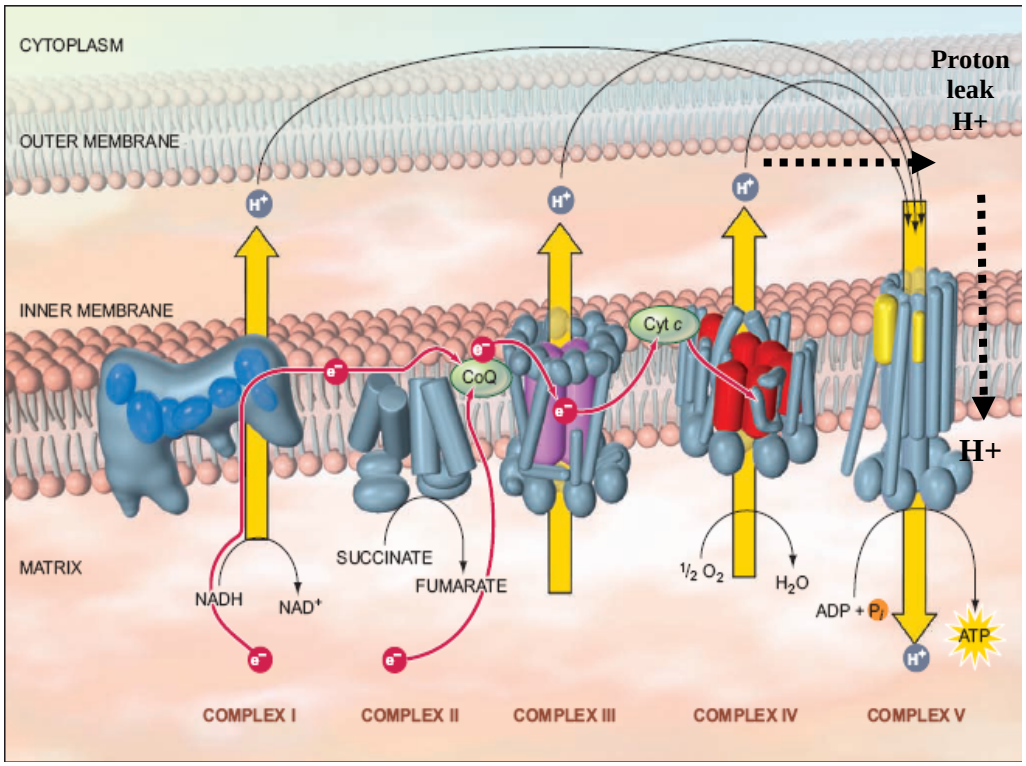

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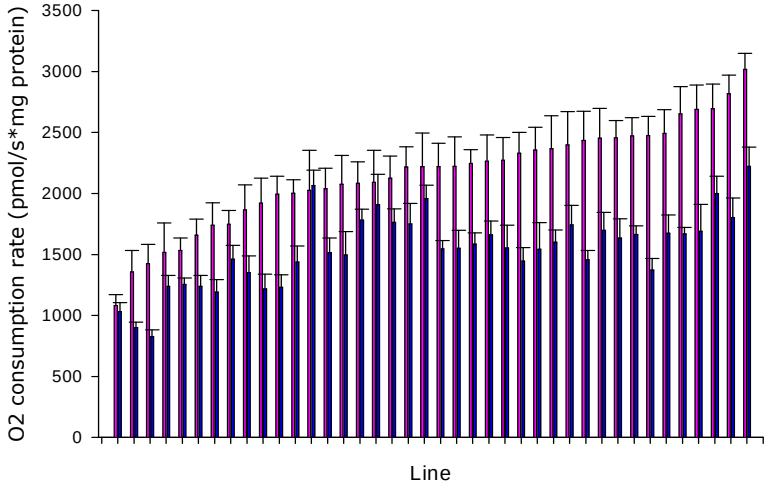
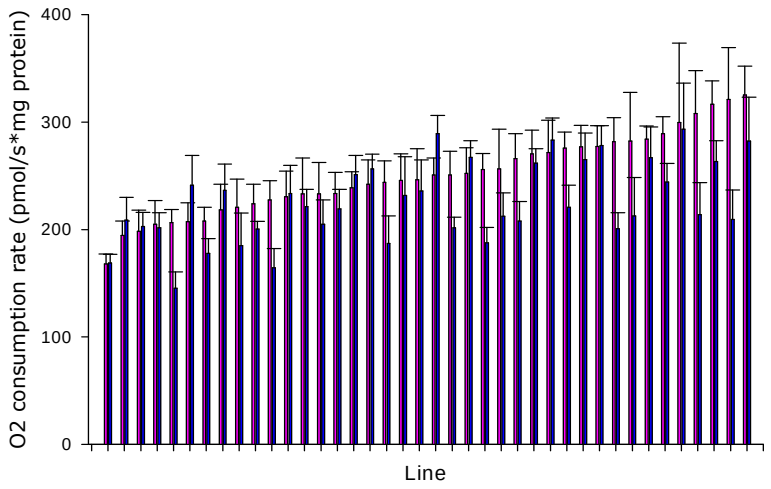
Figure 2. **Variation in organismal mitochondrial bioenergetic traits among 40 wild-derived inbred lines.** Distribution of line means for mitochondrial state 3 (Panel A) and state 4 (Panel B) respiration rates in pmol/second/mg protein, ADP/O (Panel C), respiratory control (Panel D) ratios for females (pink bars) and males (blue bars). Data represent means ± SEM for $n = 7$ independent replicates.

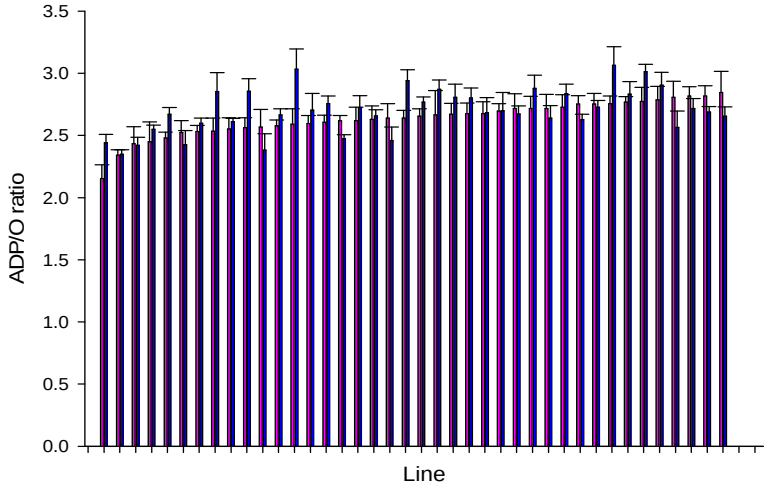
A


O2 consumption rate (pmol/s*mg protein)
3500
3000
2500
2000
1500
1000
500
0
Line

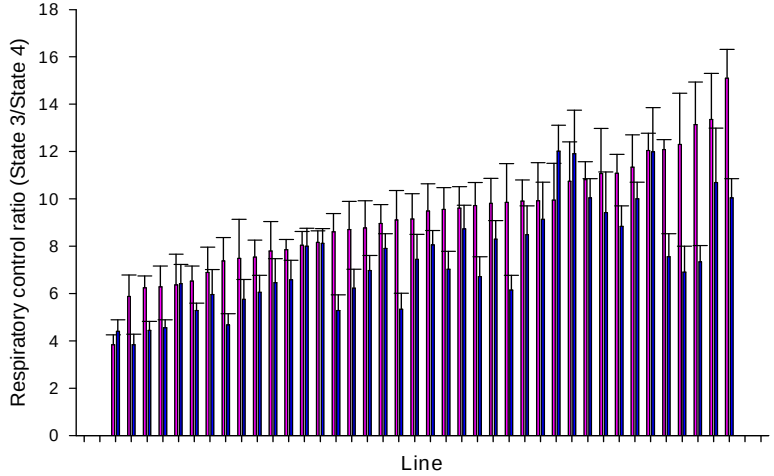
B


O2 consumption rate (pmol/s*mg protein)
400
300
200
100
0
Line

C


ADP/O ratio
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Line

D

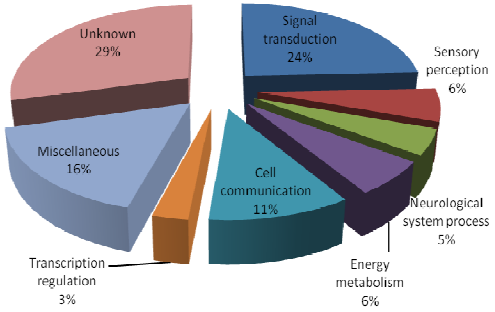

Respiratory control ratio (State 3/State 4)
18
16
14
12
10
8
6
4
2
0
Line

.

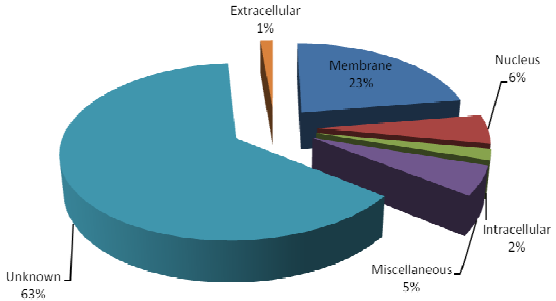
Figure 3. Gene ontology enrichment analysis of co-expression genetic networks. Gene ontology biological processes, cellular components and molecular functions for mitochondrial state 3 respiration rate (Panel A), mitochondrial state 4 respiration rate (Panel B), and mitochondrial ADP/O ratio (Panel C). According to the Gene Ontology Consortium website (http://www.geneontology.org/index.shtml): a biological process represents a series of events (not equivalent to a pathway) accomplished by one or more ordered assemblies of molecular functions; a cellular component is a component of a cell, (i.e. a part of some larger structure) which may be an anatomical structure (e.g. rough endoplasmic reticulum or nucleus) or a gene product group (e.g. ribosome, proteasome or a protein dimer), and molecular function describes activities, such as catalytic or binding activities, occurring at the molecular level.

A

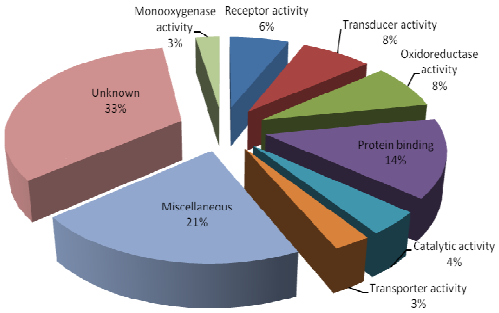
Biological processes



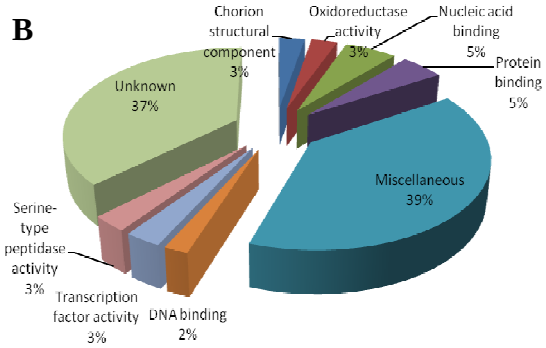
Cellular components

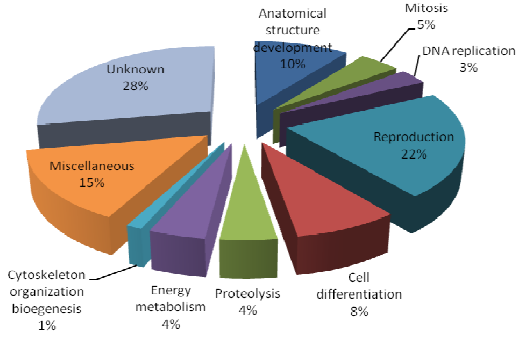


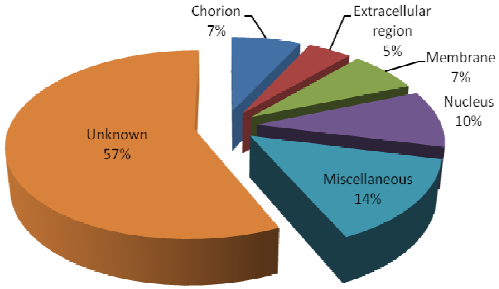
Molecular Functions



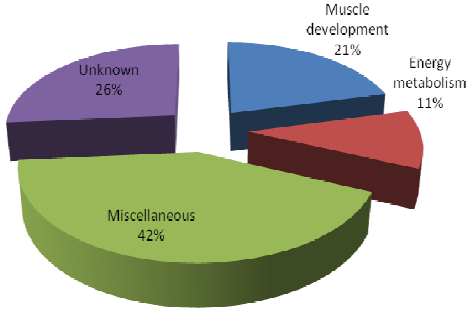
B

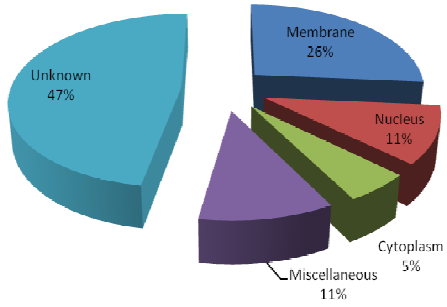






C





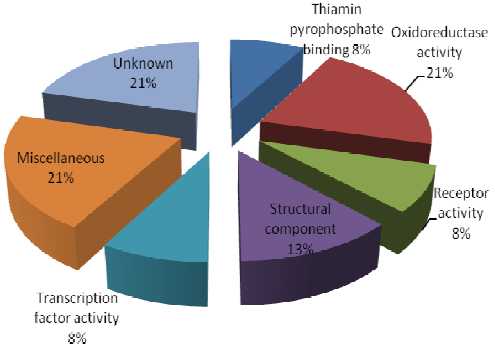


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Figure 4. Genetic networks underlying variation in mitochondrial bioenergetic traits. (A) Clustering of the 293 transcripts significantly associated with variation in mitochondrial state 3 respiration rate into 20 modules. (B) Clustering of the 105 transcripts significantly associated with variation in mitochondrial state 4 respiration rate into 8 modules. Clusterings are represented as heatmaps with a color scale spanning from red (positive correlation), green (no correlation) to blue (negative correlation).

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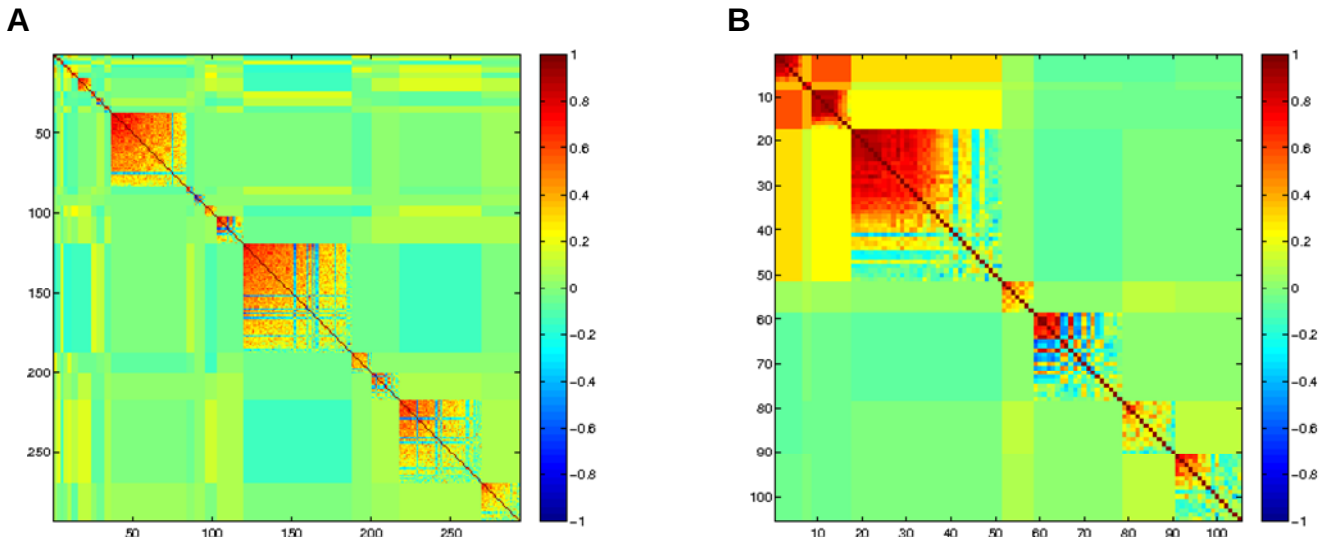
A
B

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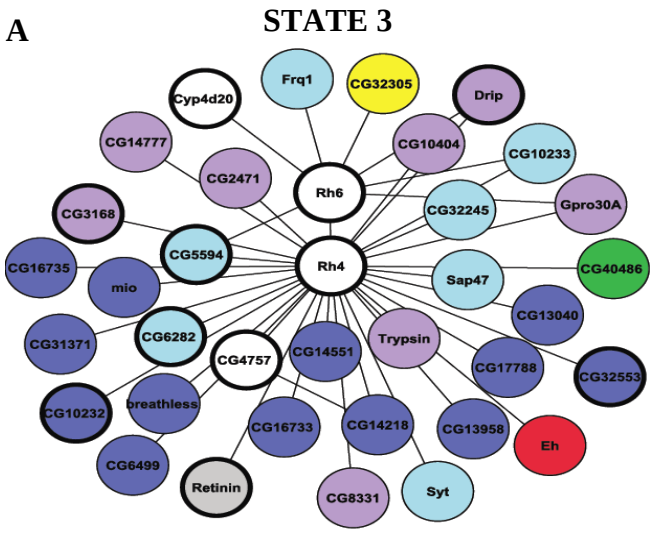
Figure 5. Genetic networks underlying variation in mitochondrial state 3 and 4 respiration rates. (A) Interaction network ($|r| \geq 0.5$) for mitochondrial state 3 module 10 enriched for genes involved in photoreception. Each node represents a gene and each edge a significant correlation between a pair of genes. Module 10 "hub" genes (white) highly interact with other "hub" genes in module 7 (green), 8 (red), 11 (yellow), 15 (gray), 16 (blue), 19 (purple), 20 (light-blue). Nodes shown as bold represent those genes previously identified as circadian- or sleep-regulated [62,73]. (B) Tissue-specific expression of transcripts comprising module 10 and 17 in the mitochondrial state 3 co-expression network. (C) Interaction network ($|r| \geq 0.6$) for mitochondrial state 3 module 17 enriched for genes involved in circadian rhythm and organismal behavior. Each node represents a gene and each edge a significant correlation between a pair of genes. Module 17 hub genes (white) highly interact with other hub genes in module 5 (orange), 6 (pink), 7 (green), 9 (red), 11 (yellow), 12 (brown), 14 (dark green), 16 (blue), 18 (gray), 19 (purple), 20 (light-blue). Nodes shown as bold represent those genes previously identified as circadian- or sleep-regulated [62,73]. (D) Interaction network ($|r| \geq 0.5$) for mitochondrial state 4 module 4. Each node represents a gene and each edge a significant correlation between a pair of genes. Nodes shown as white represent the hub genes from module 4. Module 4 hub genes highly interact with other hub genes in module 1 (blue), 2 (green), 3 (yellow), 6 (red) and 7 (light-blue). Nodes shown as bold represent those genes previously identified as circadian- or sleep-regulated [62,73].


A
STATE 3
Frq1
CG32305
Drip
Cyp4d20
CG14777
CG10404
CG10233
CG2471
Rh6
CG32245
Gpro30A
CG3168
CG5594
Rh4
CG16735
mio
Sap47
CG40486
CG13040
CG31371
CG6282
CG4757
CG14551
Trypsin
CG17788
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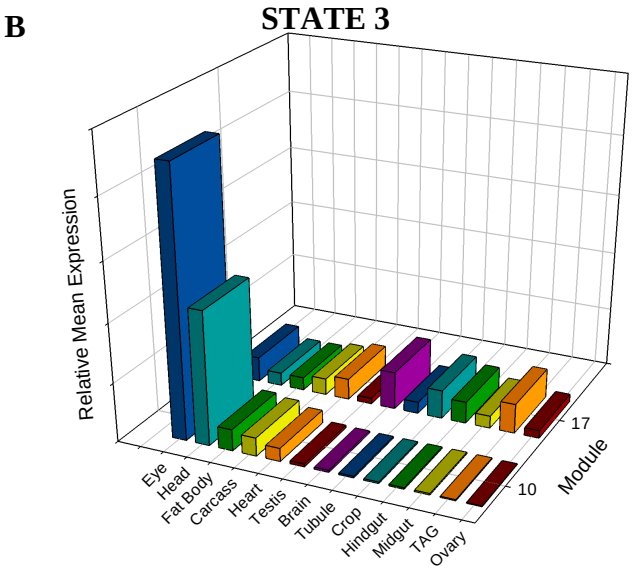
CG14218
CG13958
Eh
CG6499
Retinin
CG8331
Syt


B
STATE 3
Relative Mean Expression
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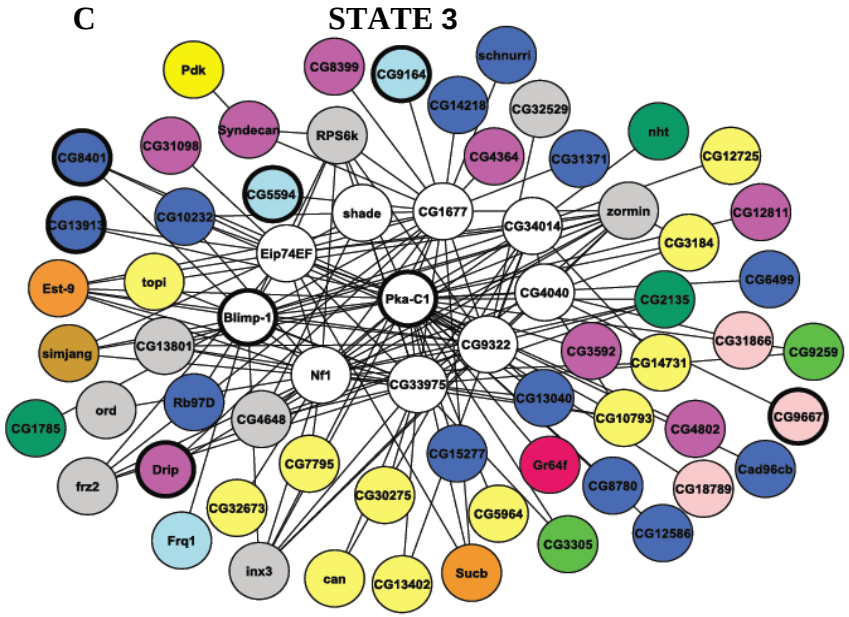
Head
Fat Body
Carcass
Heart
Testis
Brain
Tubule
Crop
Hindgut
Midgut
TAG
Ovary
10
17
Module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STATE 3
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CG9164
schnurri
CG14218
CG32529
Syndecan
RPS6k
nht
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CG12725
CG8401
CG5594
shade
CG1677
CG34014
zormin
CG12811
CG13913
CG10232
Eip74EF
CG3184
Est-9
topi
Pka-C1
CG4040
CG2135
CG6499
Blimp-1
simjang
CG13801
CG9322
CG3592
CG14731
CG31866
CG9259
Nf1
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CG4802
CG9667
CG1785
CG4648
CG15277
Gr64f
Cad96cb
frz2
Drip
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CG32673
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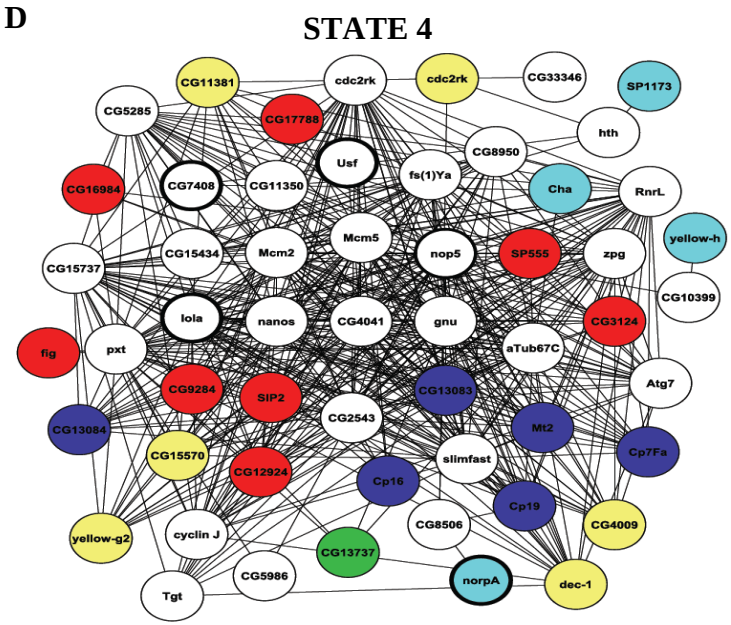
Frq1
CG3305
CG12586
inx3
can
CG13402
Sucb


D
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cdc2rk
CG33346
SP1173
CG5285
CG17788
hth
Usf
CG8950
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CG7408
CG11350
fs(1)Ya
Cha
RnrL
yellow-h
CG15434
Mcm2
Mcm5
nop5
SP555
zpg
CG15737
CG10399
lola
nanos
CG4041
gnu
CG3124
fig
pxt
aTub67C
Atg7
CG9284
SIP2
CG13083
CG13084
CG2543
Mt2
Cp7Fa
CG15570
CG12924
slimfast
Cp16
Cp19
CG4009
yellow-g2
cyclin J
CG8506
CG13737
CG5986
norpA
dec-1
Tgt

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CHAPTER IV:

A ROLE FOR *DROSOPHILA SYNDECAN* IN THE REGULATION OF WHOLE-BODY ENERGY METABOLISM AND SLEEP

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ABSTRACT

Syndecans are a family of type-I transmembrane proteins involved in cell-matrix adhesion, migration, neuronal development, inflammation, and feeding behavior. Our recent analysis of gene co-expression networks was the first to identify *Drosophila syndecan* (*dSdc)* as one of the quantitative trait transcripts regulating mitochondrial state 3 respiration rate among a highly interactive network of cycling/photoperiodic genes. Previous reports have linked *syndecan* expression to circadian rhythm in mammals and recent evidence has suggested a role for *dSdc* in sleep, a circadian-regulated behavior. Thus, we sought to independently verify the effect of the *dSdc* gene on mitochondrial respiration and sleep by measuring these traits in flies that were homozygous for the insertional mutation $Sdc^{BG02774}$ and non-mutant flies from the co-isogenic control line. As predicted, flies homozygous for the *dSdc* mutation displayed significantly lower mitochondrial ADP-stimulated (state 3) respiration, with no effect on mitochondrial ADP-independent (state 4) respiration. They also slept longer compared with homozygous wild-type flies. Moreover, real time-quantitative PCR (RT-qPCR) experiments showed a significant reduction in the mRNA levels of the *Drosophila* homolog of *PGC-1* (*CG9809*), an important co-activator of mitochondrial biogenesis and function, in $Sdc^{BG02774}$ flies compared to controls. These results strongly confirm a central role for a member of the syndecan family in the control of mitochondrial respiration and sleep in *Drosophila*.

INTRODUCTION

The *Drosophila syndecan* (*dSdc*) gene encodes a member of the *syndecan (SDC)* gene family [1], a group of type-I transmembrane proteins present on the surface of all adherent cells. While *Drosophila* appears to have only one syndecan protein ubiquitously distributed [2], mammals have four syndecan proteins encoded by four separate genes. Three of these genes, *SDC1*, *SDC2*, and *SDC3*, are expressed in a tissue-specific manner, whereas the fourth, *SDC4*, is expressed in a variety of cell types [3]. Despite apparent duplication and divergent evolution of *Drosophila* single gene to four distinct genes in mammals, syndecan protein structure is evolutionarily conserved. It comprises a core protein composed of an extracellular domain (ectodomain), a single hydrophobic membrane-spanning domain, and a short intracellular domain. The ectodomain provides attachment sites for heparan sulphate polysaccharide chains that mediate interactions with extracellular matrix (ECM) components [4], heparan-sulfate growth factors [5], cell adhesion molecules [6], lipases [7], chemokines, cytokines and their receptors [8], and pathogens [9]. As a result, syndecans function as co-receptors modulating signal transduction pathways initiated by growth factors and are involved in cell proliferation, adhesion and migration, lipid metabolism, and inflammation [3]. Syndecans have also been shown to play a key role in signal transduction from the ECM to the intracellular space interacting with cytoplasmic proteins via their intracellular domains. This allows them to control focal adhesion, cell spreading, and cytoskeletal organization [3].

To gain insights into the genetic basis of natural variation in mitochondrial respiration traits, we previously used a system genetics approach to identify gene-expression networks underlying variation in mitochondrial state 3 respiration, state 4 respiration, and mitochondrial efficiency (ADP/O ratio) using 40 wild-type inbred lines of *D. melanogaster* (see **Chapter III**: Systems Genetics Analyses of Mitochondrial Bioenergetic Traits in *Drosophila melanogaster*). This study identified *Drosophila Sdc* (*dSdc)* as one of the quantitative transcript traits regulating mitochondrial state 3 respiration rate among a highly interactive network of circadian-regulated genes. A previous report in rodent models described *SDC2* also as a circadian-regulated gene [10]. Moreover, Harbison *et al*. [11] recently reported a significant correlation between genetic variants in the *dSdc* gene and day sleep, a complex trait that displays a circadian organization in both vertebrate and invertebrate model systems [12], in a natural population of *D. melanogaster*.

Attempts to link *SDC* to organismal energy balance are exemplified by previous transgenic and knockout studies in rodent models that have demonstrated a role for syndecans in feeding behavior [13]. Ectopic over-expression of *SDC1* in the mouse hypothalamus leads to hyperphagia and increased levels of leptin, insulin, and glucose [14]. Similarly, genetic disruption of *SDC3*, endogenously expressed in the mouse hypothalamus, leads to decreased sensitivity to food deprivation [15]. Similar to sleep, feeding rhythms are under the control of the coupling action of central and peripheral clocks in metabolic tissues [16]. However, ours is the first study linking differential expression of *SDC* to natural variations in mitochondrial function. Such variations may underlie the predisposition to metabolic diseases such as obesity, insulin resistance and

type 2 diabetes mellitus which underscores the role of *SDC* as a candidate gene in metabolic disorders.

To independently verify the effect of the *dSdc* gene on mitochondrial respiration rate and sleep, in the present study, we measured these traits in flies homozygous for a hypomorphic mutation of *dSdc* ($Sdc^{BG02774}$) and flies homozygous for a wild-type *dSdc* allele (but otherwise genetically identical to the $Sdc^{BG02774}$ flies).

MATERIALS AND METHODS

Fly stocks

The *Sdc*^{BG02774} lines were established by the Berkeley *Drosophila* Gene Disruption (BDGD) Project via *P*-element insertion into the second intron of the *dSdc* gene in the *w*^{1118}*;Canton S* strain [17]. To control for larval density, we allowed the parents of the experimental flies to mate for 3 hours to generate egg collections on apple juice/agar medium in laying plates. After 24 hours, we picked groups of 100 first-instar larvae from the surface of the medium and put them into replicate vials. To minimize the influence of genetic variation in reproduction on energy metabolism, we performed all the phenotypic assays on virgin flies that were randomly collected from the replicate vials for each line on days 10 to 16 under brief CO_2 exposure. We reared flies in vials containing 10 ml of standard cornmeal, agar, sugar, and yeast medium at a constant temperature of 25°C, 60–75% relative humidity, and a 12-hr light-dark cycle.

Mitochondrial respiration rate

After removing wings and legs, we placed 20 live flies into 200μl of ice-cold isolation buffer [250 mM sucrose, 5 mM Tris-HCl, 2 mM EGTA, 1% (w/v) bovine serum albumin (BSA), pH 7.4 at 4°C] supplemented with protease inhibitors (leupeptin 1mg/ml, aprotinin 1mg/ml and pepstatin 1mg/ml) in a 1.5 ml Eppendorf tube. The samples were pounded gently 126 times over a 2 minute period, using a motorized micromortar, and filtered through a 5 μm nylon mesh. We then increased the volume to 400 μl by washing the nylon membrane with additional isolation buffer. After centrifugation of the filtered solution for 10 min at 3000 g, at 4°C, the pellet was re-

suspended in 100μl of isolation buffer. Protein concentrations in the mitochondrial fractions were determined using a Lowry assay. We performed mitochondrial respiration assays using freshly isolated mitochondrial fraction (0.1 mg/ml) by measuring oxygen consumption in a two-chamber polarographic oxygen sensor (Oroboros oxygraph, OROBOROS® INSTRUMENTS**,** Innsbruck, Austria). We measured state 3 and state 4 respiration rates using NAD^+-linked substrates, pyruvate 5 mM/proline 5 mM to feed electrons to mitochondrial complex I, along with ADP 400 □M to elicit ADP-dependent state 3 and ADP-independent state 4 respiration once ADP is exhausted. The concentrations of substrates were chosen to achieve maximal state 3 respiration rates. Respiratory control ratio was obtained as state 3/state 4. All assays were performed within 3 hours of mitochondrial isolation. Data was analyzed using the software *DatLab* Version 4.1.0.8.

Sleep and waking activity

These experiments were performed under similar laboratory conditions at North Carolina State University, NC. Adult virgins were maintained at 30 flies to a single-sex vial to ensure that each line was exposed to identical levels of social interaction [18] and had equal access to food. Sleep parameters for each fly were measured with the *Drosophila* Activity Monitoring System (Trikinetics, Waltham, MA), which counts the number of times a given fly crosses an infrared beam during a specified time interval. Here, we used one-minute intervals to record activity counts. Seven continuous days of sleep and activity were recorded for each experimental block. Sleep was defined as any period 5 minutes or longer without an activity count [19]. An in-house C^{++} program was used to calculate duration of sleep in minutes, numbers of sleep bouts, average sleep bout

duration in minutes, and the number of activity counts per waking minute (waking activity).

Quantitative RT-PCR

Total RNA was isolated using the TriPure RNA isolation kit (Roche). Isolated RNA was then used to make cDNA, using the First Strand Synthesis kit (Invitrogen^{TM}, CA, US). We performed RT-qPCR using a Syber Green Master mix and 50 ng total of cDNA per reaction and run in a Stratagene Mx3000P® qPCR machine. *Drosophila ribosomal protein 49* (*rp49*) mRNA levels were used to normalized *dSdc* and *CG9809* mRNA data.

RESULTS

Homozygous $Sdc^{BG02774}$ flies have reduced mitochondrial function

To verify the effect of the *dSdc* gene on mitochondrial bioenergetics, we used a viable mutant allele of the gene, $Sdc^{BG02774}$. $Sdc^{BG02774}$ is a mutant generated in the w^{1118};*Canton S (B)* [*CS (B)*] background strain by the insertion of a *p[GT1]*-element in the second intron of *dSdc* (Figure 1A). We examined the effects of this *P*-element insertion on *dSdc* transcription in adult flies by performing RT-qPCR experiments using RNA isolated from three body parts (head, thorax, and abdomen). We found that the overall expression of *dSdc* is significantly reduced in the three body parts of $Sdc^{BG02774}$ flies (Figure 1B).

We next assessed respiration rates of $Sdc^{BG02774}$ mitochondria and *CS (B)* mitochondria during metabolism of the NADH-linked complex I substrates, pyruvate and proline. Because no differences were observed between male and female flies in respiration rates, we pooled male and female data for statistical analysis. We found that the ADP-dependent state 3 respiration rate was reduced by approximately 15% in $Sdc^{BG02774}$ compared with the controls (Figure 2A). No difference was observed in ADP-independent state 4 respiration rate (Figure 2B). Thus, these data indicate that *dSdc* is essential for normal mitochondrial function and energy metabolism, which is in good agreement with our previous data proposing *dSdc* as a candidate gene regulating variations in mitochondrial state 3, but not state 4 respiration rates (see **Chapter III**: Systems Genetics Analyses of Mitochondrial Bioenergetic Traits in *Drosophila melanogaster*).

In mammals, PGC-1α and PGC-1β play a pivotal role in the control of energy homeostasis [20,21]. In addition, they play an essential role regulating mitochondria physiology and biogenesis [22] which may then become potential routes by which mitochondrial function is regulated. We therefore compared the transcript levels of the *Drosophila* homolog of *PGC-1* gene, *CG9809* [23], in the whole-body of $Sdc^{BG02774}$ and control flies. As predicted, we found that mRNA levels of *CG9809* were reduced by 36% in $Sdc^{BG02774}$ female flies and by 65% in $Sdc^{BG02774}$ flies relative to controls (Figure 2C).

dSdc plays a role in sleep behavior

We next assessed the effect of the *dSdc* mutation on a circadian-regulated behavior, such as sleep as it was identified as one of the quantitative trait transcripts regulating mitochondrial state 3 respiration among a highly interactive network of cycling genes. Therefore, we investigated the sleep-wake cycle in $Sdc^{BG02774}$ and *CS (B)* flies over the course of a 24 h day. We found that sleep was increased by 32% during night-time and by 76% during daytime in $Sdc^{BG02774}$ female flies compared to controls (Figure 3A). A 17% increase in daytime sleep was also observed in males (Figure 3B). This increased sleep length is due largely to increases in the average duration of sleep bouts rather than their number (data not shown).

DISCUSSION

In this study, we confirmed the role of *dSdc* as a candidate gene regulating variations in pyruvate plus proline-induced state 3 respiration rate. Our system genetics analyses of mitochondrial bioenergetic traits in 40 inbred lines of *D melanogaster* (*Jumbo et al*, manuscript in preparation; see **Chapter III**) provide key insights into the potential mechanisms underlying our findings. Indeed, our previous analysis showed that the expression of the *dSdc*, among various other genes, is highly correlated to *RPS6-p70-protein kinase* transcript abundance (Figure 4), a downstream effector of the target of rapamycin (TOR) signaling pathway. Activation of TOR signaling pathway in mammals is associated with transcriptional repression of PGC-1α and mitochondrial dysfunction [24]. PGC-1α is the master regulator of mitochondrial biogenesis and function. Consistent with these observations, we found that the $Sdc^{BG02774}$ flies were also characterized by a reduced expression of the *Drosophila* homolog of *PGC-1* (*CG9809*).

Further analysis of the mitochondrial state 3 co-regulated transcriptional network revealed other potential molecular mechanisms underlying *dSdc*-mediated regulation of mitochondrial function. Indeed, we found a high correlation between *dSdc* and *pyruvate dehydrogenase kinase* (*Pdk*) transcript levels (Figure 4; **Supplementary Table 4**). As in mammals, *Pdk* plays a critical role in the regulation of oxidative glucose metabolism in *Drosophila* [25]. *Pdk* phosphorylates and inhibits pyruvate dehydrogenase. This inhibition leads to suppression of glucose oxidation [26]. Thus, variations in the metabolism of pyruvate, the respiratory substrate used in our *ex-vivo* mitochondrial respiration experiments, may affect the availability of reducing equivalents feeding into

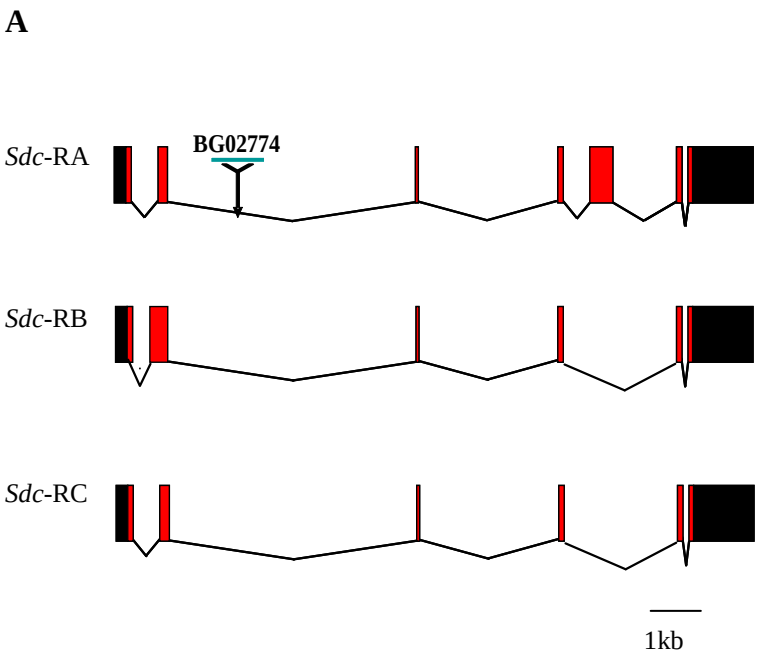
the mitochondrial electron transport chain. In this context, it will be important to elucidate the transcriptional co-expression networks associated to variations in mitochondrial bioenergetics using other physiological substrates such as α-glycerophosphate. This substrate has been shown to be rapidly oxidizable, to elicit extremely high respiratory and phosphorylative activities in *Drosophila* mitochondria [27], and to have no dependence on *Pdk* for its metabolism.

If there is previous independent evidence suggesting that *SDC* expression is under circadian regulation [10] and is linked to sleep regulation [28], we tested sleep behavior in our *dSdc* mutants. Here, we corroborated that *dSdc* affects sleep, confirming the finding of a recent study that reported significant correlations between two genetic variants in the *dSdc* gene and day sleep in 40 wild-derived *Drosophila* lines [28]. Although sleep displays a circadian organization, sleep behavioral modifications have been shown to provide timing information to the central clock [29]. Misalignment among central and peripheral clocks derived from sleep disorders may underlie the deleterious impact of jetlag and shift work on organismal health. Disruption of biological rhythms associated to shift work has been repeatedly linked to obesity, insulin resistance and cardiovascular disease [30-36]. *Drosophila* replicates clinical findings in humans. Indeed, a fly model of human insomnia exhibits increased adiposity accompanied by a differential expression of genes involved in lipid metabolism [37]. Previous microarray analyses of fly heads revealed three genes with predicted functions in lipid metabolism that increased expression during sleep [38]. Furthermore, *P*-element insertions in metabolic pathway genes impacted sleep duration and bout number [39]. Like these recent studies, our results demonstrate a molecular link between energy metabolism and

sleep. Though the nature of that link has yet to be elucidated, we speculate that the increased sleep in $Sdc^{BG02774}$ mutants in combination with reduced mitochondrial respiration may be indicative of a strategy to conserve energy, an idea long postulated as a possible function of sleep [40].

Attempts to provide an explanation to our findings may derive from a previous study by Reizes et al. [41] who recently suggested that the involvement of *SDC* in feeding behavior, another circadian-regulated trait, may be linked to the role of syndecans in neuronal development and synaptic organization in the hypothalamus. Since *dSdc* has been reported to participate in normal axon guidance and neuronal development via regulation of the Slit/Robo signaling [42], it is possible that improper wiring of the central nervous system might be responsible for sleep disorders (and differences in feeding behavior) in the *dSdc* mutant as a result of the disruption of the circadian output pathways. Even though this hypothesis needs to be tested in future studies, evidence supports the existence of high signaling trafficking between central and peripheral oscillators [43]. Such multi-oscillatory organization may have been evolved to an even more complex circadian system in mammals, which underscores the relevance of studying neuronal development and organization in the control of behavior and physiology in *Drosophila*.

Figure 1. ***Drosophila syndecan*** **gene and** ***Sdc^{BG02774}***. (A) Schematic representation of *dSdc* gene region on the second chromosome at cytological position 57E1-57E6. The *dSdc* gene contains seven exons, which generate three alternatively spliced transcripts: *Sdc*-RA, *Sdc*-RB, and *Sdc*-RC (NCBI accession no. AE013599.4). The isoform, *Sdc*-RA, has a unique exon located between exon four and five of the isoforms *Sdc*-RB and *Sdc*-RC. Moreover, *Sdc*-RA differs in the length of its first intron and second exon compared to *Sdc*-RB. Exons of the *dSdc* gene are represented by red boxes. Untranslated regions are represented by black boxes. The location of the *p[GT1]* insertion site that creates the *Sdc^{BG02774}* mutation is indicated with an arrowhead. (B) Insertion of *p[GT1]*-element in the *dSdc* of the *CS (B)* strain (*Sdc^{BG02774}*) results in a significant reduction of the expression of *dSdc* gene. *dSdc* mRNA levels analyzed by RT-qPCR on cDNA (n = 6) using primers that encompass a common region of alternative transcripts. RNAs were extracted from three body parts of homozygous *Sdc^{BG02774}* and control, *CS (B)*, flies. Levels of *dSdc* mRNA were normalized to *Drosophila ribosomal protein49* (*rp49*) mRNA levels. Statistical significance was determined by two-tailed Student's *t* test with unequal variance. In all panels, error bars represent SEM. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.


A
Sdc-RA
BG02774
Sdc-RB
Sdc-RC
1kb

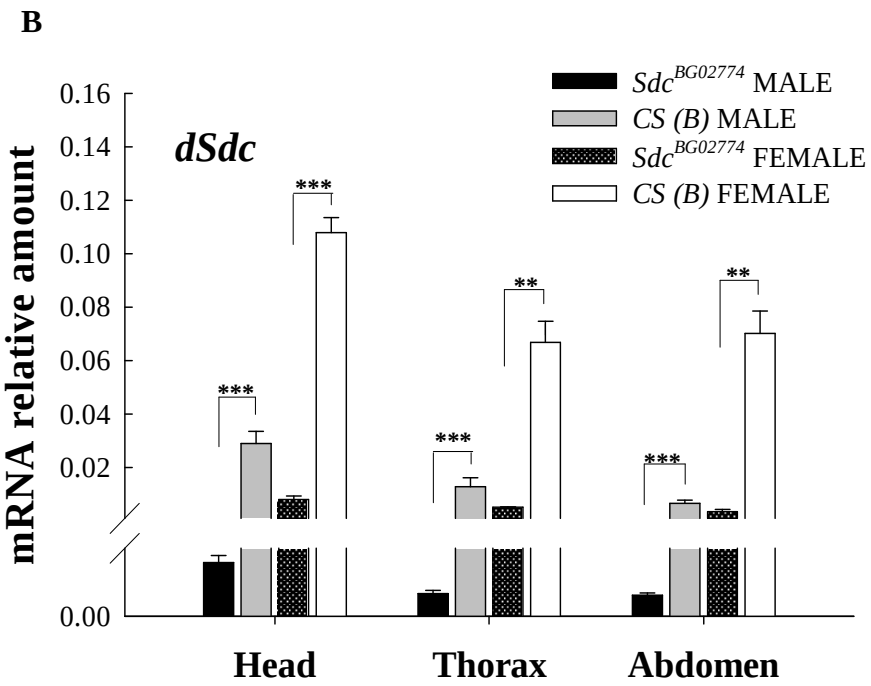

B
dSdc
mRNA relative amount
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Sdc^BG02774 MALE
CS (B) MALE
Sdc^BG02774 FEMALE
CS (B) FEMALE
***
***
***
**
***
**
Head
Thorax
Abdomen

Figure 2. Mitochondrial respiration rates and *CG9809* mRNA levels in whole body of $Sdc^{BG02774}$ and *CS (B)* flies. (A and B) Whole-fly mitochondrial respiration rates were assayed by measuring oxygen consumption rate in a polarographic oxygen sensor. State 3 and 4 respiration rates were measured with NADH-linked substrates, a mixture of pyruvate 5mM/proline 5mM. Values represent average of female and male pooled data of twenty independent replicates. Statistical significance was determined by two-tailed Student's *t* test with unequal variance. In all panels, error bars represent SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (C) Gene expression levels were measured by RT-qPCR on cDNA produced using mRNA extracted from the whole body of $Sdc^{BG02774}$ and *CS (B)* flies ($n = 6$). CG9809 mRNA levels were normalized to *rp49*. Statistical significance was determined by two-tailed Student's *t* test with unequal variance.

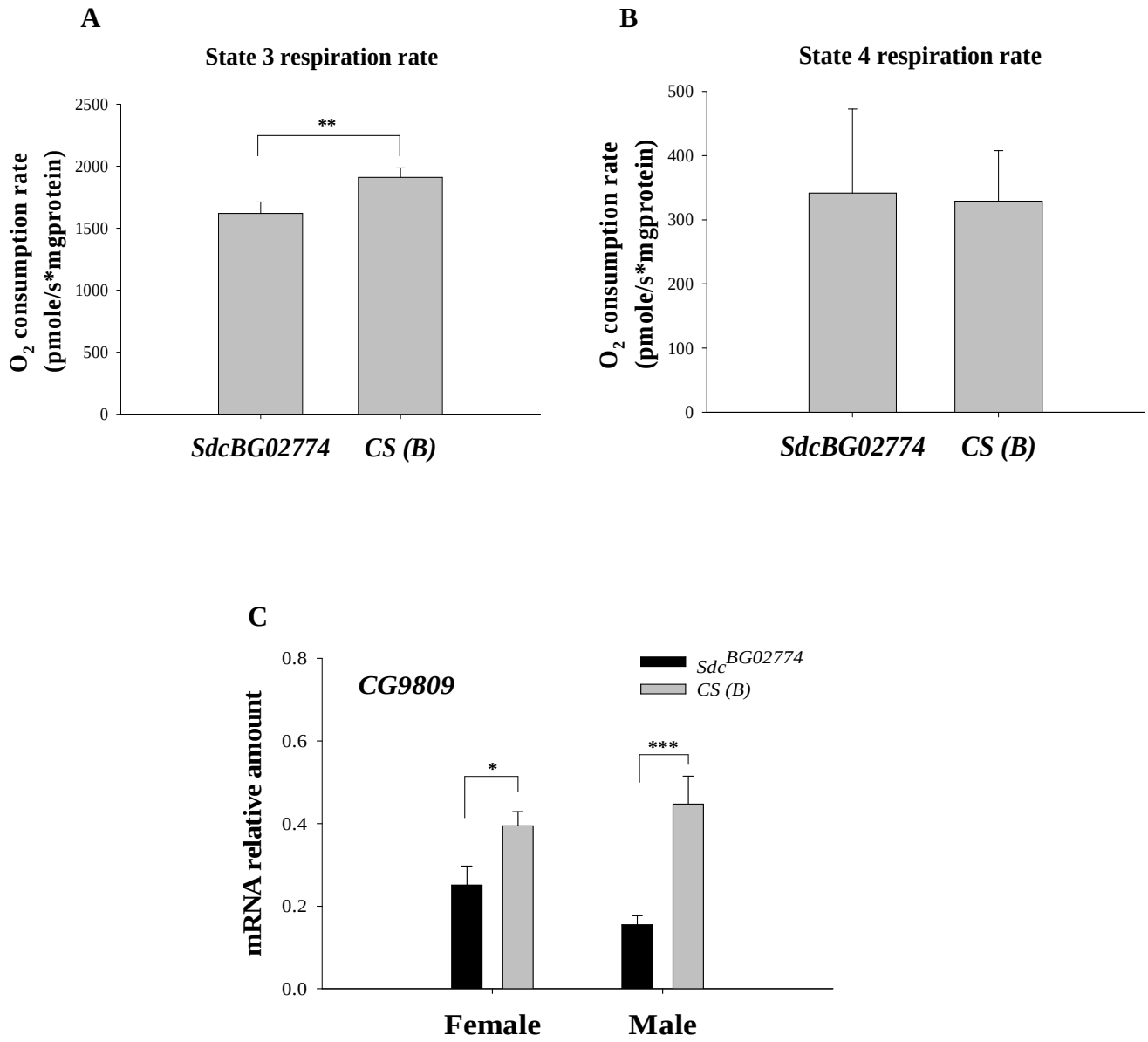

A
State 3 respiration rate
O_2 consumption rate (pmole/s*mgprotein)
2500
2000
1500
1000
500
0
**
SdcBG02774
CS (B)
B
State 4 respiration rate
O_2 consumption rate (pmole/s*mgprotein)
500
400
300
200
100
0
SdcBG02774
CS (B)
C
CG9809
mRNA relative amount
0.8
0.6
0.4
0.2
0.0
Sdc^BG02774
CS (B)
*
***
Female
Male

Figure 3. Sleep behavior in *Sdc*^{BG02774} and *CS (B)* flies. (A-B) sleep parameters were measured counting the number of times a given fly crosses an infrared beam during a one-minute interval. Sleep was defined as any period 5 minutes or longer without an activity count. In (A) and (B) values represent average hours of sleep in female and male flies, respectively, of two independent replicates of 16 flies. Statistical significance was determined by Wilcoxon T-test. In all panels, error bars represent SEM. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

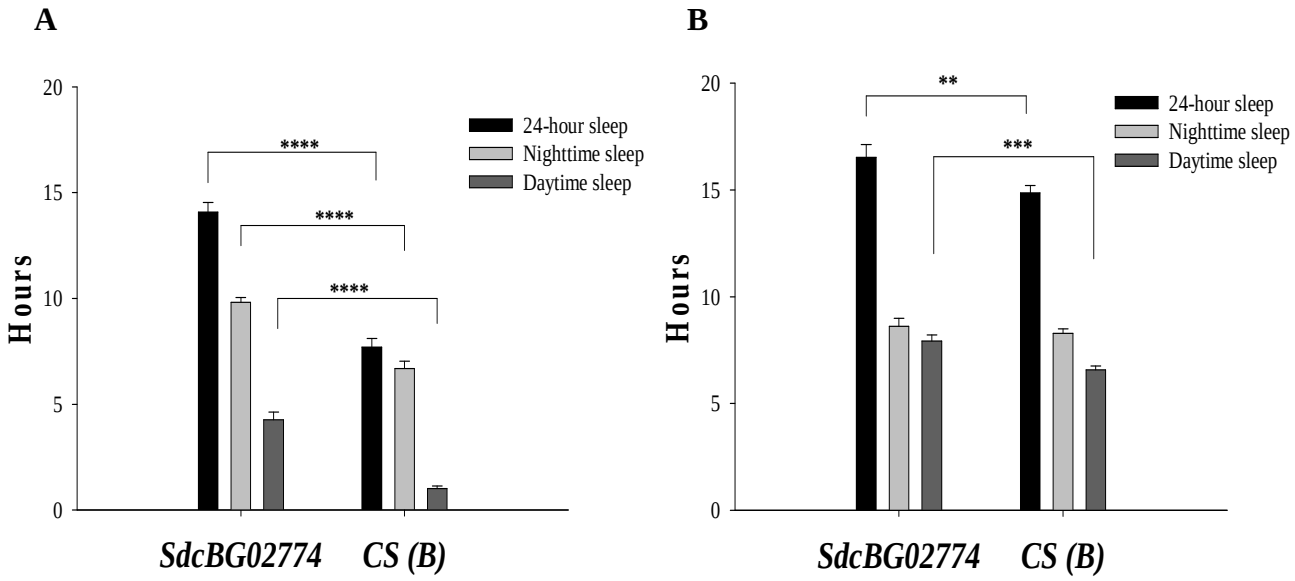
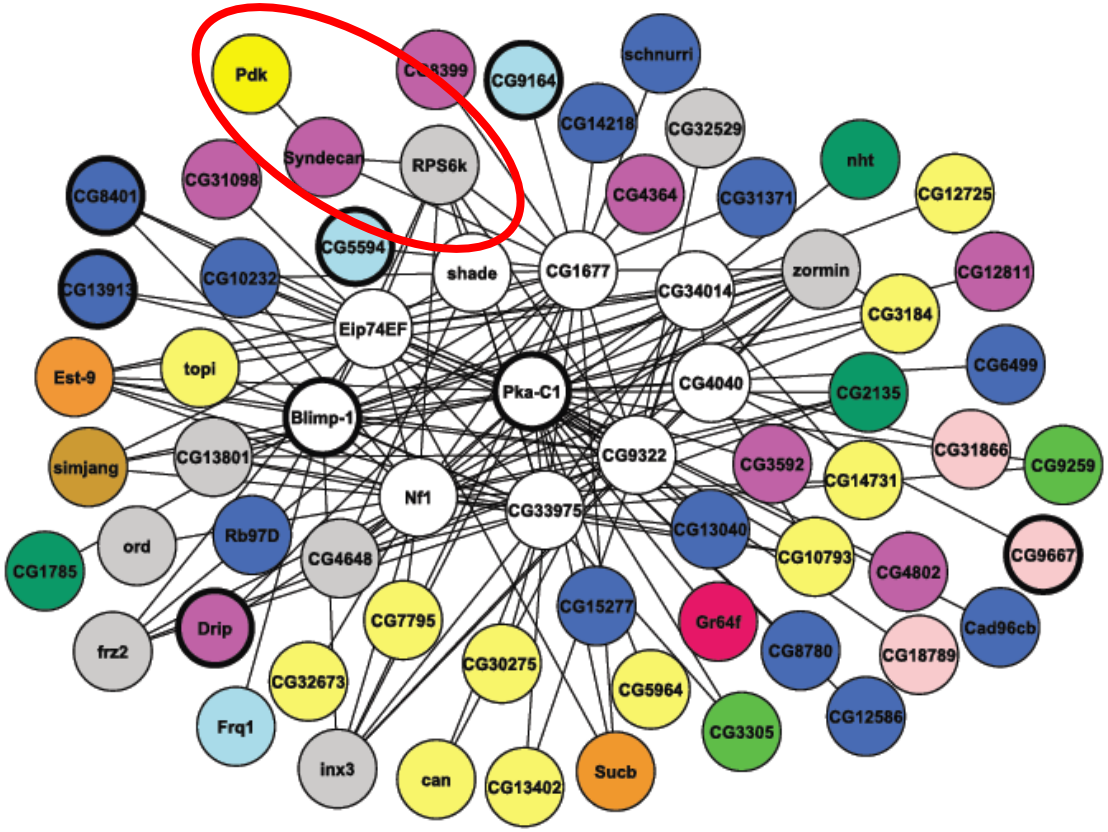

A
B
Hours
20
15
10
5
0
24-hour sleep
Nighttime sleep
Daytime sleep
****
****
****
**
***
SdcBG02774
CS (B)

Figure 4. Interaction network (|r|≥0.6) for mitochondrial state 3 module 17 enriched for genes involved in circadian rhythm and organismal behavior. Each node represents a gene and each edge a significant correlation between a pair of genes. Module 17 hub genes (white) highly interact with other hub genes in module 5 (orange), 6 (pink), 7 (green), 9 (red), 11 (yellow), 12 (brown), 14 (dark green), 16 (blue), 18 (gray), 19 (purple), 20 (light-blue). Nodes shown as bold represent those genes previously identified as circadian- or sleep-regulated [62,73]. Interaction between *dSdc*, *Pdk* and *RPS6-p70-protein kinase* is highlighted.


Pdk
CG8399
CG9164
schnurri
CG14218
CG32529
Syndecan
RPS6k
nht
CG31098
CG8401
CG4364
CG31371
CG12725
CG5594
shade
CG1677
zormin
CG12811
CG13913
CG10232
CG34014
CG3184
Eip74EF
Est-9
topi
CG4040
CG6499
Pka-C1
CG2135
Blimp-1
CG31866
simjang
CG13801
CG9322
CG3592
CG9259
CG14731
Nf1
CG33975
ord
Rb97D
CG13040
CG9667
CG10793
CG1785
CG4648
CG4802
CG15277
Gr64f
CG7795
Drip
Cad96cb
frz2
CG8780
CG18789
CG30275
CG32673
CG5964
CG12586
Frq1
CG3305
inx3
can
CG13402
Sucb

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CHAPTER V:

CONCLUSION

As complex trait disorders, both obesity and type 2 diabetes mellitus (T2DM) respond to the action and interaction of environmental and genetic susceptibility factors. Quantitative genetic analyses in humans and animal models have provided compelling evidence that multiple genes underlie the genetic architecture of obesity [103-105] and other complex diseases, including T2DM [4]. Despite the fact that obesity-related traits are highly heritable, the genetic basis underlying their natural variation and the loci playing pleiotropic roles among organismal traits have not been fully elucidated.

The overall goals of these present studies were: to shed light on the architecture of the genetic co-expression networks regulating variations in obesity-related traits, elucidate the extent to which they are regulated by pleiotropic loci, and identify pleiotropic alleles between metabolism and life-history traits to provide key insights into why different alleles are allowed to persist in natural populations, despite the fact that some of them confer susceptibility to metabolic disorders.

The findings of Chapter II confirmed the high heritability of energy metabolism traits and highlighted the relevance of genes involved in non-metabolic pathways, such as in immune response, neurogenesis and neuronal function, cell growth, food processing and water balance, as key regulators of organismal energy balance. The use of mutant stocks to independently test some of the candidate genes affecting total glycerol, triacylglycerol and glycogen storage corroborated the validity of the methodological approach used in

the study. Furthermore, the elucidation of pleiotropic transcriptional modules provided a key insight into the molecular basis of the well established trade-offs between body weight, reproduction, and survival of food deprivation.

Our second study (**Chapter III**) showed that similar to body weight, metabolic rate and body composition traits, there is segregating variation in mitochondrial respiration rates in this population of *D. melanogaster*. Ours is the first study reporting heritability for mitochondrial bioenergetic traits. The gene-co-expression network analysis revealed that highly complex and interactive nuclear-encoded transcriptional networks underlie natural variations in mitochondrial state 3 and 4 respiration rates. On the other hand, only a few nuclear-encoded genes were identified to affect variation in mitochondrial ADP/O ratio (i.e. efficiency), highlighting the relevance of mitochondrial-encoded genes and mito-nuclear interactions as key players influencing mitochondrial efficiency. Analysis of pleiotropic loci between mitochondria bioenergetic and body composition traits suggests a coordinated regulation between mitochondrial function and energy balance. In addition, the data from this study strongly indicate that molecular regulation of mitochondrial respiration plays a critical role in mediating life history trade-offs in natural populations, and underscore the relevance of the target of rapamycin signaling pathway as the key regulator of mitochondrial function.

In our last study we intended to independently verify the effect of the *dSdc* gene on mitochondrial respiration rate and circadian-regulated behaviors such as sleep. We corroborated the role of *dSdc* as a candidate gene regulating variations in mitochondrial state 3 respiration rate. Indeed, flies homozygous for the *dSdc* mutation displayed significantly lower mitochondrial ADP-stimulated (state 3) respiration. Our data further

confirmed changes in sleep, a circadian-regulated behavior known to impact organismal energy balance.

In conclusion, our results confirm that the genetic basis of natural variation in body weight and energy metabolism traits involves highly interactive co-regulated transcriptional networks, and identify several pleiotropic alleles underlying evolutionarily conserved trade-offs among obesity-related and organismal life-history traits. Such trade-offs establish inter-individual differences in survival and reproduction and underlie the basis for the perpetuation of alleles that confer susceptibility to metabolic disorders among individuals from a natural population.

Future directions

Our study sheds light on the genetic architecture of body weight and energy metabolism traits and identifies several candidate genes and genetic networks affecting these traits. Identification of these new alleles will likely provide new models for human obesity and type 2 diabetes mellitus that can be further tested in other populations. Furthermore, the completion of the sequencing of the whole mtDNA genome of the 40 Raleigh lines currently underway will elucidate the extent to which variations in mitochondrial genome and mitonuclear interactions contribute to differences in mitochondrial respiration traits and subsequently in other metabolic traits. Additionally, the completion of the sequencing of the whole nuclear genome of these 40 lines will provide further insight into the potential genetic variants underlying variations in obesity-related traits and having pleiotropic effects on body composition, energy metabolism and life-history traits. If the impact of some environmental variables on body weight regulation manifests itself only on certain genotypes, the identification of disease risk alleles will allow targeting efforts

towards recognition and counseling of susceptible individuals to prevent obesity at a public health level. Finally, further studies are warranted on the molecular and genetic mechanisms underlying the role of *Drosophila syndecan* gene on organismal energy balance.

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APPENDIX A
SUPPLEMENTARY TABLE 1

Analysis of modules of correlated transcripts associated with each of the body composition and energy metabolism traits. The *p*-values are from the regression analyses. Degree = the average correlation of a transcript with all other transcripts in its module. Avge Degree = the average correlation of all transcripts in the module.

Trait	Probe Set ID	Gene Name	*P*-Value	Module	Degree	Avge Degree
Wet Body Weight	1635549_at	Turandot	4.76E-04	1	0.81038	0.81038
	1639323_at	Turandot C	5.00E-03	1	0.81038	0.81038
	1641419_at	attacin	4.85E-05	2	0.6947	0.65825
	1638235_at	diptericin-like protein	1.29E-04	2	0.73493	0.65825
	1627613_at	metchnikowin	2.40E-04	2	0.68338	0.65825
	1636490_at	PGRP-SB1	1.13E-03	2	0.6453	0.65825
	1633545_at	PGRP-SD	7.00E-04	2	0.53293	0.65825
	1623790_at	CG31901	1.66E-03	3	0.53796	0.62547
	1633385_at	Karl	2.70E-05	3	0.73246	0.62547
	1628715_a_at	Karl	9.96E-06	3	0.60599	0.62547
	1632719_at	Cecropin	6.16E-04	4	0.5706	0.55413
	1626530_at	Cecropin	5.23E-05	4	0.53122	0.55413
	1623871_at	CG18563	1.91E-04	4	0.67763	0.55413
	1625698_at	CG6639	9.55E-04	4	0.62715	0.55413
	1636946_at	CG6687	1.66E-03	4	0.46507	0.55413
	1636333_a_at	Mth-like 2	3.10E-06	4	0.45312	0.55413
	1624236_at	CG30002	1.57E-03	5	0.41989	0.55043
	1632430_at	Transferrin	2.49E-03	5	0.64793	0.55043
	1639442_a_at	Transferrin	1.55E-03	5	0.58348	0.55043
	1631691_at	CG16772	4.68E-03	6	0.37903	0.51296
	1636293_at	CG2217	1.85E-04	6	0.53127	0.51296
	1631370_at	CG30026	8.72E-03	6	0.47434	0.51296
	1628387_s_at	CG30080	1.95E-04	6	0.64298	0.51296
	1627759_at	CG30080	5.23E-04	6	0.64099	0.51296
	1640478_at	CG31777	8.60E-03	6	0.51913	0.51296
	1636682_at	CG31778	2.80E-03	6	0.45554	0.51296
	1637370_at	yellow f	3.52E-03	6	0.46043	0.51296
	1629474_at	CG11368	5.27E-04	7	0.53025	0.46714
	1628567_at	CG13565	2.85E-03	7	0.38466	0.46714
	1632539_at	Glutathione S transferase E4	2.52E-03	7	0.32585	0.46714

	1638066_at	Olfactory-specific E	9.52E-03	7	0.53267	0.46714
	1641200_at	Olfactory-specific-F	5.49E-04	7	0.56226	0.46714
	1636213_at	---	2.23E-03	8	0.50846	0.39277
	1637382_at	---	4.26E-03	8	0.40294	0.39277
	1638114_at	CG13022	7.26E-03	8	0.38916	0.39277
	1631631_at	CG13222	8.33E-03	8	0.35087	0.39277
	1622894_at	CG15120	3.61E-04	8	0.33067	0.39277
	1624213_at	CG15535	1.95E-03	8	0.43496	0.39277
	1633251_at	CG15829	1.09E-03	8	0.2769	0.39277
	1631650_a_at	CG2056	1.14E-03	8	0.42982	0.39277
	1625763_at	CG2789	4.00E-03	8	0.47799	0.39277
	1623732_at	CG31410	8.01E-04	8	0.27595	0.39277
	1623963_at	CG32115	2.28E-03	8	0.1817	0.39277
	1640757_at	CG33462	5.22E-04	8	0.56761	0.39277
	1631521_at	CG33469	3.73E-03	8	0.44809	0.39277
	1638733_at	CG3984	1.16E-04	8	0.22902	0.39277
	1640035_at	fat body protein P6	7.26E-03	8	0.44765	0.39277
	1628742_at	molting defective	3.56E-04	8	0.37562	0.39277
	1638338_a_at	Protein-1	3.61E-03	8	0.54964	0.39277
	1641239_at	---	5.43E-03	9	0.21919	0.31385
	1624517_at	beta-galactosidase	2.14E-03	9	0.28127	0.31385
	1638580_at	capricious	7.43E-03	9	0.35759	0.31385
	1640221_at	CG10479	6.81E-03	9	0.32754	0.31385
	1625430_at	CG14022	2.62E-03	9	0.3363	0.31385
	1634510_at	CG17324	8.21E-03	9	0.20525	0.31385
	1623632_s_at	CG6327	3.72E-03	9	0.35969	0.31385
	1633357_at	CG7910	3.13E-03	9	0.39096	0.31385
	1630302_at	CG8562	7.56E-03	9	0.20296	0.31385
	1637754_at	CG9458	9.56E-03	9	0.40001	0.31385
	1628345_at	cytochrome P450 related AF4	2.49E-04	9	0.25657	0.31385
	1623068_at	Cytochrome P450-4e3	3.41E-03	9	0.1925	0.31385
	1631498_a_at	fru-satori	4.28E-03	9	0.18466	0.31385
	1637990_s_at	fumble	5.28E-03	9	0.42874	0.31385
	1632392_s_at	giant slob	1.63E-04	9	0.40927	0.31385
	1636407_at	goliath	6.13E-04	9	0.35982	0.31385
	1639622_at	Proctolin	1.62E-03	9	0.26707	0.31385
	1634742_at	Sorbitol dehydrogenase-2	5.75E-03	9	0.19292	0.31385
	1639230_at	v(2)k05816	3.83E-03	9	0.46396	0.31385
	1639273_s_at	vrille	4.08E-03	9	0.44075	0.31385
	1630043_a_at	---	8.86E-03	10	0.39869	0.3108

	1624531_s_at	---	9.63E-03	10	0.39396	0.3108
	1638496_at	---	1.16E-03	10	0.18241	0.3108
	1639729_s_at	---	1.71E-04	10	0.14334	0.3108
	1636249_at	7B2	6.43E-03	10	0.44822	0.3108
	1637321_at	AChR protein of Drosophila	7.43E-03	10	0.40767	0.3108
	1636451_a_at	Activating transcription factor-2	3.69E-03	10	0.34567	0.3108
	1628647_at	antennal protein 5	3.17E-03	10	0.24557	0.3108
	1633914_at	beta-galactosidase-1	6.19E-03	10	0.25235	0.3108
	1624417_a_at	CG10186	3.65E-03	10	0.46002	0.3108
	1630106_at	CG10362	6.00E-03	10	0.34565	0.3108
	1640845_at	CG10581	9.21E-03	10	0.20135	0.3108
	1633777_at	CG11221	3.26E-03	10	0.2825	0.3108
	1634165_at	CG11378	9.99E-03	10	0.21969	0.3108
	1631307_at	CG11438	1.48E-03	10	0.16819	0.3108
	1629996_at	CG11910	8.66E-03	10	0.40039	0.3108
	1624143_a_at	CG12071	3.43E-03	10	0.39192	0.3108
	1641513_at	CG12239	4.19E-03	10	0.46718	0.3108
	1625883_at	CG13253	7.75E-03	10	0.14347	0.3108
	1637150_at	CG13928	7.49E-03	10	0.47175	0.3108
	1632424_at	CG13995	9.66E-03	10	0.41104	0.3108
	1624834_at	CG14015	8.14E-03	10	0.29423	0.3108
	1624772_at	CG14186	1.97E-04	10	0.44021	0.3108
	1639504_at	CG14855	7.70E-03	10	0.15486	0.3108
	1633589_a_at	CG1552	8.07E-04	10	0.35997	0.3108
	1637593_at	CG15537	2.79E-03	10	0.24272	0.3108
	1633816_at	CG17181	2.34E-03	10	0.25162	0.3108
	1625951_at	CG17778	2.24E-03	10	0.40892	0.3108
	1640006_at	CG18609	5.81E-03	10	0.29072	0.3108
	1632023_s_at	CG1909	3.30E-04	10	0.47431	0.3108
	1627499_at	CG2016	4.45E-03	10	0.41127	0.3108
	1625314_at	CG2022	1.99E-03	10	0.27143	0.3108
	1636414_at	CG30004	4.83E-03	10	0.26689	0.3108
	1635271_at	CG30431	6.76E-03	10	0.17544	0.3108
	1634631_at	CG3105	6.89E-03	10	0.31021	0.3108
	1622929_at	CG31222	7.33E-03	10	0.14527	0.3108
	1632653_at	CG31555	7.31E-03	10	0.39378	0.3108
	1635041_at	CG32225	1.60E-04	10	0.28344	0.3108
	1636608_at	CG32843	8.07E-04	10	0.29204	0.3108
	1637374_at	CG33779	9.92E-04	10	0.26041	0.3108
	1628381_at	CG3579	8.17E-03	10	0.20145	0.3108

	1634462_at	CG3822	2.05E-03	10	0.21571	0.3108
	1634993_at	CG40498	7.89E-03	10	0.30752	0.3108
	1640896_at	CG4462	2.57E-03	10	0.21304	0.3108
	1633088_at	CG4733	6.10E-03	10	0.36056	0.3108
	1623267_at	CG5237	6.81E-03	10	0.36699	0.3108
	1631532_at	CG5282	5.00E-04	10	0.32542	0.3108
	1636848_at	CG6024	1.16E-03	10	0.41073	0.3108
	1641725_at	CG7000	9.38E-04	10	0.26518	0.3108
	1631098_at	CG7194	2.64E-03	10	0.16907	0.3108
	1629428_at	CG7342	5.21E-03	10	0.19995	0.3108
	1626684_at	CG7607	1.49E-03	10	0.26073	0.3108
	1637457_at	CG7646	6.66E-03	10	0.4075	0.3108
	1635963_a_at	CG7990	9.25E-03	10	0.42659	0.3108
	1640058_at	CG8216	2.77E-03	10	0.33204	0.3108
	1634879_at	CG8519	1.28E-03	10	0.28488	0.3108
	1628187_s_at	CG9691	3.95E-03	10	0.32254	0.3108
	1626124_at	CG9717	4.35E-03	10	0.38973	0.3108
	1624808_at	CheA75a	5.28E-03	10	0.36572	0.3108
	1622920_at	Cosens-Manning mutant	8.72E-03	10	0.32055	0.3108
	1625859_at	CTP:phosphocholine cytidylyltransferase 2	7.08E-03	10	0.31027	0.3108
	1637870_a_at	dpr5	2.93E-04	10	0.20941	0.3108
	1634054_at	Droninac	3.28E-03	10	0.46524	0.3108
	1629066_at	Drosophila Corazonin Receptor	3.74E-03	10	0.29565	0.3108
	1638305_at	drostatin-B2	2.54E-04	10	0.2788	0.3108
	1629064_at	erect wing	1.39E-03	10	0.36503	0.3108
	1628604_at	fish-hook	4.54E-03	10	0.32772	0.3108
	1639292_at	frequenin	1.18E-03	10	0.24461	0.3108
	1631340_at	G protein gamma 1	9.13E-03	10	0.42405	0.3108
	1623048_a_at	GABA receptor beta subunit	4.30E-03	10	0.39948	0.3108
	1641618_at	guanylate cyclase 99B	5.37E-04	10	0.25941	0.3108
	1639366_at	Hormone receptor-like in 38	3.09E-03	10	0.32503	0.3108
	1627041_s_at	inebriated	3.37E-03	10	0.31405	0.3108
	1637435_at	kekkon4	7.77E-03	10	0.26682	0.3108
	1635144_at	klingon	1.74E-03	10	0.41936	0.3108
	1628699_at	Larval serum protein 1 beta	8.80E-04	10	0.14794	0.3108
	1633298_at	meiotic P22	7.14E-03	10	0.17044	0.3108
	1633061_at	Mgat2	7.97E-03	10	0.36865	0.3108
	1634438_at	Munster	4.35E-04	10	0.30822	0.3108
	1628288_s_at	Neprilysin 4	7.87E-03	10	0.2709	0.3108

	1628147_at	neuroligin	4.25E-03	10	0.46407	0.3108
	1640614_at	nightblind	4.71E-03	10	0.31896	0.3108
	1639535_at	polypeptide GalNAc transferase 2	1.20E-03	10	0.37299	0.3108
	1635928_a_at	Protein-kinase-like-72A	7.38E-03	10	0.39757	0.3108
	1638436_at	pyrokinin	2.66E-03	10	0.29212	0.3108
	1625195_s_at	quo vadis	8.30E-03	10	0.17734	0.3108
	1625431_at	reverse polarity	6.81E-03	10	0.41881	0.3108
	1634402_at	Sarcoglycan beta	2.65E-04	10	0.36665	0.3108
	1638040_at	shade	5.07E-03	10	0.18921	0.3108
	1640129_at	sloppy-paired	4.20E-03	10	0.18767	0.3108
	1631408_at	soxneuro	2.71E-03	10	0.37538	0.3108
	1638811_at	Sugar-baby	6.24E-03	10	0.18721	0.3108
	1639431_at	synaptogyrin	3.23E-03	10	0.30891	0.3108
	1639743_s_at	Syntrophin-like 2	2.06E-04	10	0.29711	0.3108
	1633696_at	Transmembrane 4 superfamily	1.66E-03	10	0.36436	0.3108
	1633383_at	twin of eyegone	2.04E-03	10	0.2931	0.3108
	1641664_at	CG10170	5.08E-03	11	0.22625	0.27627
	1636257_at	CG10688	5.26E-05	11	0.3452	0.27627
	1624945_a_at	CG10910	3.74E-04	11	0.11344	0.27627
	1640733_at	CG14321	4.55E-03	11	0.24893	0.27627
	1636085_at	CG18143	8.82E-04	11	0.39635	0.27627
	1632141_at	CG18265	3.09E-03	11	0.16701	0.27627
	1628404_at	CG2767	6.20E-03	11	0.42176	0.27627
	1626326_at	CG31436	1.49E-03	11	0.1729	0.27627
	1625710_at	CG31717	7.62E-03	11	0.25758	0.27627
	1628309_at	CG5126	8.83E-03	11	0.26533	0.27627
	1641343_at	corazonin	5.38E-05	11	0.21833	0.27627
	1641650_at	fragment D	7.26E-04	11	0.35914	0.27627
	1635740_at	hemomucin	6.84E-03	11	0.28525	0.27627
	1625463_at	Histamine-gated chloride channel subunit 2	7.03E-03	11	0.25712	0.27627
	1625217_at	Mediator complex subunit 17	2.69E-04	11	0.35235	0.27627
	1626053_at	robo3	5.33E-04	11	0.30123	0.27627
	1630643_at	sterol carrier protein X-related thiolase	6.23E-03	11	0.31596	0.27627
	1623675_at	turn on sex-specificity	2.83E-05	11	0.13202	0.27627
	1627317_a_at	tyramine-beta-hydroxylase	2.74E-03	11	0.33114	0.27627
	1624156_at	Ugt86Da	8.01E-04	11	0.35821	0.27627
	1640242_s_at	---	3.27E-03	12	0.18752	0.18922

	1625957_at	CG10051	4.63E-03	12	0.14802	0.18922
	1624454_at	CG10361	7.83E-04	12	0.14216	0.18922
	1627144_at	CG10560	6.33E-03	12	0.24603	0.18922
	1632120_at	CG15533	7.93E-03	12	0.1632	0.18922
	1638016_at	CG2993	9.12E-03	12	0.13211	0.18922
	1636567_at	CG31199	1.95E-04	12	0.23108	0.18922
	1623969_at	CG31200	3.94E-03	12	0.20537	0.18922
	1624332_s_at	CG4098 /// CG18217	5.42E-03	12	0.17076	0.18922
	1625675_a_at	CG4853	9.82E-03	12	0.14603	0.18922
	1632045_at	CG5697	3.65E-04	12	0.22943	0.18922
	1625487_at	CG6839	9.52E-03	12	0.18964	0.18922
	1630917_at	CG7889	1.50E-03	12	0.18448	0.18922
	1634515_at	CG8093	1.10E-05	12	0.22291	0.18922
	1631806_at	CG8693	7.75E-03	12	0.23524	0.18922
	1624509_at	hdl cuticle gene cluster.	2.40E-03	12	0.23204	0.18922
	1632235_at	Methionyl-tRNA synthetase	4.01E-03	12	0.15065	0.18922
	1625557_at	---	4.80E-03	13	0.2282	0.16322
	1637548_at	arginase	2.65E-06	13	0.20387	0.16322
	1623555_at	CG10131	7.37E-04	13	0.15768	0.16322
	1638322_at	CG10168	5.56E-03	13	0.15867	0.16322
	1623666_at	CG10516	5.38E-03	13	0.16351	0.16322
	1638567_at	CG1092	2.43E-05	13	0.22207	0.16322
	1638612_at	CG11142	5.09E-03	13	0.13294	0.16322
	1625828_at	CG11841	9.17E-03	13	0.16817	0.16322
	1628716_at	CG13283	5.51E-04	13	0.12675	0.16322
	1637275_a_at	CG13335	2.40E-03	13	0.19423	0.16322
	1624171_at	CG13488	8.59E-03	13	0.15256	0.16322
	1636078_at	CG13670	7.45E-04	13	0.15512	0.16322
	1622896_at	CG13848	9.34E-03	13	0.13432	0.16322
	1633481_at	CG14394	4.66E-03	13	0.16612	0.16322
	1624950_a_at	CG14823	4.75E-03	13	0.18116	0.16322
	1635446_at	CG15043	6.17E-03	13	0.1482	0.16322
	1631660_at	CG15065	5.84E-04	13	0.18279	0.16322
	1634878_at	CG15127	2.79E-03	13	0.13118	0.16322
	1640950_at	CG15739	4.10E-03	13	0.12054	0.16322
	1635812_at	CG16965	1.36E-05	13	0.12667	0.16322
	1625948_at	CG17633	6.06E-03	13	0.19382	0.16322
	1640144_at	CG18067	8.36E-03	13	0.20392	0.16322
	1633434_at	CG18327	7.67E-03	13	0.16913	0.16322
	1626503_at	CG2254	3.15E-03	13	0.12795	0.16322
	1640217_at	CG30154	2.52E-03	13	0.19943	0.16322

	1626201_at	CG31091	1.81E-04	13	0.12753	0.16322
	1634815_at	CG31104	4.54E-04	13	0.14526	0.16322
	1634477_at	CG31177	6.98E-03	13	0.14713	0.16322
	1625657_at	CG32335	4.77E-03	13	0.14563	0.16322
	1628702_at	CG32580	2.48E-03	13	0.099129	0.16322
	1634697_at	CG32667	4.45E-03	13	0.22613	0.16322
	1623000_at	CG33093	3.81E-03	13	0.16977	0.16322
	1626116_at	CG33329	4.25E-03	13	0.2071	0.16322
	1639730_at	CG34054	1.59E-04	13	0.17333	0.16322
	1635998_at	CG3672	2.94E-03	13	0.13692	0.16322
	1640045_at	CG3934	2.75E-03	13	0.13348	0.16322
	1637772_at	CG4726	6.35E-03	13	0.12873	0.16322
	1634788_at	CG4872	3.37E-03	13	0.14805	0.16322
	1632531_at	CG5770	9.79E-05	13	0.15532	0.16322
	1633460_at	CG6034	5.34E-03	13	0.13852	0.16322
	1632215_at	CG6296	9.06E-04	13	0.13513	0.16322
	1640521_at	CG6361	8.02E-03	13	0.16514	0.16322
	1639819_at	CG6385	7.04E-03	13	0.14649	0.16322
	1633009_a_at	CG6640	7.33E-03	13	0.13616	0.16322
	1626536_at	CG6776	4.35E-03	13	0.10366	0.16322
	1634702_at	CG7227	5.63E-04	13	0.13062	0.16322
	1623769_at	CG7322	6.21E-03	13	0.20303	0.16322
	1624895_at	CG7529	4.38E-03	13	0.23551	0.16322
	1624821_at	CG8586	8.56E-04	13	0.17002	0.16322
	1630723_a_at	CG8708	2.57E-03	13	0.11411	0.16322
	1635744_at	CG9342	8.50E-05	13	0.20069	0.16322
	1631405_at	CG9508	3.82E-05	13	0.1269	0.16322
	1633401_s_at	Cyp12d1-p /// Cyp12d1-d	2.82E-03	13	0.152	0.16322
	1624939_at	Cyp6a21	3.00E-05	13	0.12969	0.16322
	1627844_at	Cytochrome P450-4e2	9.41E-03	13	0.18934	0.16322
	1625023_a_at	Dbeta3	1.12E-03	13	0.20002	0.16322
	1630515_s_at	glutactin	3.98E-05	13	0.21608	0.16322
	1630258_at	Glutathione S transferase D2	7.75E-03	13	0.18687	0.16322
	1626253_at	Glutathione S transferase D4	4.53E-03	13	0.17636	0.16322
	1624793_at	Glutathione S transferase D7	9.11E-03	13	0.16856	0.16322
	1636174_at	Glutathione S transferase D9	2.57E-03	13	0.12739	0.16322
	1640360_at	Immune induced molecule 2	8.13E-03	13	0.14129	0.16322
	1626052_at	Melanization Protease 1	3.03E-03	13	0.22917	0.16322

	1632705_at	neither inactivation nor afterpotential D	1.22E-04	13	0.10582	0.16322
	1627250_at	nesprin	2.14E-03	13	0.10866	0.16322
	1625140_at	NtR	4.28E-03	13	0.14295	0.16322
	1632984_s_at	putative noncoding RNA 007:3R /// CG6503	6.24E-03	13	0.22451	0.16322
	1631821_at	Scavenger Receptor	1.96E-04	13	0.18288	0.16322
	1628884_at	semmelweis	1.64E-06	13	0.24716	0.16322
	1641359_at	Serine Protease 2	2.27E-03	13	0.18227	0.16322
	1639892_at	Sorbitol dehydrogenase like	7.50E-03	13	0.1609	0.16322
	1629072_at	suppressor of white-apricot	2.89E-05	13	0.1078	0.16322
	1634552_at	Thiolester containing protein IV	1.84E-04	13	0.2366	0.16322
Glycogen storage	1626093_at	Jonah 65A	5.17E-03	1	0.90887	0.90887
	1628800_at	Jonah 65A	1.04E-03	1	0.90887	0.90887
	1626251_at	rhodopsin	7.90E-05	2	0.84963	0.84963
	1640642_at	rhodopsin	1.12E-03	2	0.84963	0.84963
	1623229_at	CG7881	9.41E-03	3	0.5924	0.5924
	1640249_at	CG33173	2.69E-03	3	0.5924	0.5924
	1629308_at	CG17192	2.61E-03	4	0.58055	0.58055
	1635868_at	CG6295	4.07E-03	4	0.58055	0.58055
	1632032_at	CG13188	3.31E-03	5	0.57627	0.57627
	1639351_at	CG15884	6.76E-03	5	0.57627	0.57627
	1631765_at	puckered	3.24E-03	6	0.56778	0.43833
	1637107_at	CG10809	5.87E-03	6	0.49723	0.43833
	1637916_at	CG33013	2.83E-03	6	0.49624	0.43833
	1641326_at	CG30118	4.55E-04	6	0.45364	0.43833
	1626369_at	CG12269	3.26E-03	6	0.40209	0.43833
	1638675_at	CG10680	2.54E-03	6	0.39247	0.43833
	1634949_at	hemipterous	8.76E-03	6	0.36643	0.43833
	1639498_a_at	Xasta	2.64E-03	6	0.33079	0.43833
	1638004_at	calbindin	5.23E-03	7	0.4997	0.36279
	1631340_at	G protein gamma 1	2.57E-03	7	0.47713	0.36279
	1639028_a_at	CG30296	9.98E-04	7	0.47684	0.36279
	1637321_at	AChR protein of Drosophila	6.95E-03	7	0.46873	0.36279
	1634452_s_at	endophilin	9.65E-03	7	0.45516	0.36279
	1640767_s_at	CG14591	3.46E-03	7	0.45276	0.36279
	1636846_at	---	6.14E-03	7	0.44769	0.36279
	1640284_at	choline acetyltransferase	6.71E-03	7	0.44577	0.36279

	1635928_a_at	Protein-kinase-like-72A	9.21E-03	7	0.44132	0.36279
	1640201_at	undertaker	3.54E-03	7	0.43716	0.36279
	1625921_at	---	4.60E-03	7	0.43392	0.36279
	1630129_at	CG32355	4.21E-04	7	0.41377	0.36279
	1640534_s_at	calmodulin-dependent kinase	9.19E-03	7	0.41289	0.36279
	1636345_s_at	Synaptotagmin	4.85E-04	7	0.41177	0.36279
	1624833_a_at	muscarinic receptor	5.90E-03	7	0.39662	0.36279
	1635360_at	CG10830	5.93E-03	7	0.3833	0.36279
	1636608_at	CG32843	5.77E-03	7	0.36867	0.36279
	1637666_at	CG15651	8.38E-03	7	0.35335	0.36279
	1637294_at	CG4641	9.89E-04	7	0.34376	0.36279
	1632490_at	CG8008	7.32E-03	7	0.34154	0.36279
	1631417_s_at	CG6282	5.48E-03	7	0.3415	0.36279
	1624033_at	beta-amyloid-protein-precursor-like	8.52E-03	7	0.33293	0.36279
	1631371_s_at	Phosphodiesterase 8	3.93E-03	7	0.33095	0.36279
	1633020_at	unkempt	9.63E-03	7	0.32789	0.36279
	1634184_a_at	junctophilin	8.43E-03	7	0.31954	0.36279
	1628888_at	CG7180	6.17E-05	7	0.31642	0.36279
	1638193_at	CG5612	4.42E-03	7	0.30896	0.36279
	1629091_at	CG5177	2.00E-03	7	0.27911	0.36279
	1639469_a_at	GTP cyclohydrolase	9.66E-05	7	0.25662	0.36279
	1638424_at	CG10514	5.90E-03	7	0.23298	0.36279
	1624577_at	acetylcholinesterase	4.57E-03	7	0.23267	0.36279
	1637717_at	sevenless	2.64E-03	7	0.20525	0.36279
	1635047_s_at	trio	3.04E-03	7	0.19786	0.36279
	1632325_at	tiptop	7.43E-03	7	0.19045	0.36279
	1640755_at	cytochrome P450 related AF5	7.81E-03	8	0.40161	0.34363
	1635455_at	CG14529	5.85E-03	8	0.37601	0.34363
	1622946_at	CG6908	3.52E-03	8	0.3689	0.34363
	1626253_at	Glutathione S transferase D4	4.95E-03	8	0.34049	0.34363
	1626350_at	fragment K	2.53E-03	8	0.23113	0.34363
	1623943_at	CG17556	2.89E-03	9	0.25449	0.16014
	1634936_at	CG14972	9.98E-04	9	0.25003	0.16014
	1636532_at	CG13958	4.71E-03	9	0.238	0.16014
	1631692_at	CG16798	6.51E-04	9	0.23617	0.16014
	1636546_s_at	CG1640	4.73E-03	9	0.23361	0.16014
	1635287_at	CG15366	1.49E-03	9	0.22756	0.16014
	1629259_at	CG4186	9.07E-03	9	0.22168	0.16014
	1637002_at	CG14125	9.42E-03	9	0.21697	0.16014

	1635375_at	CG5630	7.13E-03	9	0.216	0.16014
	1634444_at	CG12736	3.52E-03	9	0.21588	0.16014
	1631573_a_at	wunen	7.07E-03	9	0.20661	0.16014
	1637875_a_at	---	9.19E-03	9	0.20622	0.16014
	1623697_s_at	CG33465	2.57E-03	9	0.20507	0.16014
	1628129_a_at	dynein /// Sdic:CG32823 /// Sdic:CG33499	3.75E-03	9	0.20139	0.16014
	1641527_at	CG4576	1.34E-03	9	0.19857	0.16014
	1637592_at	CG10073	7.53E-05	9	0.19833	0.16014
	1638777_at	Rim	9.37E-03	9	0.19827	0.16014
	1634547_at	CG32506	2.06E-03	9	0.19821	0.16014
	1627408_at	CG32107	5.27E-03	9	0.19796	0.16014
	1623683_at	mitochondrial ribosomal protein L33	1.16E-03	9	0.19428	0.16014
	1637236_at	CG41136	7.90E-03	9	0.19296	0.16014
	1633648_at	sticks and stones	2.95E-03	9	0.19239	0.16014
	1631084_at	CG12976	5.63E-03	9	0.18811	0.16014
	1629469_s_at	CG10960	4.52E-04	9	0.18612	0.16014
	1624215_s_at	klett	3.58E-04	9	0.18481	0.16014
	1641508_s_at	tetraspanin 42E	2.20E-03	9	0.18008	0.16014
	1640085_at	CG9733	7.58E-03	9	0.17552	0.16014
	1631604_at	CG9511	6.04E-03	9	0.17454	0.16014
	1633499_at	retinin	2.80E-03	9	0.17054	0.16014
	1626965_at	CG31743	2.64E-03	9	0.16801	0.16014
	1635998_at	CG3672	1.92E-03	9	0.16127	0.16014
	1631882_at	CG10264	6.47E-03	9	0.15877	0.16014
	1638862_at	CG15553	2.77E-03	9	0.15136	0.16014
	1632791_at	CG32479	8.93E-03	9	0.15116	0.16014
	1630404_at	CG34112	6.08E-03	9	0.15105	0.16014
	1641280_at	Ance-4	6.64E-03	9	0.14837	0.16014
	1636693_at	CG4330	6.66E-03	9	0.14549	0.16014
	1640521_at	CG6361	1.69E-03	9	0.14313	0.16014
	1626759_at	CG34136	1.54E-03	9	0.14261	0.16014
	1636330_at	CG17107	7.52E-04	9	0.1419	0.16014
	1640956_at	CG4725	6.39E-03	9	0.14097	0.16014
	1628989_at	---	3.29E-03	9	0.14052	0.16014
	1626536_at	CG6776	4.25E-03	9	0.14019	0.16014
	1639268_at	CG13324	6.13E-03	9	0.13881	0.16014
	1628611_at	CG11241	9.86E-03	9	0.13554	0.16014
	1636258_at	CG8550	9.70E-03	9	0.13505	0.16014
	1626431_at	CG10681	2.36E-03	9	0.13342	0.16014
	1631225_at	CG8317	9.40E-03	9	0.13319	0.16014

	1640556_at	CG14606	9.52E-04	9	0.13227	0.16014
	1631193_at	CG3014 /// rigor mortis	7.29E-03	9	0.13176	0.16014
	1638775_at	Multi drug resistance 50	6.90E-03	9	0.13147	0.16014
	1636283_at	CG14826	9.01E-03	9	0.13143	0.16014
	1630970_at	CG14973	1.92E-03	9	0.12982	0.16014
	1623666_at	CG10516	7.52E-03	9	0.12882	0.16014
	1639442_a_at	Transferrin	2.33E-03	9	0.12707	0.16014
	1639424_at	CG6910	3.67E-03	9	0.1267	0.16014
	1632120_at	CG15533	3.60E-03	9	0.12644	0.16014
	1631405_at	CG9508	9.79E-04	9	0.12555	0.16014
	1627636_at	CG14636	8.93E-03	9	0.12374	0.16014
	1638021_at	CG4757	1.06E-06	9	0.12367	0.16014
	1632045_at	CG5697	3.16E-03	9	0.12255	0.16014
	1638856_at	0.9kb transcript /// CG2650	2.89E-03	9	0.12164	0.16014
	1625129_at	CG1946	9.40E-03	9	0.11828	0.16014
	1632907_a_at	CG13204	8.37E-03	9	0.11446	0.16014
	1628271_at	CG3609	8.09E-03	9	0.11431	0.16014
	1636394_a_at	---	4.64E-03	9	0.1143	0.16014
	1638716_a_at	CG10725	2.59E-04	9	0.11189	0.16014
	1638128_at	CG30280	3.72E-03	9	0.10982	0.16014
	1627771_at	CG13075	3.22E-03	9	0.10828	0.16014
	1633147_at	CG10659	9.04E-03	9	0.1058	0.16014
	1633631_at	CG9682	5.62E-03	9	0.10372	0.16014
	1629619_at	Tryptophanyl-tRNA synthetase	5.47E-03	9	0.095895	0.16014
	1624069_at	CG7296	9.52E-03	9	0.089728	0.16014
Total proteins	1632936_at	alpha-mannosidase	9.36E-04	1	0.77387	0.75195
	1626839_s_at	bloated tubules	9.76E-03	1	0.74584	0.75195
	1625964_at	recombination-defective	1.52E-04	1	0.73615	0.75195
	1623086_at	germline transcription factor	7.67E-03	2	0.7795	0.66942
	1639435_at	Ulp1	8.54E-03	2	0.7682	0.66942
	1627999_s_at	Barren	5.47E-03	2	0.74605	0.66942
	1637456_at	CG8878	2.09E-03	2	0.74088	0.66942
	1632309_at	Drosophila Angelman syndrome	9.88E-03	2	0.69949	0.66942
	1629306_at	X11L	6.21E-03	2	0.66384	0.66942
	1624252_s_at	CG12592 /// slender lobes	7.88E-03	2	0.65512	0.66942
	1624803_at	CG34013	8.57E-03	2	0.30229	0.66942
	1624301_at	lethal (3) 01640	2.21E-03	3	0.76252	0.6548
	1634849_at	CG9247	4.67E-03	3	0.74123	0.6548

	1631919_at	CG17078	9.33E-03	3	0.73612	0.6548
	1636774_at	Drosophila cleavage and polyadenylation stimulation factor-160	2.73E-03	3	0.7314	0.6548
	1630213_at	CG1078	9.18E-03	3	0.7292	0.6548
	1641644_s_at	swollen-antenna	8.83E-03	3	0.7283	0.6548
	1628853_at	CG8915	8.09E-03	3	0.71433	0.6548
	1629111_at	Mediator complex subunit 1	2.03E-03	3	0.70674	0.6548
	1627331_at	TBP-associated factor 80	8.33E-03	3	0.69233	0.6548
	1630683_at	CG5208	5.53E-03	3	0.69092	0.6548
	1627383_at	abnormal spindle	8.28E-03	3	0.68908	0.6548
	1625564_at	CG9613	7.15E-04	3	0.68121	0.6548
	1632194_at	Nup98	5.80E-03	3	0.65846	0.6548
	1633331_at	domino	3.87E-03	3	0.6565	0.6548
	1637905_s_at	CG2924	8.76E-03	3	0.65479	0.6548
	1628324_at	CG14442	2.23E-03	3	0.65183	0.6548
	1635618_at	Downstream of kinase	2.39E-03	3	0.62794	0.6548
	1638463_at	CG11986	9.17E-04	3	0.47745	0.6548
	1638400_at	CG8503	8.41E-03	3	0.40478	0.6548
	1636632_at	CG9784	2.06E-03	3	0.3608	0.6548
	1629662_at	CG3527	8.97E-03	4	0.68149	0.53242
	1634572_at	CG6459	2.53E-04	4	0.669	0.53242
	1631718_at	mitochondrial ribosomal protein L12	3.66E-03	4	0.65473	0.53242
	1635825_a_at	mitochondrial transcription factor A	2.81E-03	4	0.64586	0.53242
	1634230_s_at	grapes	8.06E-03	4	0.63625	0.53242
	1638237_at	CG5412	6.99E-03	4	0.61817	0.53242
	1639392_at	lethal (2) 09851	3.29E-03	4	0.61286	0.53242
	1625271_at	CG15438	7.72E-03	4	0.58297	0.53242
	1625665_at	mitochondrial ribosomal protein L39	4.97E-03	4	0.57806	0.53242
	1638146_at	Rtc1	6.87E-03	4	0.56175	0.53242
	1632689_at	hypothetical 23.1kd-like protein	4.94E-03	4	0.54438	0.53242
	1627926_at	CG8315	5.25E-03	4	0.54384	0.53242
	1625902_at	CG11655	7.73E-03	4	0.54265	0.53242
	1626444_at	Fibrillarin	9.99E-03	4	0.49393	0.53242
	1624181_at	CG8062	9.83E-03	4	0.39395	0.53242
	1632656_at	CG12112	1.77E-03	4	0.33854	0.53242
	1638297_at	CG12056	6.58E-03	4	0.31622	0.53242
	1626431_at	CG10681	7.14E-03	4	0.16893	0.53242
	1629688_at	Oseg4	5.31E-03	5	0.50715	0.50715

	1637857_at	Lectin24A	9.89E-03	5	0.50715	0.50715
	1634786_at	Lectin28C	5.13E-04	6	0.50456	0.50456
	1637958_at	CG33510	2.09E-03	6	0.50456	0.50456
	1630884_at	Rad21	4.98E-03	7	0.6192	0.44141
	1623702_at	CG3226	8.22E-03	7	0.61836	0.44141
	1627027_at	CG15168	1.75E-03	7	0.61719	0.44141
	1628486_a_at	d4	5.11E-03	7	0.61282	0.44141
	1640689_a_at	Heterochromatin Protein 1A	3.41E-03	7	0.60697	0.44141
	1630841_at	CG8668	4.24E-03	7	0.60502	0.44141
	1635385_at	CG10225	2.23E-03	7	0.60388	0.44141
	1636164_s_at	CG3107	6.40E-03	7	0.59222	0.44141
	1625845_s_at	Chromosomal protein D1	5.09E-03	7	0.58886	0.44141
	1641170_a_at	Glorund	6.56E-03	7	0.58878	0.44141
	1640358_at	CG4968	8.77E-03	7	0.58818	0.44141
	1640377_s_at	anon-fast-evolving-1H4	5.96E-03	7	0.58533	0.44141
	1640510_at	CG8600	8.94E-03	7	0.58506	0.44141
	1632821_a_at	CG3287	8.50E-03	7	0.58386	0.44141
	1634182_at	CG11593	8.21E-03	7	0.58095	0.44141
	1638651_a_at	CG3508	9.22E-03	7	0.58084	0.44141
	1629941_a_at	CG8044	4.96E-03	7	0.57961	0.44141
	1634055_at	Mat1	5.46E-03	7	0.578	0.44141
	1629949_at	CG14647	5.53E-03	7	0.57702	0.44141
	1630991_at	CG11007	8.22E-03	7	0.57396	0.44141
	1638147_at	CG7772	6.00E-03	7	0.57346	0.44141
	1638591_at	Tif-IA	2.71E-03	7	0.57169	0.44141
	1636628_at	CG11866	5.49E-03	7	0.57034	0.44141
	1626522_at	Nucleosome remodeling factor	7.33E-03	7	0.5665	0.44141
	1632105_a_at	ciboulot	4.42E-03	7	0.56395	0.44141
	1637554_a_at	lethal(2)49Fk	7.03E-03	7	0.56263	0.44141
	1641115_at	CG10055	7.26E-03	7	0.5607	0.44141
	1628922_s_at	vegetable	8.79E-03	7	0.55997	0.44141
	1630817_s_at	CG17528	2.86E-03	7	0.55862	0.44141
	1627488_at	twinfillin	8.39E-03	7	0.5585	0.44141
	1631775_at	CG11137	2.18E-03	7	0.55728	0.44141
	1630369_a_at	CG3847	6.84E-03	7	0.55387	0.44141
	1640375_at	bonsai	6.10E-03	7	0.5534	0.44141
	1627473_at	CG8090	8.39E-03	7	0.55281	0.44141
	1634683_at	Mothers against Decapentaplegic	3.90E-03	7	0.55237	0.44141
	1635448_at	Mitochondrial sorting protein 1	7.40E-03	7	0.55022	0.44141

	1633767_at	heterogeneous nuclear ribonucleoprotein M	4.68E-03	7	0.5497	0.44141
	1630246_at	CG14199	8.19E-03	7	0.54627	0.44141
	1632236_at	CG10221	5.28E-03	7	0.54448	0.44141
	1629695_at	CG4686 /// GA18356	4.74E-03	7	0.54428	0.44141
	1627154_at	CG10904	7.07E-03	7	0.54385	0.44141
	1624396_at	CG7927	2.56E-03	7	0.54352	0.44141
	1634112_a_at	CG5174	6.13E-03	7	0.54279	0.44141
	1640643_a_at	CG7609	8.61E-04	7	0.54181	0.44141
	1634037_s_at	Sequence-specific single-stranded DNA-binding protein	7.09E-03	7	0.54108	0.44141
	1641058_at	CG1319	3.61E-03	7	0.54013	0.44141
	1640311_s_at	Batman	5.72E-03	7	0.53929	0.44141
	1639625_at	Vacuolar protein sorting 25	4.09E-03	7	0.53652	0.44141
	1637282_at	Anon-becker2 /// CG6171	4.59E-03	7	0.53248	0.44141
	1622981_at	Ultraspiracle	4.64E-03	7	0.52762	0.44141
	1639162_at	---	7.77E-03	7	0.52439	0.44141
	1637190_at	CG9149	6.93E-03	7	0.52395	0.44141
	1625855_at	Mediator complex subunit 30	2.45E-03	7	0.52282	0.44141
	1638507_at	mitochondrial ribosomal protein L46	7.76E-03	7	0.52261	0.44141
	1627641_s_at	CG17494	3.52E-03	7	0.52215	0.44141
	1627794_a_at	Translin associated factor X	7.73E-03	7	0.52165	0.44141
	1639411_at	lethal (1) G0148	6.50E-03	7	0.52034	0.44141
	1625260_at	CG11802	6.27E-04	7	0.5202	0.44141
	1634193_at	CG13142	8.69E-03	7	0.52004	0.44141
	1627894_s_at	Chip	8.15E-03	7	0.51995	0.44141
	1633486_at	mitochondrial ribosomal protein L3	3.80E-03	7	0.51905	0.44141
	1623506_s_at	Protein phosphatase X	9.04E-03	7	0.51858	0.44141
	1639768_at	CG2813	4.64E-03	7	0.51838	0.44141
	1637148_at	CG13366	9.84E-03	7	0.51489	0.44141
	1631073_at	Chloride intracellular channel	2.31E-04	7	0.50978	0.44141
	1638782_at	CG8195	2.48E-03	7	0.50937	0.44141
	1634359_a_at	Borealin	6.06E-03	7	0.50904	0.44141
	1639052_a_at	CG12024	6.50E-03	7	0.50566	0.44141
	1626621_at	CG1407	9.53E-03	7	0.50377	0.44141
	1631460_s_at	Painting of fourth	8.11E-03	7	0.50194	0.44141
	1625722_at	CG2774	2.65E-03	7	0.50144	0.44141
	1623826_at	CHIP	1.07E-03	7	0.49833	0.44141

	1637417_at	CG6565	3.32E-03	7	0.49661	0.44141
	1633840_a_at	CG8026	9.90E-03	7	0.49551	0.44141
	1630370_at	CG9796	1.25E-03	7	0.48617	0.44141
	1639520_at	CG1298	1.24E-03	7	0.48577	0.44141
	1633506_s_at	CG6091	3.58E-03	7	0.48485	0.44141
	1632586_at	CG9951 /// GA22148	7.00E-03	7	0.48311	0.44141
	1627749_at	CG6412	9.30E-03	7	0.48173	0.44141
	1640633_s_at	CG32425	2.31E-03	7	0.47989	0.44141
	1632776_at	JUN activation domain-binding protein-1	1.39E-03	7	0.47975	0.44141
	1631712_at	CG13398	2.59E-03	7	0.47749	0.44141
	1631006_a_at	CG10171	1.20E-03	7	0.47724	0.44141
	1627323_at	CG8388	7.29E-03	7	0.47625	0.44141
	1640900_at	Arc42	4.50E-03	7	0.47599	0.44141
	1631367_at	CG7828	6.45E-03	7	0.47471	0.44141
	1629537_s_at	CG30394	5.38E-03	7	0.47275	0.44141
	1630340_at	CG11927	5.84E-03	7	0.47183	0.44141
	1635647_at	CG2617	8.14E-03	7	0.47102	0.44141
	1631648_at	Srp9	7.60E-03	7	0.47059	0.44141
	1626387_s_at	mei-217 /// transcript c	5.70E-03	7	0.47043	0.44141
	1638447_s_at	CG11141	9.57E-03	7	0.46933	0.44141
	1636125_a_at	granny smith	6.94E-03	7	0.46515	0.44141
	1638963_s_at	mrj	2.68E-03	7	0.46483	0.44141
	1625324_at	CG18549	1.58E-03	7	0.46355	0.44141
	1628869_s_at	CG11504	9.18E-03	7	0.45813	0.44141
	1627465_at	NF-YC-like	8.20E-03	7	0.45694	0.44141
	1625875_at	CG10635	7.02E-03	7	0.44622	0.44141
	1633980_at	CG14969	7.70E-03	7	0.44547	0.44141
	1634299_at	CG7810	8.33E-03	7	0.43948	0.44141
	1629820_at	CG14353	4.60E-03	7	0.43872	0.44141
	1641183_a_at	Phosphoethanolamine cytidylyltransferase	3.48E-03	7	0.43737	0.44141
	1640163_at	Fasciclin II	3.63E-03	7	0.43644	0.44141
	1636068_a_at	Argonaute2	7.33E-03	7	0.42904	0.44141
	1637545_at	CG1837	1.93E-03	7	0.42622	0.44141
	1629821_at	CG30055	2.88E-03	7	0.42315	0.44141
	1638113_at	beta4GalNAcTA	3.68E-03	7	0.42314	0.44141
	1630227_a_at	CG8176	4.02E-03	7	0.42259	0.44141
	1631513_at	CG30000	3.68E-03	7	0.4207	0.44141
	1632182_at	CG12096	9.93E-03	7	0.41972	0.44141
	1637738_at	Srp14	1.57E-03	7	0.41704	0.44141
	1625017_at	CG3732	4.03E-05	7	0.41688	0.44141

	1625288_at	CG33177	9.39E-03	7	0.41641	0.44141
	1636210_at	CG10191	1.53E-03	7	0.41332	0.44141
	1640734_a_at	CG16718	9.02E-03	7	0.39908	0.44141
	1633032_s_at	CG8177	5.49E-03	7	0.37483	0.44141
	1623191_at	Rab9	4.93E-03	7	0.36826	0.44141
	1624923_s_at	---	3.85E-03	7	0.36449	0.44141
	1630912_at	CG7789	8.13E-03	7	0.36394	0.44141
	1636076_a_at	turtle	2.76E-03	7	0.36388	0.44141
	1625602_at	---	2.39E-03	7	0.35889	0.44141
	1636633_at	---	7.86E-03	7	0.35476	0.44141
	1630298_at	CG30496	1.73E-04	7	0.35235	0.44141
	1625840_at	CG12001	3.13E-03	7	0.35111	0.44141
	1627405_at	CG14966	5.90E-03	7	0.35048	0.44141
	1639048_a_at	CG8229	5.08E-03	7	0.34768	0.44141
	1639193_a_at	CG1971	4.63E-04	7	0.33453	0.44141
	1641659_s_at	CG7834	1.45E-03	7	0.33297	0.44141
	1641370_s_at	CG4199	1.88E-03	7	0.3291	0.44141
	1623759_at	CG13428	2.75E-03	7	0.31938	0.44141
	1623904_a_at	Rpt3R	9.06E-03	7	0.29901	0.44141
	1638884_at	CG4238	2.63E-03	7	0.29344	0.44141
	1624689_at	---	3.37E-03	7	0.28164	0.44141
	1623137_s_at	dpr8	8.54E-03	7	0.27874	0.44141
	1626894_at	CG13165	3.58E-03	7	0.26575	0.44141
	1633869_at	deformed	5.42E-03	7	0.26175	0.44141
	1635200_at	CG11261	1.95E-03	7	0.23811	0.44141
	1626891_at	CG15414	4.69E-03	7	0.22995	0.44141
	1624797_at	CG1637	9.33E-04	7	0.21574	0.44141
	1625833_at	CG9896	5.00E-05	7	0.21552	0.44141
	1625582_at	CG32187	3.12E-03	7	0.21396	0.44141
	1626547_at	---	5.41E-03	7	0.2043	0.44141
	1623773_at	CG32082	3.79E-04	7	0.20236	0.44141
	1634388_at	CG30197	5.05E-03	7	0.19612	0.44141
	1635410_at	CG12766	4.20E-03	7	0.19223	0.44141
	1629609_at	hormone receptor	7.81E-04	7	0.18944	0.44141
	1625918_a_at	CG14346	2.36E-03	7	0.16768	0.44141
	1638074_at	CG6673	1.14E-03	7	0.16653	0.44141
	1635223_at	CG30285	7.04E-03	7	0.16604	0.44141
	1635006_at	CG7077	4.45E-03	7	0.13282	0.44141
	1635893_at	fragment I	1.21E-03	7	0.12304	0.44141
	1633631_at	CG9682	5.82E-05	7	0.10769	0.44141
	1632400_at	CG3841 /// nemo	9.00E-03	7	0.10004	0.44141

	1639906_a_at	CG7759	1.04E-03	7	0.095559	0.44141
	1624512_at	CG14496	2.70E-03	7	0.089315	0.44141
	1635793_at	CG11413	1.43E-03	7	0.086256	0.44141
	1633684_at	aldose 1-epimerase /// CG32444	8.84E-03	7	0.085185	0.44141
	1637804_at	CG18673	3.36E-03	7	0.082677	0.44141
	1631334_at	CG10260	2.80E-03	8	0.55583	0.44017
	1628969_s_at	CG8485	8.20E-03	8	0.50999	0.44017
	1623495_at	inhibitor-2	5.88E-03	8	0.46855	0.44017
	1635488_at	CG31660	7.07E-04	8	0.37632	0.44017
	1625744_at	Glutathione S transferase E6	1.02E-05	8	0.29014	0.44017
	1631761_at	CG31313	8.92E-03	9	0.48055	0.39396
	1639232_s_at	SP1029	4.86E-03	9	0.47341	0.39396
	1636338_at	CG15021	1.75E-03	9	0.39818	0.39396
	1638104_a_at	---	8.78E-03	9	0.36751	0.39396
	1640743_at	Dipeptidase C	3.57E-03	9	0.35734	0.39396
	1636853_at	CG13214	6.80E-04	9	0.28674	0.39396
	1627533_at	straightjacket	9.44E-03	10	0.46109	0.36538
	1623151_a_at	CG2010	1.91E-03	10	0.32983	0.36538
	1639490_at	Rim	3.41E-03	10	0.30522	0.36538
	1626099_s_at	Ste20-like protein kinase	6.25E-03	11	0.47153	0.36499
	1625806_at	Protein phosphatase 1alpha at 96A	6.24E-03	11	0.46641	0.36499
	1624631_x_at	---	4.07E-04	11	0.31628	0.36499
	1640625_at	CG15654	6.30E-03	11	0.30362	0.36499
	1634162_at	CG40198	8.78E-03	11	0.26712	0.36499
	1627396_a_at	lethal3Des	4.11E-03	12	0.4336	0.34787
	1633788_s_at	CG15186	4.96E-04	12	0.32848	0.34787
	1628258_at	CG14526	1.59E-04	12	0.28153	0.34787
	1640035_at	fat body protein P6	1.15E-03	13	0.47271	0.34055
	1627281_at	CG3117	8.94E-03	13	0.43384	0.34055
	1624717_s_at	CG32647	6.23E-04	13	0.40563	0.34055
	1637382_at	---	5.91E-03	13	0.34088	0.34055
	1638611_at	---	9.28E-04	13	0.29556	0.34055
	1632720_at	Lysozyme X	2.26E-04	13	0.28181	0.34055
	1630503_at	CG6045	3.89E-04	13	0.27039	0.34055
	1639402_a_at	scribble	1.36E-03	13	0.22356	0.34055
	1640627_at	dachsous	3.57E-03	14	0.45951	0.32966
	1640586_at	CG1537	5.42E-03	14	0.36088	0.32966
	1630963_at	Pheromone-binding protein-related protein 4	9.01E-04	14	0.30531	0.32966
	1641389_at	CG14959	6.85E-03	14	0.29766	0.32966

	1639530_at	CG14528	9.33E-03	14	0.22495	0.32966
	1634027_at	CG12715	2.54E-03	15	0.50404	0.3121
	1629815_at	CG33679	1.17E-03	15	0.46854	0.3121
	1626278_at	CG6180	5.73E-06	15	0.41424	0.3121
	1629802_at	CG15484	7.47E-04	15	0.40587	0.3121
	1641680_at	---	4.54E-04	15	0.40256	0.3121
	1633135_s_at	CG18854	4.29E-04	15	0.35749	0.3121
	1629752_at	CG5525	5.22E-03	15	0.35065	0.3121
	1626625_at	---	2.88E-03	15	0.34002	0.3121
	1625550_at	CR33294	6.43E-04	15	0.33463	0.3121
	1630302_at	CG8562	4.25E-05	15	0.29825	0.3121
	1631497_at	tetraspanin 42E	3.32E-05	15	0.28869	0.3121
	1640642_at	rhodopsin	2.74E-03	15	0.28233	0.3121
	1640219_at	---	5.95E-03	15	0.27416	0.3121
	1627080_at	CG17752	2.68E-04	15	0.26214	0.3121
	1639933_at	---	9.35E-03	15	0.2525	0.3121
	1636495_at	---	1.53E-04	15	0.2502	0.3121
	1639147_s_at	CG30438	3.87E-03	15	0.2305	0.3121
	1623208_at	Sox100B	5.57E-03	15	0.22374	0.3121
	1633196_at	fragment B	5.02E-03	15	0.15478	0.3121
	1634409_at	CG31775	7.74E-04	15	0.14668	0.3121
	1626554_at	CG16777	9.07E-03	16	0.4127	0.2782
	1631222_at	pou domain motif 3	2.37E-03	16	0.27601	0.2782
	1638227_at	SP1070	8.82E-04	16	0.26301	0.2782
	1628406_s_at	---	3.12E-03	16	0.25282	0.2782
	1639495_at	Cytochrome P450-9b1	2.17E-03	16	0.18645	0.2782
	1626429_at	Larval serum protein 1 gamma	7.95E-04	17	0.34054	0.25605
	1631631_at	CG13222	3.85E-03	17	0.30998	0.25605
	1641039_at	CG1397	7.31E-03	17	0.27096	0.25605
	1638063_at	CG3950	6.61E-03	17	0.23477	0.25605
	1628310_at	---	3.38E-04	17	0.20248	0.25605
	1624729_at	longitudinals absent	4.60E-03	17	0.1776	0.25605
	1625409_at	CG17086	1.70E-03	18	0.29137	0.19925
	1639418_at	Acp65Aa	9.38E-03	18	0.27105	0.19925
	1634842_a_at	CG11160	1.08E-03	18	0.27071	0.19925
	1623867_at	CG14168	1.00E-03	18	0.26888	0.19925
	1630261_a_at	troponin T	5.26E-03	18	0.24587	0.19925
	1630252_at	Ribosomal protein S11	9.15E-03	18	0.22866	0.19925
	1624137_at	CG11911	2.52E-03	18	0.22481	0.19925
	1624092_at	Jonah 99C Alpha	7.83E-03	18	0.20723	0.19925
	1627881_at	spalt	1.22E-03	18	0.19444	0.19925

	1639703_s_at	CG10936	6.16E-03	18	0.19416	0.19925
	1628547_at	CG5167	6.88E-03	18	0.18466	0.19925
	1638207_at	CG15617	1.28E-03	18	0.18425	0.19925
	1639469_a_at	GTP cyclohydrolase	3.96E-05	18	0.18394	0.19925
	1635757_at	CG11440	2.88E-03	18	0.17159	0.19925
	1629098_at	CG7631	7.69E-03	18	0.16749	0.19925
	1641629_at	CG7402	4.83E-03	18	0.1666	0.19925
	1629961_s_at	Fatty acyl CoA synthetase	3.53E-04	18	0.15455	0.19925
	1636275_a_at	CG33528	9.17E-03	18	0.14816	0.19925
	1639395_at	CG31269	9.53E-03	18	0.14473	0.19925
	1635590_a_at	CG34109	7.08E-03	18	0.14173	0.19925
	1623687_at	CG15199	4.02E-03	18	0.13943	0.19925
TAG storage	1627967_a_at	mth-like 4	4.35E-03	1	0.88682	0.88682
	1641282_at	mth-like 4	6.36E-03	1	0.88682	0.88682
	1627472_at	Annexin X	1.31E-03	2	0.80489	0.74537
	1636173_s_at	p38a MAP kinase	2.31E-03	2	0.80172	0.74537
	1625158_at	tipE homolog 4	2.81E-03	2	0.78096	0.74537
	1633971_at	CG31036	8.04E-03	2	0.75471	0.74537
	1631051_at	CG9323	8.54E-03	2	0.58456	0.74537
	1641037_at	CG32702	1.88E-04	3	0.75487	0.70415
	1623953_at	CG11592	9.03E-04	3	0.72917	0.70415
	1633789_at	Hand	7.76E-03	3	0.71469	0.70415
	1629723_at	CG14714	9.64E-03	3	0.61788	0.70415
	1629252_at	CG14657	7.34E-03	4	0.49264	0.32596
	1637194_at	wing morphogenesis defect	8.52E-03	4	0.48938	0.32596
	1629916_at	deborg	8.56E-03	4	0.47785	0.32596
	1640352_at	CG13779	7.92E-03	4	0.44752	0.32596
	1638783_at	mitochondrial ribosomal protein L48	6.75E-03	4	0.44529	0.32596
	1634899_a_at	CG6512	7.34E-03	4	0.43549	0.32596
	1634078_at	Sirt7	4.98E-03	4	0.4301	0.32596
	1624267_at	CG7182	7.64E-03	4	0.42353	0.32596
	1631632_s_at	Bicaudal-C	1.28E-03	4	0.42267	0.32596
	1635939_a_at	CG9641	4.76E-03	4	0.41611	0.32596
	1628536_s_at	CG11880	7.25E-04	4	0.40469	0.32596
	1632338_a_at	MSP protein	5.61E-03	4	0.40364	0.32596
	1639023_at	NEM-sensitive fusion protein 2	3.13E-03	4	0.37767	0.32596
	1629287_at	CG14130	5.63E-04	4	0.37539	0.32596
	1623281_s_at	His2B:CG33868 /// His2B:CG33870 ///	5.13E-03	4	0.37061	0.32596

		His2B:CG33872 /// His2B:CG33874 /// His2B:CG33876 /// His2B:CG33878 /// His2B:CG33880 /// His2B:CG33882 /// His2B:CG33884 /// His2B:CG33886 /// His2B:CG33888 /// His2B:CG33890 /// His2B:CG33892 /// His2B:CG33894 /// His2B:CG33896 /// His2B:CG33898 /// His2B:CG33900 /// His2B:CG33902 /// His2B:CG33904 /// His2B:CG33906 /// His2B:CG33908 /// His2B:CG33910 /// His2B:CG40461 /// His2B:CG17949				
	1631137_at	viral IAP-associated factor	8.97E-03	4	0.36996	0.32596
	1639727_at	CG7601	7.12E-03	4	0.36916	0.32596
	1639264_at	Dead-box-1	8.13E-03	4	0.35419	0.32596
	1640220_a_at	CG11779	3.72E-03	4	0.34509	0.32596
	1628382_at	Srp54	3.07E-03	4	0.34367	0.32596
	1624180_at	Psf3	2.97E-03	4	0.33667	0.32596
	1627898_at	dRing1	4.30E-03	4	0.32654	0.32596
	1632303_a_at	Trc8	4.18E-03	4	0.32556	0.32596
	1630705_at	CG4511	9.50E-03	4	0.31524	0.32596
	1637893_at	Vps28	1.47E-03	4	0.31128	0.32596
	1629352_at	UDP-glucose dehydrogenase	6.60E-03	4	0.27021	0.32596
	1632717_a_at	CG14341	7.88E-03	4	0.26901	0.32596
	1630842_s_at	CG32641 /// CG32640	2.95E-04	4	0.26722	0.32596
	1641486_at	CG32108	7.25E-03	4	0.26691	0.32596
	1631627_at	CG5335 /// Protein-1	6.58E-03	4	0.26366	0.32596
	1640872_at	CG6463 /// CG33493	6.36E-03	4	0.25906	0.32596
	1625027_a_at	GXIVsPLA2	6.92E-03	4	0.25689	0.32596
	1639767_at	Transient receptor potential A1	1.25E-03	4	0.25512	0.32596
	1637835_at	CG12177	9.13E-04	4	0.25302	0.32596
	1623378_at	Olig family	3.67E-03	4	0.24662	0.32596
	1637468_at	CG7516	5.71E-03	4	0.24408	0.32596
	1637051_at	RhoGAP71E	6.98E-04	4	0.24361	0.32596
	1638231_at	CG4393	9.18E-03	4	0.23788	0.32596

	1639943_at	acyl co-enzyme A oxidase	9.66E-03	4	0.22953	0.32596
	1629398_at	CG10383	7.44E-03	4	0.21243	0.32596
	1632715_at	---	1.91E-04	4	0.20343	0.32596
	1641590_at	timeless 2	8.97E-03	4	0.20277	0.32596
	1639666_at	S1P	1.59E-03	4	0.19307	0.32596
	1634445_at	CG32750	2.87E-03	4	0.15775	0.32596
	1634487_at	tau	1.55E-04	5	0.18121	0.13136
	1637928_at	Organic anion transporting polypeptide 58Db	8.27E-03	5	0.17913	0.13136
	1627292_at	CG2898	1.97E-03	5	0.17438	0.13136
	1626247_at	CG4785	1.04E-04	5	0.16928	0.13136
	1637941_at	Ribosomal protein S10a	7.42E-03	5	0.16481	0.13136
	1631666_s_at	CG9766	9.88E-03	5	0.16174	0.13136
	1623710_at	CG5847	9.85E-03	5	0.16125	0.13136
	1626632_at	---	7.15E-03	5	0.16096	0.13136
	1640774_a_at	Mob1	6.30E-03	5	0.15792	0.13136
	1629898_s_at	Gene 1	1.66E-03	5	0.15351	0.13136
	1640073_at	CG14164	1.79E-04	5	0.15207	0.13136
	1626606_at	CG10630	2.59E-03	5	0.15193	0.13136
	1629385_s_at	---	2.23E-04	5	0.14919	0.13136
	1641055_at	Protein 23	4.46E-03	5	0.14911	0.13136
	1626747_at	20s proteasome	4.60E-03	5	0.14784	0.13136
	1632149_at	CG17930	8.70E-03	5	0.14759	0.13136
	1623612_at	CG7722	2.19E-03	5	0.14399	0.13136
	1625108_a_at	lethal(2)37Bd	3.62E-03	5	0.14346	0.13136
	1636818_at	CG13133	9.72E-03	5	0.14138	0.13136
	1628353_at	CG10045 Glutathione S-transferase	8.84E-03	5	0.14076	0.13136
	1627833_at	Cellular retinaldehyde binding protein	5.56E-03	5	0.13989	0.13136
	1633356_s_at	Dalpha2	8.72E-03	5	0.13906	0.13136
	1635573_at	CG12488	3.00E-05	5	0.13896	0.13136
	1634187_x_at	---	4.60E-03	5	0.13767	0.13136
	1637732_at	Gustatory receptor 98a	6.21E-04	5	0.13689	0.13136
	1634255_at	CG12721	4.91E-03	5	0.13606	0.13136
	1637374_at	CG33779	5.76E-03	5	0.13577	0.13136
	1639436_at	protein phosphatase from PCR fragment D6	5.76E-03	5	0.13478	0.13136
	1628594_at	rutabaga	7.52E-04	5	0.13428	0.13136
	1630827_s_at	---	9.72E-03	5	0.13178	0.13136
	1634144_at	CG12958	5.30E-03	5	0.131	0.13136
	1640540_at	CG30273	8.84E-04	5	0.13086	0.13136

	1634140_a_at	CG11155	1.45E-03	5	0.12934	0.13136
	1630797_at	CG32582	5.95E-03	5	0.12926	0.13136
	1627080_at	CG17752	6.42E-03	5	0.12762	0.13136
	1630359_at	CG31810	7.26E-03	5	0.12693	0.13136
	1637103_at	CG9640	1.48E-03	5	0.12651	0.13136
	1625491_at	brain washing	3.25E-04	5	0.12645	0.13136
	1635674_at	CG6901	4.19E-03	5	0.12606	0.13136
	1629184_at	CG3819	3.96E-04	5	0.12579	0.13136
	1638856_at	0.9kb transcript /// CG2650	9.01E-03	5	0.12578	0.13136
	1625341_at	alpha1,3-fucosyltransferase	4.01E-03	5	0.12552	0.13136
	1629619_at	Tryptophanyl-tRNA synthetase	2.55E-03	5	0.12286	0.13136
	1624393_at	white	4.27E-03	5	0.12264	0.13136
	1631882_at	CG10264	2.98E-03	5	0.1223	0.13136
	1626641_s_at	Hemoglobin	1.94E-03	5	0.12193	0.13136
	1629667_at	CG14298 /// GA12887	3.52E-03	5	0.12157	0.13136
	1625785_at	lethal (1) G0196	3.44E-04	5	0.12135	0.13136
	1634674_at	CG9989	6.37E-03	5	0.11974	0.13136
	1640411_at	CG18284	9.52E-03	5	0.11954	0.13136
	1634525_at	CG7738	3.36E-03	5	0.11952	0.13136
	1640556_at	CG14606	2.50E-03	5	0.11617	0.13136
	1639638_a_at	CG14502	3.36E-03	5	0.11587	0.13136
	1634064_at	CG13311	4.05E-03	5	0.11554	0.13136
	1629716_a_at	CG13284	2.94E-03	5	0.11551	0.13136
	1637788_at	CG31176	7.91E-03	5	0.11491	0.13136
	1628100_at	CG8957	3.17E-03	5	0.11442	0.13136
	1639934_at	CG31272	5.24E-03	5	0.11411	0.13136
	1629551_s_at	CG12268	2.04E-03	5	0.11262	0.13136
	1629540_a_at	Plum	1.18E-03	5	0.11126	0.13136
	1623486_at	CG7900	6.29E-03	5	0.11025	0.13136
	1627745_s_at	---	1.01E-03	5	0.10892	0.13136
	1638253_at	CG5361	7.34E-03	5	0.10802	0.13136
	1628373_at	CG32085	7.32E-03	5	0.10768	0.13136
	1634619_at	Inositol 1,4,5-triphosphate kinase 1	3.33E-03	5	0.10734	0.13136
	1626434_s_at	pol	1.04E-04	5	0.10687	0.13136
	1622946_at	CG6908	9.37E-03	5	0.1041	0.13136
	1641280_at	Ance-4	7.25E-03	5	0.098878	0.13136
	1641242_at	Ugt86Di	4.14E-04	5	0.098596	0.13136
	1634521_at	zetaCOP	6.89E-03	5	0.094663	0.13136

Total glycerol	1632557_s_at	Rab30	1.73E-03	1	0.87088	0.82532
	1623650_a_at	PNGase	7.34E-03	1	0.84645	0.82532
	1639398_at	Hormone receptor-like in 96	4.16E-03	1	0.82799	0.82532
	1627150_at	CG3056	6.87E-04	1	0.81576	0.82532
	1638438_a_at	protein tyrosine phosphatase	6.11E-03	1	0.76551	0.82532
	1631460_s_at	Painting of fourth	6.44E-03	2	0.81789	0.77278
	1630870_s_at	septin interacting protein 2	4.43E-03	2	0.80573	0.77278
	1626387_s_at	mei-217 /// transcript c	9.24E-03	2	0.79632	0.77278
	1630207_at	CG16972	6.93E-03	2	0.76393	0.77278
	1625201_s_at	CG17230	3.84E-03	2	0.74847	0.77278
	1629427_at	CG5660	1.41E-03	2	0.70436	0.77278
	1639435_at	Ulp1	2.84E-03	3	0.81496	0.7651
	1634461_at	DISCO Interacting Protein 2	6.58E-03	3	0.81171	0.7651
	1640127_at	CG6650	5.06E-03	3	0.79382	0.7651
	1625845_s_at	Chromosomal protein D1	1.52E-03	3	0.79225	0.7651
	1638130_at	CG7379	7.37E-03	3	0.7722	0.7651
	1633767_at	heterogeneous nuclear ribonucleoprotein M	1.44E-03	3	0.75761	0.7651
	1639309_at	CG12333	8.68E-03	3	0.74828	0.7651
	1630677_at	CG15100	7.52E-03	3	0.73681	0.7651
	1630023_at	Daxx-like protein	5.39E-04	3	0.71332	0.7651
	1625658_at	CG5181	7.89E-03	3	0.71001	0.7651
	1636002_at	CG4281	9.77E-03	4	0.8211	0.74327
	1635290_at	CG11418	9.75E-03	4	0.78087	0.74327
	1629756_at	CG11092	7.25E-03	4	0.76869	0.74327
	1639553_at	CG9987	2.99E-03	4	0.75883	0.74327
	1629974_at	TBP-associated factor 150kD	4.81E-03	4	0.70923	0.74327
	1626680_at	CG9305	9.67E-03	4	0.70509	0.74327
	1622968_at	p115	8.95E-03	4	0.70352	0.74327
	1638457_at	CG18347	9.73E-03	4	0.69886	0.74327
	1633274_at	CG10158	4.87E-03	5	0.82192	0.73409
	1626985_a_at	CG7263	7.45E-03	5	0.80111	0.73409
	1624559_a_at	Myelodysplasia/myeloid leukemia factor	8.04E-03	5	0.77092	0.73409
	1626823_a_at	CG7945	6.44E-03	5	0.76413	0.73409
	1638456_at	CG8531	7.99E-03	5	0.75755	0.73409
	1623353_at	abstract	2.01E-03	5	0.74651	0.73409
	1635279_at	dpontin	6.83E-03	5	0.74337	0.73409
	1636709_at	CG8710	8.60E-03	5	0.73682	0.73409

	1636299_at	CG4707	6.28E-03	5	0.73625	0.73409
	1636877_at	CG1134	6.29E-03	5	0.72271	0.73409
	1627527_s_at	CG9992	6.92E-03	5	0.71671	0.73409
	1631367_at	CG7828	4.71E-03	5	0.70986	0.73409
	1630416_at	kin17	4.49E-04	5	0.70241	0.73409
	1625568_a_at	CG15111	2.07E-03	5	0.68059	0.73409
	1640661_at	Ack	4.67E-03	5	0.66871	0.73409
	1632482_at	CG5554	9.75E-03	5	0.66592	0.73409
	1635668_at	thoc5	5.26E-03	6	0.80487	0.73386
	1633346_at	CG7351	8.78E-03	6	0.80241	0.73386
	1628006_at	Developmental embryonic B	6.91E-03	6	0.79265	0.73386
	1628557_at	CG2063	2.27E-03	6	0.78161	0.73386
	1641160_s_at	Mediator complex subunit 31	1.63E-03	6	0.76821	0.73386
	1631853_at	CG6610	1.03E-03	6	0.76523	0.73386
	1629288_s_at	CG12608 /// CG9123	1.00E-03	6	0.76213	0.73386
	1636401_a_at	N-myristoyl transferase	5.89E-04	6	0.75534	0.73386
	1632663_at	CG7407	8.59E-03	6	0.7499	0.73386
	1633602_at	Eaf6	6.80E-03	6	0.72514	0.73386
	1625989_at	CG6144	3.67E-03	6	0.7152	0.73386
	1623726_at	CG11755	2.08E-03	6	0.71031	0.73386
	1636656_at	CG13379	2.96E-03	6	0.69596	0.73386
	1639108_at	CG31184	7.55E-03	6	0.68819	0.73386
	1637038_at	tex	4.37E-04	6	0.68702	0.73386
	1625855_at	Mediator complex subunit 30	5.39E-03	6	0.68671	0.73386
	1628943_at	Transferrin 2	3.39E-03	6	0.67686	0.73386
	1636741_s_at	Hsc70Cb	2.26E-03	6	0.64168	0.73386
	1635523_a_at	CG6479	1.97E-03	7	0.79954	0.71685
	1634209_at	pimples	3.82E-03	7	0.79687	0.71685
	1631344_at	msb1l	7.69E-03	7	0.79102	0.71685
	1627325_at	Aurora-A	7.58E-03	7	0.77087	0.71685
	1627707_at	CG3313	1.55E-03	7	0.76105	0.71685
	1624191_at	CENP-meta	2.91E-03	7	0.76078	0.71685
	1634359_a_at	Borealin	4.98E-04	7	0.75588	0.71685
	1634385_at	CG15524	7.01E-03	7	0.75297	0.71685
	1639059_s_at	exuperantia	4.61E-03	7	0.74426	0.71685
	1631249_at	CG12713	5.95E-03	7	0.74044	0.71685
	1630786_at	Chd3	4.60E-03	7	0.73502	0.71685
	1641382_at	Pms2	1.67E-04	7	0.73016	0.71685
	1628321_at	CG4788	7.68E-03	7	0.72835	0.71685
	1627897_at	CG12265	1.46E-03	7	0.72802	0.71685

	1635322_a_at	CG13322	9.14E-03	7	0.7277	0.71685
	1636533_at	Lethal hybrid rescue	1.14E-03	7	0.72649	0.71685
	1638326_at	CG7685	6.82E-03	7	0.72422	0.71685
	1641713_a_at	CG11120	3.78E-03	7	0.71846	0.71685
	1641708_at	CG34133	7.19E-03	7	0.71562	0.71685
	1631876_at	CG15266	3.29E-03	7	0.70695	0.71685
	1627340_at	lionette	2.54E-03	7	0.70563	0.71685
	1629441_at	Fermitin 2	3.67E-04	7	0.69401	0.71685
	1627532_at	Rpb4	8.12E-03	7	0.68491	0.71685
	1633231_a_at	TBP-like factor	6.04E-03	7	0.68212	0.71685
	1639037_at	CG13588	6.82E-03	7	0.6768	0.71685
	1629948_at	CG5194	1.76E-04	7	0.67155	0.71685
	1638539_at	Gene 3	2.34E-03	7	0.61184	0.71685
	1635811_at	roughex	9.77E-03	7	0.57656	0.71685
	1623250_at	CG18591	4.31E-03	7	0.57066	0.71685
	1636847_s_at	Makorin 1	2.43E-03	8	0.63484	0.48745
	1628974_at	CG32711	6.80E-03	8	0.61274	0.48745
	1632271_a_at	anon-fast-evolving-1A7	7.23E-03	8	0.60267	0.48745
	1640412_at	dNicastrin	7.54E-03	8	0.59022	0.48745
	1624353_at	CG32654	7.83E-03	8	0.58365	0.48745
	1636265_s_at	fickle	9.96E-03	8	0.56186	0.48745
	1629795_at	Protein tyrosine phosphatase-ERK/Enhancer of Ras1	9.56E-03	8	0.5534	0.48745
	1630227_a_at	CG8176	4.52E-03	8	0.52414	0.48745
	1625978_at	CG15141	8.72E-03	8	0.49672	0.48745
	1627770_at	Cyclin-dependent kinase subunit 30A	3.36E-04	8	0.46189	0.48745
	1637123_a_at	CG33281	7.82E-04	8	0.11661	0.48745
	1634418_at	CG33281	1.14E-03	8	0.11067	0.48745
	1627403_s_at	platelet-activating factor acetylhydrolase alpha subunit homolog	2.68E-03	9	0.63914	0.45811
	1627027_at	CG15168	8.74E-03	9	0.61273	0.45811
	1635429_at	CG33095	6.22E-03	9	0.60825	0.45811
	1627934_at	CG11723	6.38E-03	9	0.59384	0.45811
	1633940_at	CG9393	1.49E-03	9	0.58914	0.45811
	1625747_at	RhoGAP92B	3.87E-04	9	0.58034	0.45811
	1640619_at	CG7028	9.00E-03	9	0.57918	0.45811
	1641017_at	separation anxiety	1.83E-03	9	0.57672	0.45811
	1631049_at	Actin-related protein 14D	8.96E-03	9	0.57304	0.45811
	1641058_at	CG1319	8.98E-03	9	0.5564	0.45811
	1623722_at	no-on transient A gene	4.13E-03	9	0.55336	0.45811

	1636541_at	CG33052	7.91E-04	9	0.54283	0.45811
	1631362_at	CG11279	2.04E-03	9	0.51239	0.45811
	1627641_s_at	CG17494	1.99E-03	9	0.50574	0.45811
	1639797_at	CG34109	4.06E-03	9	0.16981	0.45811
	1638074_at	CG6673	8.95E-05	9	0.14591	0.45811
	1631882_at	CG10264	7.46E-03	9	0.14302	0.45811
	1641618_at	guanylate cyclase 99B	2.42E-03	9	0.14001	0.45811
	1634143_at	Cyp6w1	3.98E-03	9	0.082326	0.45811
	1628243_at	debra	3.72E-03	10	0.53376	0.41001
	1625757_at	GST-containing FLYWCH zinc-finger protein	8.71E-03	10	0.51203	0.41001
	1636182_a_at	CG10948	9.15E-05	10	0.49098	0.41001
	1638709_at	Apaf-1 related killer	8.97E-03	10	0.47501	0.41001
	1641281_at	CG12391	3.86E-03	10	0.43286	0.41001
	1627270_at	CG15893	4.88E-03	10	0.37852	0.41001
	1641603_s_at	gp150-like	2.38E-03	10	0.35223	0.41001
	1633927_a_at	beat-IIIc	8.91E-03	10	0.10469	0.41001
	1627605_at	E2F transcription factor 2	8.55E-03	11	0.51314	0.31354
	1631479_at	lethal(3)73Ah	1.04E-03	11	0.50875	0.31354
	1637053_at	CAS/CSE1 segregation protein	9.51E-03	11	0.50197	0.31354
	1625169_at	CG14667	7.89E-03	11	0.50181	0.31354
	1626049_at	CG13089	8.34E-03	11	0.49664	0.31354
	1624858_at	CG6693	5.10E-03	11	0.4919	0.31354
	1637716_a_at	RNA-binding protein 3	4.49E-03	11	0.48849	0.31354
	1629941_a_at	CG8044	8.95E-03	11	0.4877	0.31354
	1635003_at	CG6697	8.21E-03	11	0.48401	0.31354
	1631286_at	CG7857	3.64E-03	11	0.48228	0.31354
	1637618_at	heparan-sulfate-2-sulfotransferase	6.77E-03	11	0.48049	0.31354
	1623487_at	dalmatian	6.25E-03	11	0.47735	0.31354
	1636606_at	slalom	3.33E-04	11	0.47327	0.31354
	1627473_at	CG8090	2.02E-03	11	0.46857	0.31354
	1637191_at	Coproporphyrinogen oxidase	9.80E-03	11	0.45904	0.31354
	1636972_at	CG8003	9.62E-04	11	0.45873	0.31354
	1636929_at	aveugle	7.71E-04	11	0.44881	0.31354
	1638929_at	CG8920	4.11E-03	11	0.44877	0.31354
	1639715_at	mitochondrial ribosomal protein L11	6.54E-03	11	0.4463	0.31354
	1628418_at	abnormal oocyte	9.69E-03	11	0.4461	0.31354
	1638237_at	CG5412	9.97E-03	11	0.44237	0.31354

	1637736_a_at	polypeptide GalNAc transferase 7	1.89E-04	11	0.43908	0.31354
	1625422_at	CG4925	5.99E-03	11	0.43548	0.31354
	1635939_a_at	CG9641	5.57E-03	11	0.42884	0.31354
	1635468_a_at	CG7730	7.50E-04	11	0.42668	0.31354
	1636388_at	CG14220	2.14E-03	11	0.42509	0.31354
	1635329_at	CG7638	1.81E-03	11	0.42239	0.31354
	1635681_at	CG4615	8.00E-03	11	0.42219	0.31354
	1628560_at	CG9344	8.57E-03	11	0.41576	0.31354
	1630527_at	CG8436	1.43E-03	11	0.41549	0.31354
	1633754_at	CG5626	2.98E-03	11	0.40801	0.31354
	1632262_at	CG6833	2.57E-03	11	0.406	0.31354
	1636309_at	CG4089	4.97E-03	11	0.40047	0.31354
	1624418_s_at	bhringi	9.88E-03	11	0.39987	0.31354
	1624429_at	CG3967	8.13E-03	11	0.39412	0.31354
	1630803_at	TBP-associated factor 16kD	6.97E-03	11	0.38848	0.31354
	1633629_at	CG11030	6.22E-03	11	0.38549	0.31354
	1636636_at	CG11771	1.27E-03	11	0.36696	0.31354
	1632280_at	mitochondrial ribosomal protein S35	3.92E-03	11	0.36362	0.31354
	1631704_at	CG5805	3.38E-03	11	0.35922	0.31354
	1635299_at	Rev1	6.29E-03	11	0.33743	0.31354
	1629866_at	Suv4-20	2.04E-03	11	0.3244	0.31354
	1623285_at	CG1637	1.09E-03	11	0.32079	0.31354
	1630762_at	CG9918	6.61E-03	11	0.3119	0.31354
	1625459_at	CG3520	5.30E-03	11	0.30917	0.31354
	1637994_at	mars	6.53E-03	11	0.30032	0.31354
	1635848_at	myo-inositol-1-phosphate-synthase	4.79E-03	11	0.28556	0.31354
	1624237_at	CG15099	3.60E-03	11	0.27091	0.31354
	1633869_at	deformed	1.01E-04	11	0.26385	0.31354
	1641463_at	CG6583	5.44E-04	11	0.23872	0.31354
	1624211_at	CG9005	1.91E-03	11	0.22553	0.31354
	1637012_at	E(spl) region transcript m2	7.18E-03	11	0.22552	0.31354
	1637733_at	CG31063	9.72E-03	11	0.21987	0.31354
	1626165_at	CG3754	6.74E-03	11	0.21823	0.31354
	1641152_at	CG1718	8.53E-03	11	0.20267	0.31354
	1641265_at	tweety	4.69E-03	11	0.20016	0.31354
	1626328_at	CG32259	6.64E-03	11	0.19926	0.31354
	1641451_at	Dihydropteridine reductase	9.37E-03	11	0.15951	0.31354
	1630746_at	CG31807	3.24E-03	11	0.1593	0.31354

	1627596_at	CG4446	2.29E-03	11	0.14761	0.31354
	1631498_a_at	fru-satori	7.77E-03	11	0.13587	0.31354
	1634106_at	CG11686	1.87E-03	11	0.13175	0.31354
	1627744_at	CG15209	1.19E-03	11	0.12659	0.31354
	1632215_at	CG6296	9.37E-03	11	0.12641	0.31354
	1632260_s_at	bruno-2	3.45E-03	11	0.12566	0.31354
	1637306_at	CG31720	2.91E-03	11	0.12428	0.31354
	1628888_at	CG7180	9.68E-05	11	0.11995	0.31354
	1625728_at	CG8866	6.90E-04	11	0.11927	0.31354
	1632688_s_at	CG11594	7.96E-03	11	0.11757	0.31354
	1636407_at	goliath	7.24E-03	11	0.11301	0.31354
	1633145_at	PRGP-like	9.22E-03	11	0.11004	0.31354
	1634339_at	CG32694	4.08E-03	11	0.10846	0.31354
	1634515_at	CG8093	6.60E-03	11	0.10805	0.31354
	1623675_at	turn on sex-specificity	6.43E-03	11	0.10563	0.31354
	1640918_at	omega	7.36E-03	11	0.10394	0.31354
	1636015_s_at	CG32850	6.94E-03	11	0.1006	0.31354
	1626552_at	CG3568	7.08E-03	11	0.095984	0.31354
	1633089_a_at	longitudinals absent	1.41E-03	11	0.085364	0.31354
	1641268_at	CG13313	3.47E-03	11	0.080521	0.31354
	1626947_s_at	pleiohomeotic	4.85E-03	12	0.40288	0.22207
	1633204_a_at	CG10971	6.91E-03	12	0.3917	0.22207
	1635501_at	p120 catenin	7.09E-03	12	0.38878	0.22207
	1635863_at	CG7185	6.08E-03	12	0.37382	0.22207
	1641403_at	CG14967	8.91E-04	12	0.37095	0.22207
	1628422_s_at	CG31650	4.25E-03	12	0.36878	0.22207
	1640237_a_at	Chorion-factor-4	6.25E-03	12	0.36754	0.22207
	1632332_at	CG5447	3.60E-03	12	0.36248	0.22207
	1631677_at	Brahma associated protein 111kD	5.99E-03	12	0.34294	0.22207
	1623985_at	lethal (3) j2D3	9.68E-03	12	0.3415	0.22207
	1631784_at	CG13663	4.51E-04	12	0.3413	0.22207
	1629284_a_at	CG32387	9.34E-03	12	0.32433	0.22207
	1631006_a_at	CG10171	1.20E-03	12	0.32179	0.22207
	1635972_at	CG15014	1.66E-04	12	0.30691	0.22207
	1625566_a_at	CG9293	6.22E-03	12	0.30116	0.22207
	1638113_at	beta4GalNAcTA	6.23E-03	12	0.29694	0.22207
	1625549_at	CG5970	2.50E-03	12	0.29439	0.22207
	1624837_at	CG7506	4.85E-04	12	0.28536	0.22207
	1625602_at	---	8.84E-04	12	0.28393	0.22207
	1635908_at	CG11071	4.34E-03	12	0.28259	0.22207
	1628601_at	Kinesin-like protein at	6.16E-03	12	0.2802	0.22207

	64D				
1639155_at	---	2.28E-03	12	0.2783	0.22207
1632123_at	CG12050	7.47E-03	12	0.27794	0.22207
1630063_a_at	Eukaryotic initiation factor 3 p66 subunit	8.71E-03	12	0.27733	0.22207
1639202_a_at	CG7023	5.67E-03	12	0.27345	0.22207
1639046_at	CG5454	6.11E-04	12	0.27152	0.22207
1625495_at	CG12807	2.32E-03	12	0.27136	0.22207
1626657_a_at	wolfram syndrome 1	7.45E-03	12	0.27106	0.22207
1637336_at	CG5377	7.74E-03	12	0.27102	0.22207
1630539_at	Topoisomerase I-interacting protein	5.36E-03	12	0.26956	0.22207
1627602_at	turtle	4.50E-03	12	0.26859	0.22207
1637180_at	CG17977	8.94E-03	12	0.26771	0.22207
1639856_at	CG5727	3.06E-03	12	0.26381	0.22207
1638265_s_at	---	1.05E-03	12	0.2638	0.22207
1631051_at	CG9323	9.79E-03	12	0.26146	0.22207
1625066_at	CG13230	4.67E-03	12	0.25959	0.22207
1623375_at	CG17081	7.07E-03	12	0.25613	0.22207
1636257_at	CG10688	2.73E-03	12	0.25464	0.22207
1641279_at	bendless	4.29E-03	12	0.25095	0.22207
1631692_at	CG16798	7.50E-03	12	0.24979	0.22207
1639016_at	CG8206	3.38E-03	12	0.24517	0.22207
1635449_s_at	Dipeptidase B	9.36E-03	12	0.24387	0.22207
1629372_a_at	sec71	2.40E-03	12	0.24086	0.22207
1630112_at	CG18731	2.28E-03	12	0.23919	0.22207
1625949_at	5' gene	7.66E-04	12	0.23709	0.22207
1637032_at	beta4GalNAcTB	3.40E-03	12	0.21708	0.22207
1629924_at	CG10238	8.13E-03	12	0.20954	0.22207
1630512_at	CG8316	6.65E-03	12	0.20933	0.22207
1635137_a_at	CG5946	7.35E-03	12	0.20112	0.22207
1636764_at	CG31075	3.58E-03	12	0.19659	0.22207
1636487_at	CG7079	3.95E-04	12	0.19354	0.22207
1627935_a_at	Odorant-binding protein 59a	4.77E-03	12	0.18971	0.22207
1625883_at	CG13253	1.57E-03	12	0.18289	0.22207
1624203_s_at	gliotactin	2.51E-03	12	0.16572	0.22207
1633492_at	homogentisate 1,2-dioxygenase	4.28E-03	12	0.16396	0.22207
1635181_at	CG3337	1.32E-03	12	0.16273	0.22207
1633036_s_at	CG32495 /// Glutathione Synthetase	2.07E-04	12	0.15598	0.22207
1641675_at	medusa	7.48E-03	12	0.14817	0.22207
1637374_at	CG33779	5.06E-04	12	0.13944	0.22207

	1627785_at	tetraspanin 42E	1.69E-03	12	0.13551	0.22207
	1635611_a_at	---	4.14E-03	12	0.13546	0.22207
	1631801_at	CG15044	1.53E-03	12	0.13345	0.22207
	1640923_at	Juvenile hormone esterase duplication	3.97E-03	12	0.13264	0.22207
	1626326_at	CG31436	6.00E-03	12	0.13059	0.22207
	1633812_at	CG9080	8.27E-04	12	0.1285	0.22207
	1624509_at	hdl cuticle gene cluster.	3.43E-03	12	0.12715	0.22207
	1636750_s_at	CG13737	8.34E-03	12	0.12481	0.22207
	1625369_at	CG10877	9.12E-03	12	0.11941	0.22207
	1640045_at	CG3934	2.25E-03	12	0.1177	0.22207
	1635694_at	CG12907	1.90E-03	12	0.11616	0.22207
	1633031_at	CG8299	4.29E-03	12	0.11107	0.22207
	1628927_at	yellow-b	3.99E-04	12	0.11098	0.22207
	1634623_a_at	Cyp6a14	8.94E-03	12	0.10979	0.22207
	1637575_at	CG8028	2.51E-03	12	0.10795	0.22207
	1635467_a_at	CG7381	2.60E-03	12	0.10727	0.22207
	1640758_at	CG32613	4.12E-04	12	0.10454	0.22207
	1633862_at	CG12934	9.27E-03	12	0.10418	0.22207
	1635764_at	CG14696	5.24E-04	12	0.10351	0.22207
	1641131_at	CG5621	6.88E-03	12	0.1034	0.22207
	1623371_at	CG11126	2.29E-04	12	0.099917	0.22207
	1637687_at	cut	1.63E-03	12	0.09901	0.22207
	1637154_at	CG7470	4.34E-03	12	0.095645	0.22207
	1628657_at	Glutathione S transferase E9	4.49E-03	12	0.087924	0.22207
	1624695_at	CG3999	8.61E-03	12	0.077185	0.22207
	1638680_at	CG17282	6.43E-04	13	0.27156	0.17088
	1631196_at	Mediator complex subunit 10	2.74E-03	13	0.27102	0.17088
	1625233_at	dPI 3-kinase	9.36E-03	13	0.26257	0.17088
	1638133_at	KH domain encompassing protein 1	4.90E-03	13	0.2572	0.17088
	1632488_a_at	---	6.33E-03	13	0.25651	0.17088
	1625147_at	CG7866	1.33E-03	13	0.25428	0.17088
	1639567_at	frataxin-like	8.64E-05	13	0.2517	0.17088
	1638414_a_at	Bmcp	5.41E-03	13	0.2487	0.17088
	1628976_at	CG14222	8.01E-04	13	0.24751	0.17088
	1633418_at	CG9666	3.35E-03	13	0.24424	0.17088
	1635293_s_at	CG8336	6.21E-03	13	0.23977	0.17088
	1623383_at	dynein	2.09E-03	13	0.23677	0.17088
	1631687_at	tre oncogene-related protein	4.93E-03	13	0.23622	0.17088
	1640835_a_at	Glutamate	7.28E-03	13	0.23454	0.17088

		dehydrogenase				
	1634859_at	r2d2	7.39E-03	13	0.23348	0.17088
	1631465_at	CG3919	4.41E-03	13	0.2315	0.17088
	1637327_at	CG11376	8.69E-03	13	0.23076	0.17088
	1637473_s_at	CG18789 /// CG18787	8.93E-04	13	0.2275	0.17088
	1628990_at	columbus	8.51E-03	13	0.22612	0.17088
	1641504_s_at	windpipe	5.85E-03	13	0.22571	0.17088
	1637085_at	Cyclin-dependent kinase 5	5.46E-03	13	0.224	0.17088
	1624259_at	CG32040	1.16E-03	13	0.22378	0.17088
	1636665_at	---	8.11E-03	13	0.22221	0.17088
	1624289_at	CG10133	3.01E-03	13	0.21963	0.17088
	1640084_a_at	CG8712	7.07E-03	13	0.21852	0.17088
	1637304_at	CG14911	6.40E-03	13	0.21848	0.17088
	1626278_at	CG6180	1.24E-03	13	0.21695	0.17088
	1636076_a_at	turtle	4.98E-03	13	0.2161	0.17088
	1624380_at	CG1359	6.88E-03	13	0.21356	0.17088
	1624163_at	---	8.59E-03	13	0.2129	0.17088
	1637402_at	CG32109	2.49E-03	13	0.21171	0.17088
	1631003_at	CG31857	2.80E-03	13	0.20732	0.17088
	1641740_at	CG13499	6.10E-03	13	0.20212	0.17088
	1639296_at	RCC GEF-related protein	7.54E-03	13	0.20042	0.17088
	1635315_at	---	1.70E-04	13	0.20041	0.17088
	1631627_at	CG5335 /// Protein-1	7.71E-03	13	0.19546	0.17088
	1641526_a_at	CG31302	3.81E-03	13	0.1881	0.17088
	1640790_at	CG11293	2.10E-03	13	0.18712	0.17088
	1632847_at	CG13484	1.58E-03	13	0.18621	0.17088
	1639787_at	CG6791	7.55E-05	13	0.17987	0.17088
	1636869_at	---	5.43E-03	13	0.17856	0.17088
	1633106_at	CG6662	6.42E-05	13	0.17473	0.17088
	1627982_at	CG31077	2.30E-05	13	0.17293	0.17088
	1631435_at	CG7988	4.64E-03	13	0.16838	0.17088
	1638227_at	SP1070	2.61E-03	13	0.16249	0.17088
	1631059_at	protein kinase C	5.01E-04	13	0.16181	0.17088
	1629520_at	CG8997	4.29E-05	13	0.1594	0.17088
	1632145_a_at	CG31720	1.78E-03	13	0.15826	0.17088
	1624681_at	---	8.91E-04	13	0.15705	0.17088
	1626547_at	---	9.70E-04	13	0.15366	0.17088
	1625688_at	CG6293	1.39E-05	13	0.15339	0.17088
	1630676_at	CG30116	8.06E-03	13	0.14979	0.17088
	1637403_at	CG7953	4.58E-04	13	0.1496	0.17088

	1623869_at	C-terminal Binding Protein	5.94E-03	13	0.1474	0.17088
	1624056_a_at	CG12455	1.63E-04	13	0.14278	0.17088
	1623817_at	CG7916	1.70E-06	13	0.14235	0.17088
	1625374_at	CG9466	5.77E-03	13	0.14144	0.17088
	1633957_s_at	CG5316	4.90E-04	13	0.14045	0.17088
	1625349_at	beat-IIIc	7.86E-03	13	0.13946	0.17088
	1627816_a_at	CG32264	7.64E-03	13	0.13819	0.17088
	1625780_a_at	CG9812	7.35E-04	13	0.1381	0.17088
	1625082_a_at	CG7882	7.72E-03	13	0.13795	0.17088
	1627129_at	---	5.22E-03	13	0.1379	0.17088
	1625828_at	CG11841	1.45E-03	13	0.13696	0.17088
	1627144_at	CG10560	2.44E-03	13	0.1358	0.17088
	1638583_at	CG31103	7.67E-03	13	0.1319	0.17088
	1628476_at	PP2A B' subunit	6.13E-04	13	0.12974	0.17088
	1632498_at	CG15629	2.39E-03	13	0.12968	0.17088
	1640109_at	UDP-glycosyltransferase 37b1	2.32E-03	13	0.12967	0.17088
	1626664_at	CG3285	3.77E-03	13	0.12836	0.17088
	1628013_at	CG3597	5.69E-03	13	0.12771	0.17088
	1633474_x_at	CG32603	1.31E-03	13	0.12413	0.17088
	1640773_at	CG14821	5.18E-03	13	0.12213	0.17088
	1626949_at	CG11158	6.00E-03	13	0.11916	0.17088
	1639427_at	CG13333	1.54E-03	13	0.1188	0.17088
	1632076_a_at	CG6933	5.29E-03	13	0.11792	0.17088
	1626414_at	JH-epoxide hydrolase	3.69E-03	13	0.11778	0.17088
	1629467_at	CG32005	9.91E-04	13	0.11725	0.17088
	1634267_at	CG30495	3.26E-03	13	0.11699	0.17088
	1627837_at	CG5527	3.98E-03	13	0.11573	0.17088
	1638393_at	---	3.68E-03	13	0.11459	0.17088
	1623289_at	Pseudogene 83e	5.39E-03	13	0.11208	0.17088
	1630066_at	CG4302	5.63E-04	13	0.11114	0.17088
	1628907_at	Type III alcohol dehydrogenase	2.24E-03	13	0.10996	0.17088
	1638388_at	CG10559	1.45E-03	13	0.10968	0.17088
	1636398_at	CG10086	6.61E-03	13	0.10895	0.17088
	1630064_at	N-like	3.56E-03	13	0.10831	0.17088
	1630289_at	CG6435	3.67E-03	13	0.10702	0.17088
	1626764_at	CG10182	8.87E-03	13	0.10681	0.17088
	1635590_a_at	CG34109	8.34E-03	13	0.10565	0.17088
	1634716_s_at	CG32843	1.28E-03	13	0.099114	0.17088
	1637577_at	Zinc/iron regulated transporter-related	6.71E-03	13	0.097815	0.17088

		protein 3				
	1628284_at	CG3690	3.14E-03	13	0.096265	0.17088
	1637941_at	Ribosomal protein S10a	7.35E-03	13	0.094732	0.17088
	1634831_at	antdh	9.99E-03	13	0.092703	0.17088
Metabolic Rate	1637649_at	CG1707	2.85E-03	1	0.69113	0.56314
	1624821_at	CG8586	6.36E-04	1	0.65199	0.56314
	1623466_at	bric-a-brac	3.47E-04	1	0.64449	0.56314
	1638758_at	CG3016	6.97E-03	1	0.63836	0.56314
	1641359_at	Serine Protease 2	6.69E-03	1	0.54038	0.56314
	1623066_at	Inwardly rectifying potassium channel 3	6.43E-03	1	0.41643	0.56314
	1637146_at	---	3.22E-03	1	0.3592	0.56314
	1625270_at	CG31259	1.23E-03	2	0.53328	0.47045
	1630679_at	CG1961	2.45E-03	2	0.52025	0.47045
	1635769_at	CG8773	4.13E-03	2	0.51828	0.47045
	1638240_s_at	CG8785	9.78E-03	2	0.50996	0.47045
	1625778_at	CG4562	4.46E-03	2	0.50573	0.47045
	1638661_at	Machete	1.97E-03	2	0.46178	0.47045
	1623398_at	CG4830	9.76E-04	2	0.44596	0.47045
	1628800_at	Jonah 65A	1.35E-03	2	0.44523	0.47045
	1629566_at	CG8834	3.93E-03	2	0.44081	0.47045
	1637829_at	CG4020	3.22E-03	2	0.42715	0.47045
	1637660_at	CG5150	6.24E-03	2	0.3665	0.47045
	1641688_at	CG18136	5.86E-03	3	0.51501	0.45706
	1640736_at	Oregon-R glutamate decarboxylase	8.89E-03	3	0.46368	0.45706
	1634139_at	Cyp301a1	4.06E-06	3	0.39248	0.45706
	1630917_at	CG7889	3.93E-03	4	0.42995	0.36684
	1626182_a_at	CG5026	7.31E-03	4	0.42607	0.36684
	1627592_at	CG3295	7.13E-04	4	0.35027	0.36684
	1628018_at	hemolectin	7.72E-03	4	0.26107	0.36684
	1639401_at	hdl cuticle gene cluster.	1.52E-03	5	0.37694	0.30278
	1623521_at	CG11909	3.39E-03	5	0.36891	0.30278
	1624914_at	CG8690	1.75E-03	5	0.36351	0.30278
	1623666_at	CG10516	3.37E-04	5	0.3455	0.30278
	1625835_at	yellow-f2	8.69E-03	5	0.33398	0.30278
	1628541_at	Tak1-like 1	4.82E-03	5	0.33395	0.30278
	1631523_at	CG10824	5.46E-03	5	0.32236	0.30278
	1628503_at	CG30424	3.13E-03	5	0.31673	0.30278
	1636383_at	CG13856	4.92E-04	5	0.25425	0.30278
	1641681_s_at	CG5288	8.09E-03	5	0.23513	0.30278

	1635110_at	Cyp6a13	7.44E-03	5	0.19973	0.30278
	1636800_at	---	3.63E-03	5	0.18239	0.30278
	1639282_at	CG9220	2.68E-03	6	0.25648	0.15653
	1631148_at	CG9673	3.40E-03	6	0.2359	0.15653
	1632936_at	alpha-mannosidase	4.20E-03	6	0.23356	0.15653
	1632154_at	CG14060	6.83E-03	6	0.23347	0.15653
	1623229_at	CG7881	2.47E-03	6	0.23042	0.15653
	1639890_at	CG17508	1.75E-03	6	0.21517	0.15653
	1627277_s_at	CG2930	8.50E-03	6	0.20898	0.15653
	1636657_at	CheA87a	3.30E-03	6	0.20804	0.15653
	1628984_s_at	HMG protein Z	7.40E-03	6	0.20656	0.15653
	1631746_a_at	CG8774	3.64E-03	6	0.20329	0.15653
	1626749_a_at	CG18135	5.70E-03	6	0.19507	0.15653
	1627448_at	Updo	8.80E-04	6	0.19429	0.15653
	1630217_at	CG13650	3.92E-03	6	0.18693	0.15653
	1626837_a_at	CG32245	8.26E-03	6	0.18451	0.15653
	1635348_at	CG8086	6.03E-03	6	0.18252	0.15653
	1632100_s_at	anon-fast-evolving-2H7	3.48E-03	6	0.18112	0.15653
	1633366_at	CG12455	9.48E-03	6	0.17868	0.15653
	1636888_a_at	Alkaline phosphatase	6.77E-03	6	0.17697	0.15653
	1636728_at	Ptc-related Disp-like	4.52E-03	6	0.17189	0.15653
	1628173_at	CG17189	5.31E-03	6	0.17153	0.15653
	1624932_at	Odorant-binding protein 49a	6.13E-03	6	0.16722	0.15653
	1640249_at	CG33173	7.67E-03	6	0.16293	0.15653
	1641241_at	---	6.89E-03	6	0.15349	0.15653
	1633425_at	CG2191	9.72E-04	6	0.15325	0.15653
	1624857_at	CG17510	1.52E-03	6	0.15284	0.15653
	1637236_at	CG41136	2.17E-03	6	0.15213	0.15653
	1637620_s_at	CG9186	1.10E-03	6	0.15183	0.15653
	1634503_at	CG15695	6.99E-03	6	0.15053	0.15653
	1627040_at	CG1344	7.72E-03	6	0.14627	0.15653
	1632808_at	CG6012	8.21E-04	6	0.14315	0.15653
	1624117_at	CG18397	9.03E-04	6	0.14116	0.15653
	1637939_at	Serine protease inhibitor 1	9.21E-03	6	0.13534	0.15653
	1632791_at	CG32479	9.84E-03	6	0.13467	0.15653
	1629374_at	CG31739	7.91E-03	6	0.13333	0.15653
	1628594_at	rutabaga	5.57E-04	6	0.13142	0.15653
	1626536_at	CG6776	5.90E-03	6	0.13065	0.15653
	1628177_at	juvenile hormone acid methyltransferase	5.34E-03	6	0.13036	0.15653
	1634170_a_at	filamin	3.69E-03	6	0.12988	0.15653

	1638716_a_at	CG10725	2.88E-03	6	0.12964	0.15653
	1627604_at	CG34109	3.50E-03	6	0.12854	0.15653
	1637535_at	---	5.95E-03	6	0.12804	0.15653
	1628496_at	dpr16	1.54E-03	6	0.12605	0.15653
	1627841_at	CG11170	7.99E-04	6	0.12585	0.15653
	1635065_at	CG7025	8.54E-03	6	0.12575	0.15653
	1628150_a_at	CG9449	6.73E-03	6	0.12523	0.15653
	1628087_s_at	CG10200	1.22E-03	6	0.12488	0.15653
	1633749_at	CG6337	1.28E-03	6	0.11774	0.15653
	1640073_at	CG14164	4.83E-03	6	0.11636	0.15653
	1638330_at	Dorothy	1.78E-03	6	0.11633	0.15653
	1639982_at	CG15369	9.97E-03	6	0.11339	0.15653
	1635548_s_at	CG15281	3.07E-03	6	0.10997	0.15653
	1637103_at	CG9640	1.77E-03	6	0.109	0.15653
	1634305_at	CheB42b	9.23E-03	6	0.10613	0.15653
	1630585_s_at	---	8.51E-03	6	0.10297	0.15653
	1634276_a_at	Adenosine deaminase acting on RNA	5.10E-03	6	0.10255	0.15653
	1633300_at	CG13282	1.19E-03	6	0.10164	0.15653

APPENDIX B

SUPPLEMENTARY TABLE 2

Effects of *P[GT1]* and *PiggyBac* transposon insertional mutations in candidate genes affecting triacylglycerol storage and total glycerol. Measurements given as deviations from the co-isogenic control line (Canton S A, B or F or *w1118*). *a* is one half of the difference between the homozygous mutant and the control line. *P*-values are from analyses of variance comparing the mutant lines to their control.

Trait	Line (Background)	Candidate gene	Cytological Location	*a* (♀)	*a* (♂)	P(L)	P(S)	P(SL)	P(L)♀	P(L)♂	*P[GT1]* insertion site	*PiggyBac* transposon insertion site
TAG storage	BG01863 (B)	***Ddx1***	79D3-79D3	-0.236	-0.005	9.26E-02	**2.66E-02**	1.04E-01	**3.25E-02**	9.67E-01	1283 bp at *Ddx1* 5' side	
	BG01624 (A)	***RhoGAP71E***	71E1-71E2	-0.102	0.189	3.64E-01	1.79E-01	1.95E-01	8.67E-01	**2.53E-02**	608 bp at *RhoGAP71E* isoform C 3' side	
	BG02747 (B)	***rut***	12F4-12F4	0.417	-0.207	**1.70E-03**	7.82E-02	**4.17E-02**	**1.70E-03**	3.50E-01	24534 bp at *rut* 5' side	
	BG01214 (B)	***sgl***	65D4-65D5	-0.248	-0.204	**9.00E-04**	2.85E-01	5.84E-01	**2.60E-03**	**4.50E-02**	234 bp at *sgl* 5' side	
	19053	***SIRT7***	98F6-98F6	-0.863	-0.102	**<1.00E-04**	8.84E-01	7.86E-01	**7.00E-04**	**<1.00E-04**		98F6, at 3R:24,884,693; 5' terminus to the left.
	18363	***GXIVsPLA2***	72B2-72B2	-1.662	-1.484	**<1.00E-04**	1.18E-01	7.95E-02	**<1.00E-04**	**<1.00E-04**		72B2, at 3L:15,949,324; 5' terminus to the left.
Total glycerol	BG02565 (B)	***b4GalNAcTA***	50E6-50E6	0.132	0.494	**2.56E-02**	1.40E-01	4.63E-01	2.35E-01	**1.35E-02**	112 bp at *b4GalNAcTA* 3' side	
	BG02518 (B)	***CG8920***	56F16-56F16	2.007	0.526	**<1.00E-04**	4.23E-01	6.51E-01	**3.90E-03**	**1.80E-03**	530 bp at *CG8920* isoform B 3' side	
	BG01607 (B)	***Gli***	35D4-35D4	0.486	-0.074	**5.40E-03**	4.78E-01	3.54E-01	**2.13E-02**	2.06E-01	3082 bp at *Gli* 5' side	
	BG02181 (B)	***Gdh***	95C13-95D1	0.033	0.173	**2.00E-04**	**1.20E-03**	3.18E-01	**2.84E-02**	**1.10E-02**	4874 bp at *Gdh* 5' side	

	BG00349 (F)	***tty***	19F4-19F5	1.744	-0.198	**<1.00E-04**	2.23E-01	1.27E-01	**7.00E-04**	7.18E-01	2039 bp at *tweety* isoform B 5' side	
	BG02420 (B)	***CG5946***	68E1-68E1	-0.107	0.041	8.64E-01	6.09E-01	7.01E-01	7.68E-01	7.69E-01	intron 2 of *CG5946* isoform B and D	
Glycogen	BG00720 (F)	***Appl***	1B9-1B10	6.534	5.583	**3.00E-04**	**<1.00E-04**	7.58E-01	**1.55E-02**	**7.40E-03**	intron 2 of *Appl* isoform A	
storage	18382	***Cbp53E***	53E4-53E4	16.8202	10.751	**<1.00E-04**	6.53E-01	8.46E-01	**<1.00E-04**	**1.81E-02**		53E4, at 2R: 12,872,142; 5' terminus to the left.
	18838	***transferrin 1***	17A9-17A9	-1.2438	-4.1169	2.69E-01	2.83E-01	9.91E-01	5.50E-01	8.00E-01		17A9, at X: 18,281,636; 5' terminus to the left.
	18545	***sevenless***	10A4-10A4	13.2397	13.2998	**9.00E-04**	1.96E-01	3.11E-01	8.72E-02	**3.00E-04**		10A4, at X: 10,975,465; 5' terminus to the right.
	19205	***junctophilin***	30B10-30B12	22.4639	13.2998	**2.00E-04**	5.00E-01	3.52E-01	**<1.00E-04**	**5.70E-03**		30B12, at 2L:9,560,489; 5' terminus to the right.

APPENDIX C

SUPPLEMENTARY TABLE 3

Over-representation of Gene Ontology (GO) Categories (BP= biological processes; CC= cellular component; MF= molecular function), KEGG Pathways and Keywords for transcripts associated with quantitative traits. Count = the number of genes in the annotation category. % = the number of genes in the annotation category/total number of significant genes. The *P* value is from a modified Fisher exact test for enrichment of genes in an annotation category.

Trait	Analysis	Category	Term	Count	%	P-Value
Wet Body Weight	**Regression List**	**GO BP**	behavior	12	4.30%	4.44E-02
			response to toxin	8	2.87%	9.30E-03
			immune system process	15	5.38%	4.86E-04
			response to stimulus	46	16.49%	6.24E-06
			response to abiotic stimulus	9	3.23%	3.08E-02
			humoral immune response	7	2.51%	1.71E-02
			phototransduction	6	2.15%	5.09E-03
			visual perception	9	3.23%	1.50E-03
			detection of stimulus	7	2.51%	6.14E-03
			sensory perception	11	3.94%	6.33E-03
			immune response	13	4.66%	2.57E-04
			response to fungus	4	1.43%	5.50E-02
			response to bacterium	10	3.58%	1.93E-04
			defense response	24	8.60%	5.21E-06
			innate immune response	9	3.23%	5.57E-04
			response to chemical stimulus	16	5.73%	1.28E-02
			neurological system process	20	7.17%	6.05E-03
			response to biotic stimulus	10	3.58%	1.80E-02
			response to external stimulus	9	3.23%	2.54E-02
			G-protein coupled receptor protein signaling pathway	12	4.30%	1.11E-02
			detection of visible light	6	2.15%	9.21E-03
			detection of light stimulus	6	2.15%	9.21E-03
			detection of abiotic stimulus	6	2.15%	1.26E-02
			detection of external stimulus	7	2.51%	4.51E-03
			defense response to fungus	4	1.43%	4.49E-02
			defense response to Gram-positive bacterium	6	2.15%	4.15E-04

	defense response to bacterium	10	3.58%	8.57E-05
	defense response to Gram-negative bacterium	4	1.43%	2.43E-02
	detection of light stimulus during sensory perception	6	2.15%	8.25E-03
	ectoderm development	7	2.51%	8.80E-02
	sensory perception of light stimulus	9	3.23%	1.50E-03
	response to other organism	10	3.58%	6.49E-03
	response to light stimulus	6	2.15%	3.88E-02
	multi-organism process	12	4.30%	6.69E-03
	system process	22	7.89%	4.21E-03
	response to radiation	7	2.51%	1.97E-02
	detection of light stimulus during visual perception	6	2.15%	8.25E-03
	antibacterial humoral response	7	2.51%	3.99E-04
	detection of stimulus during sensory perception	6	2.15%	1.38E-02
	antimicrobial humoral response	7	2.51%	1.25E-02
GO CC	intrinsic to plasma membrane	15	5.38%	4.99E-05
	membrane part	44	15.77%	1.22E-03
	intrinsic to membrane	39	13.98%	1.72E-04
	extracellular region	24	8.60%	2.25E-05
	extracellular region part	7	2.51%	2.19E-02
	integral to plasma membrane	15	5.38%	4.24E-05
	extrinsic to plasma membrane	4	1.43%	3.68E-02
	plasma membrane	26	9.32%	9.84E-05
	postsynaptic membrane	4	1.43%	2.63E-02
	unlocalized protein complex	3	1.08%	4.78E-02
	plasma membrane part	22	7.89%	2.61E-06
	synapse	5	1.79%	2.76E-02
	integral to membrane	39	13.98%	1.62E-04
	synapse part	4	1.43%	2.96E-02
	receptor complex	4	1.43%	3.31E-02
	membrane	62	22.22%	8.56E-05
GO MF	neurotransmitter binding	7	2.51%	3.95E-04
	substrate specific channel activity	8	2.87%	1.78E-02
	hydrolase activity	53	19.00%	1.72E-02
	gated channel activity	8	2.87%	4.51E-03
	ligand-gated channel activity	7	2.51%	1.44E-03
	trypsin activity	7	2.51%	1.15E-02

catalytic activity	99	35.48%	4.24E-02
molecular transducer activity	25	8.96%	9.15E-04
passive transmembrane transporter activity	8	2.87%	2.31E-02
heme binding	8	2.87%	1.98E-02
tetrapyrrole binding	8	2.87%	1.98E-02
peptidoglycan binding	3	1.08%	3.94E-02
serine-type peptidase activity	14	5.02%	6.14E-03
transferase activity, transferring alkyl or aryl (other than methyl) groups	6	2.15%	2.02E-02
peptidase activity	24	8.60%	6.32E-03
transcription factor activity	13	4.66%	1.09E-02
monooxygenase activity	8	2.87%	2.20E-02
serine-type endopeptidase activity	14	5.02%	2.41E-03
extracellular ligand-gated ion channel activity	5	1.79%	1.50E-02
endopeptidase activity	17	6.09%	2.30E-02
N-acetylmuramoyl-L-alanine amidase activity	3	1.08%	3.29E-02
neurotransmitter receptor activity	7	2.51%	3.95E-04
serine hydrolase activity	14	5.02%	6.66E-03
transmembrane receptor activity	12	4.30%	2.09E-02
ligand-gated ion channel activity	7	2.51%	1.44E-03
ion channel activity	8	2.87%	1.51E-02
glutathione transferase activity	6	2.15%	3.68E-03
channel activity	8	2.87%	2.31E-02
scavenger receptor activity	4	1.43%	1.04E-02
peptide binding	4	1.43%	4.73E-02
receptor activity	21	7.53%	1.06E-04
neuropeptide hormone activity	5	1.79%	1.07E-02
signal transducer activity	25	8.96%	9.15E-04
hormone activity	5	1.79%	3.61E-02
KEGG	dme00980:Metabolism of xenobiotics by cytochrome P450	6	2.15%	2.10E-02
	dme00480:Glutathione metabolism	5	1.79%	3.38E-02
	dme00052:Galactose metabolism	4	1.43%	3.29E-02
Keywords	immune response	8	2.87%	7.76E-05
	monooxygenase	8	2.87%	1.99E-02

			antimicrobial	4	1.43%	6.89E-03
			Ionic channel	7	2.51%	1.58E-02
			Direct protein sequencing	8	2.87%	3.69E-02
			antibiotic	4	1.43%	1.24E-03
			innate immunity	8	2.87%	6.24E-05
			signal	29	10.39%	6.81E-08
			amidation	6	2.15%	5.72E-04
			Postsynaptic cell membrane	4	1.43%	2.55E-02
			Secreted	18	6.45%	1.07E-06
	Module 2	**GO BP**	response to other organism	4	80.00%	3.15E-05
			peptidoglycan catabolic process	2	40.00%	8.77E-03
			response to stimulus	5	100.00%	3.77E-04
			multi-organism process	4	80.00%	8.24E-05
			response to biotic stimulus	4	80.00%	5.17E-05
			defense response	5	100.00%	5.54E-06
			peptidoglycan metabolic process	2	40.00%	9.57E-03
			antibacterial humoral response	3	60.00%	2.07E-04
			antimicrobial humoral response	3	60.00%	7.82E-04
			defense response to bacterium	4	80.00%	5.28E-06
			carbohydrate catabolic process	2	40.00%	4.41E-02
			innate immune response	3	60.00%	7.55E-04
			response to bacterium	4	80.00%	7.20E-06
			defense response to Gram-positive bacterium	2	40.00%	1.59E-02
			humoral immune response	3	60.00%	8.93E-04
			immune system process	5	100.00%	8.00E-07
			immune response	5	100.00%	2.14E-07
			extracellular region	5	100.00%	4.20E-05
		GO MF	hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides	2	40.00%	7.39E-03
			hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds	2	40.00%	1.15E-02
			peptidoglycan binding	2	40.00%	1.63E-03
			N-acetylmuramoyl-L-alanine amidase activity	2	40.00%	1.48E-03
			pattern binding	2	40.00%	1.09E-02
		KEGG	dme00550:Peptidoglycan biosynthesis	2	40.00%	1.00E-02

	Keywords	antibiotic	2	40.00%	5.48E-03
		innate immunity	4	80.00%	4.83E-07
		immune response	4	80.00%	5.33E-07
		signal	4	80.00%	3.56E-04
		antimicrobial	2	40.00%	9.39E-03
		Secreted	4	80.00%	3.67E-05
		glycoprotein	3	60.00%	1.11E-02
Module 4	**GO BP**	antibacterial humoral response	2	33.33%	2.38E-02
		antimicrobial humoral response	2	33.33%	4.56E-02
		defense response to bacterium	2	33.33%	4.41E-02
		innate immune response	2	33.33%	4.48E-02
		defense response to fungus	2	33.33%	1.91E-02
		response to fungus	2	33.33%	2.06E-02
		response to bacterium	2	33.33%	4.87E-02
		defense response to Gram-positive bacterium	2	33.33%	1.59E-02
		humoral immune response	2	33.33%	4.87E-02
		defense response to Gram-negative bacterium	2	33.33%	1.51E-02
	Keywords	antibiotic	2	33.33%	5.48E-03
		innate immunity	2	33.33%	2.41E-02
		immune response	2	33.33%	2.49E-02
		signal	3	50.00%	1.44E-02
		antimicrobial	2	33.33%	9.39E-03
		amidation	2	33.33%	1.64E-02
Module 7	**GO BP**	response to pheromone	2	40.00%	2.20E-03
		response to chemical stimulus	2	40.00%	4.70E-02
	GO MF	pheromone binding	2	40.00%	2.40E-03
		odorant binding	2	40.00%	1.20E-02
		receptor binding	2	40.00%	4.90E-02
	Keywords	secreted	2	40.00%	3.30E-02
Module 10	**GO BP**	behavior	7	7.22%	2.21E-02
		gliogenesis	3	3.09%	2.57E-02
		calcium-mediated signaling	3	3.09%	2.80E-02
		neuropeptide signaling pathway	3	3.09%	4.97E-02
		response to abiotic stimulus	6	6.19%	9.83E-03
		transmission of nerve impulse	8	8.25%	7.60E-03

			phototransduction	4	4.12%	7.55E-03
			visual perception	6	6.19%	1.07E-03
			detection of stimulus	4	4.12%	2.16E-02
			sensory perception	6	6.19%	1.35E-02
			neurological system process	12	12.37%	8.19E-04
			ion transport	7	7.22%	3.80E-02
			response to external stimulus	6	6.19%	8.44E-03
			muscle contraction	4	4.12%	1.63E-02
			rhodopsin mediated phototransduction	3	3.09%	1.53E-02
			G-protein coupled receptor protein signaling pathway	10	10.31%	5.87E-05
			detection of visible light	4	4.12%	1.11E-02
			detection of light stimulus	4	4.12%	1.11E-02
			detection of abiotic stimulus	4	4.12%	1.35E-02
			detection of external stimulus	4	4.12%	1.83E-02
			multicellular organismal process	31	31.96%	9.82E-05
			cell surface receptor linked signal transduction	12	12.37%	7.54E-03
			organ development	14	14.43%	3.10E-02
			signal transduction	26	26.80%	1.65E-06
			cell communication	29	29.90%	6.53E-07
			detection of light stimulus during sensory perception	4	4.12%	1.03E-02
			nervous system development	11	11.34%	8.66E-03
			multicellular organismal development	21	21.65%	2.24E-02
			ectoderm development	5	5.15%	2.39E-02
			glial cell differentiation	2	2.06%	4.72E-02
			sensory perception of light stimulus	6	6.19%	1.07E-03
			response to light stimulus	4	4.12%	2.90E-02
			cell-cell signaling	8	8.25%	1.44E-02
			chemosensory behavior	4	4.12%	4.25E-02
			muscle system process	4	4.12%	1.63E-02
			intracellular signaling cascade	10	10.31%	1.58E-02
			system development	16	16.49%	3.15E-02
			hormone metabolic process	3	3.09%	2.12E-02
			system process	13	13.40%	5.45E-04
			response to radiation	5	5.15%	6.96E-03
			detection of light stimulus during visual perception	4	4.12%	1.03E-02
			learning	4	4.12%	9.56E-03
			detection of stimulus during sensory perception	4	4.12%	1.44E-02
			learning and/or memory	4	4.12%	2.05E-02

GO CC	membrane part	27	27.84%	3.28E-05
	intrinsic to membrane	22	22.68%	1.66E-04
	extracellular region part	5	5.15%	1.56E-02
	extrinsic to membrane	4	4.12%	8.18E-03
	extrinsic to plasma membrane	4	4.12%	4.05E-03
	plasma membrane	14	14.43%	9.86E-04
	plasma membrane part	12	12.37%	1.46E-04
	integral to membrane	22	22.68%	1.59E-04
	membrane	32	32.99%	1.93E-04
GO MF	neurotransmitter binding	5	5.15%	3.86E-04
	substrate specific channel activity	5	5.15%	1.48E-02
	gated channel activity	5	5.15%	6.06E-03
	ligand-gated channel activity	4	4.12%	9.74E-03
	calmodulin binding	4	4.12%	5.93E-03
	calcium ion binding	6	6.19%	2.33E-02
	neuropeptide binding	3	3.09%	1.45E-02
	sequence-specific DNA binding	5	5.15%	1.83E-02
	organic cation transmembrane transporter activity	3	3.09%	2.72E-02
	molecular transducer activity	14	14.43%	2.63E-04
	passive transmembrane transporter activity	5	5.15%	1.77E-02
	transcription regulator activity	10	10.31%	4.90E-02
	RNA polymerase II transcription factor activity	6	6.19%	3.80E-02
	transcription factor activity	9	9.28%	7.87E-04
	cation transmembrane transporter activity	7	7.22%	3.28E-02
	ion transmembrane transporter activity	10	10.31%	3.22E-03
	extracellular ligand-gated ion channel activity	3	3.09%	4.53E-02
	neurotransmitter receptor activity	5	5.15%	3.86E-04
	neuropeptide receptor activity	3	3.09%	1.45E-02
	transmembrane receptor activity	7	7.22%	1.25E-02
	ligand-gated ion channel activity	4	4.12%	9.74E-03
	ion channel activity	5	5.15%	1.33E-02
	transporter activity	13	13.40%	1.77E-02
	substrate-specific	11	11.34%	1.50E-02

			transporter activity			
			channel activity	5	5.15%	1.77E-02
			substrate-specific transmembrane transporter activity	10	10.31%	1.73E-02
			peptide receptor activity	3	3.09%	1.61E-02
			peptide binding	4	4.12%	2.89E-03
			receptor activity	10	10.31%	1.68E-03
			neuropeptide hormone activity	3	3.09%	3.81E-02
			signal transducer activity	14	14.43%	2.63E-04
		Keywords	Ionic channel	5	5.15%	5.91E-03
			receptor	6	6.19%	1.65E-02
			Homeobox	4	4.12%	1.17E-02
			calcium	5	5.15%	1.12E-02
			membrane	13	13.40%	2.83E-02
			ion transport	5	5.15%	2.64E-02
			dna-binding	8	8.25%	1.74E-02
			ion channel	2	2.06%	4.83E-02
			immunoglobulin domain	4	4.12%	3.81E-02
	Module 13	**GO BP**	response to stimulus	12	15.58%	3.03E-02
			rhodopsin biosynthetic process	2	2.60%	4.23E-02
			leukocyte activation	2	2.60%	3.40E-02
			myeloid leukocyte activation	2	2.60%	3.40E-02
			defense response	11	14.29%	2.93E-05
			proteolysis	10	12.99%	1.18E-02
			response to toxin	4	5.19%	2.26E-02
			macrophage activation	2	2.60%	3.40E-02
			immune system process	7	9.09%	1.66E-03
			immune response	5	6.49%	1.37E-02
		GO CC	intrinsic to plasma membrane	7	9.09%	2.17E-04
			intrinsic to membrane	10	12.99%	3.71E-02
			integral to plasma membrane	7	9.09%	2.00E-04
			plasma membrane	7	9.09%	3.40E-02
			plasma membrane part	7	9.09%	3.11E-03
			integrin complex	2	2.60%	4.53E-02
			integral to membrane	10	12.99%	3.65E-02
			receptor complex	3	3.90%	1.05E-02
			membrane	16	20.78%	1.38E-02
		GO MF	heme binding	4	5.19%	3.52E-02
			hydrolase activity	17	22.08%	4.19E-02
			serine-type endopeptidase	6	7.79%	1.22E-02

			activity			
			serine hydrolase activity	6	7.79%	1.96E-02
			trypsin activity	4	5.19%	1.34E-02
			scavenger receptor activity	3	3.90%	6.49E-03
			oxidoreductase activity	11	14.29%	3.76E-03
			tetrapyrrole binding	4	5.19%	3.52E-02
			endopeptidase activity	8	10.39%	1.01E-02
			glutathione transferase activity	5	6.49%	1.52E-04
			iron ion binding	5	6.49%	3.19E-02
			catalytic activity	34	44.16%	2.55E-03
			monooxygenase activity	4	5.19%	3.71E-02
			transferase activity, transferring alkyl or aryl (other than methyl) groups	5	6.49%	7.39E-04
			serine-type peptidase activity	6	7.79%	1.88E-02
			electron carrier activity	5	6.49%	2.59E-02
			peptidase activity	11	14.29%	1.99E-03
		Keywords	oxidoreductase	9	11.69%	7.64E-03
			serine protease	4	5.19%	2.68E-02
			heme	4	5.19%	3.17E-02
			iron	5	6.49%	2.95E-02
			protease	7	9.09%	4.87E-03
			monooxygenase	4	5.19%	3.17E-02
Glycogen storage	**Regression List**	**GO BP**	signal transduction	19	13.87%	1.80E-02
			neurological system process	14	10.22%	1.80E-04
			synaptic vesicle transport	4	2.92%	4.56E-02
			synaptic vesicle endocytosis	3	2.19%	3.84E-02
			system process	14	10.22%	5.11E-04
			mitochondrial DNA metabolic process	3	2.19%	2.64E-03
			multicellular organismal process	26	18.98%	4.49E-02
			acetylcholine metabolic process	2	1.46%	4.03E-02
			response to wounding	3	2.19%	1.49E-02
			mitochondrial genome maintenance	3	2.19%	3.66E-03
			cell communication	25	18.25%	8.67E-04
			response to light stimulus	4	2.92%	4.00E-02
			response to external stimulus	9	6.57%	8.06E-05
			synaptic transmission	9	6.57%	6.38E-04
			cell-cell signaling	12	8.76%	1.38E-04
			mitochondrial DNA replication	3	2.19%	2.64E-03
			response to chemical stimulus	8	5.84%	4.08E-02

			imaginal disc eversion	2	1.46%	4.03E-02
			regulation of neurotransmitter levels	6	4.38%	5.13E-03
			transmission of nerve impulse	12	8.76%	4.53E-05
			response to stimulus	17	12.41%	2.24E-02
			micropyle formation	2	1.46%	4.03E-02
		GO CC	postsynaptic membrane	3	2.19%	2.97E-02
			synapse	4	2.92%	1.19E-02
			membrane	27	19.71%	6.11E-03
			plasma membrane	10	7.30%	4.18E-02
			gamma DNA polymerase complex	3	2.19%	4.48E-04
			synapse part	3	2.19%	3.22E-02
		GO MF	hydrolase activity	27	19.71%	1.70E-02
			rhodopsin-like receptor activity	4	2.92%	2.22E-02
			molecular transducer activity	12	8.76%	1.62E-02
			neprilysin activity	3	2.19%	3.47E-02
			carboxylesterase activity	5	3.65%	3.82E-02
			signal transducer activity	12	8.76%	1.62E-02
			gamma DNA-directed DNA polymerase activity	3	2.19%	4.86E-04
			hydrolase activity, acting on ester bonds	12	8.76%	8.06E-03
			DNA-directed DNA polymerase activity	3	2.19%	4.05E-02
			metalloendopeptidase activity	4	2.92%	3.98E-02
		Keywords	membrane	17	12.41%	3.15E-03
			G-protein coupled receptor	4	2.92%	3.43E-02
			transducer	5	3.65%	1.03E-02
			hydrolase	21	15.33%	1.36E-02
	Module 6	**GO BP**	imaginal disc fusion	2	25.00%	2.40E-03
			embryonic development ending in birth or egg hatching	2	25.00%	3.76E-02
			post-embryonic development	3	37.50%	3.83E-03
			wound healing	2	25.00%	3.60E-03
			embryonic development via the syncytial blastoderm	2	25.00%	3.65E-02
			cell morphogenesis	3	37.50%	4.56E-03
			actin cytoskeleton organization and biogenesis	2	25.00%	3.61E-02
			cellular developmental process	3	37.50%	1.83E-02

			actin filament-based process	2	25.00%	3.69E-02
			response to wounding	2	25.00%	5.59E-03
			morphogenesis of an epithelium	2	25.00%	4.23E-02
			anatomical structure development	3	37.50%	3.28E-02
			stress-activated protein kinase signaling pathway	2	25.00%	1.32E-02
			response to external stimulus	2	25.00%	4.20E-02
			imaginal disc development	3	37.50%	2.91E-03
			aging	2	25.00%	1.71E-02
			eggshell chorion formation	2	25.00%	1.59E-02
			chorion-containing eggshell formation	2	25.00%	1.95E-02
			imaginal disc eversion	2	25.00%	1.20E-03
			imaginal disc morphogenesis	3	37.50%	2.02E-03
			cellular structure morphogenesis	3	37.50%	4.56E-03
			organ morphogenesis	3	37.50%	3.17E-03
			metamorphosis	3	37.50%	2.22E-03
			organ development	3	37.50%	1.56E-02
			establishment of tissue polarity	2	25.00%	1.47E-02
			micropyle formation	2	25.00%	1.20E-03
			dorsal appendage formation	2	25.00%	7.59E-03
			instar larval or pupal development	3	37.50%	3.71E-03
			larval development	3	37.50%	2.57E-03
			actin filament organization	2	25.00%	2.19E-02
			multicellular organismal development	3	37.50%	4.63E-02
			ectoderm development	2	25.00%	3.65E-02
			larval development (sensu Amphibia)	3	37.50%	2.22E-03
			MAPKKK cascade	2	25.00%	2.43E-02
			dorsal closure	2	25.00%	1.99E-02
			morphogenesis of embryonic epithelium	2	25.00%	2.35E-02
			cell differentiation	3	37.50%	1.77E-02
			multicellular organismal aging	2	25.00%	1.71E-02
			ovarian follicle cell development	2	25.00%	4.08E-02
			cell development	3	37.50%	1.25E-02
			eggshell formation	2	25.00%	1.95E-02
			JNK cascade	2	25.00%	1.28E-02
			embryonic morphogenesis	2	25.00%	3.53E-02
			anatomical structure morphogenesis	3	37.50%	1.48E-02

			protein kinase cascade	2	25.00%	3.14E-02
			morphogenesis of a polarized epithelium	2	25.00%	1.67E-02
			system development	3	37.50%	2.31E-02
			establishment of planar polarity	2	25.00%	1.47E-02
			determination of adult life span	2	25.00%	1.71E-02
	Module 7	**GO BP**	behavior	5	14.71%	6.50E-03
			transmission of nerve impulse	11	32.35%	1.35E-09
			cell morphogenesis	5	14.71%	4.92E-02
			acetylcholine metabolic process	2	5.88%	1.25E-02
			anatomical structure development	10	29.41%	7.39E-03
			neurological system process	11	32.35%	1.16E-07
			response to external stimulus	4	11.76%	9.33E-03
			G-protein coupled receptor protein signaling pathway	4	11.76%	2.33E-02
			cellular structure morphogenesis	5	14.71%	4.92E-02
			developmental process	13	38.24%	1.83E-03
			multicellular organismal process	16	47.06%	2.44E-05
			cell surface receptor linked signal transduction	6	17.65%	2.29E-02
			regulated secretory pathway	3	8.82%	3.51E-02
			energy taxis	2	5.88%	3.72E-02
			membrane invagination	4	11.76%	4.63E-02
			organ development	8	23.53%	1.10E-02
			signal transduction	11	32.35%	6.78E-04
			endocytosis	4	11.76%	4.63E-02
			neurotransmitter metabolic process	2	5.88%	4.93E-02
			cell communication	16	47.06%	1.95E-07
			biological regulation	13	38.24%	1.67E-03
			multicellular organismal development	11	32.35%	6.95E-03
			phototaxis	2	5.88%	3.72E-02
			synaptic vesicle transport	3	8.82%	2.49E-02
			regulation of neurotransmitter levels	5	14.71%	3.41E-04
			synaptic vesicle endocytosis	3	8.82%	4.03E-03
			neurotransmitter secretion	3	8.82%	3.51E-02
			synaptic transmission	8	23.53%	1.12E-06
			cell-cell signaling	11	32.35%	4.48E-09
			regulation of biological process	12	35.29%	3.07E-03
			cell development	7	20.59%	2.38E-02

			locomotory behavior	3	8.82%	3.60E-02
			anatomical structure morphogenesis	8	23.53%	9.52E-03
			intracellular signaling cascade	6	17.65%	1.03E-02
			system development	10	29.41%	2.17E-03
			system process	11	32.35%	3.15E-07
			generation of a signal involved in cell-cell signaling	3	8.82%	3.79E-02
		GO CC	postsynaptic membrane	3	8.82%	3.67E-03
			synapse	4	11.76%	5.14E-04
			membrane	12	35.29%	8.66E-03
			plasma membrane	8	23.53%	5.09E-04
			synapse part	3	8.82%	4.01E-03
		GO MF	molecular transducer activity	9	26.50%	1.10E-04
			signal transducer activity	9	26.50%	1.10E-04
			receptor activity	6	17.60%	3.20E-03
			neurotransmitter binding	3	8.80%	7.40E-03
			neurotransmitter receptor activity	3	8.80%	7.40E-03
			phospholipid binding	3	8.80%	7.80E-03
			lipid binding	3	8.80%	2.80E-02
		Keywords	synapse	3	8.80%	7.60E-03
			transmembrane protein	3	8.80%	1.30E-02
			cell junction	3	8.80%	1.50E-02
			membrane	7	20.60%	1.80E-02
			transducer	3	8.80%	2.50E-02
			neurotransmitter receptor	2	5.90%	3.30E-02
			phosphoprotein	4	11.80%	4.20E-02
			alternative splicing	5	14.70%	4.90E-02
Total proteins	**Regression List**	**GO BP**	cell projection morphogenesis	12	3.92%	4.17E-02
			cell part morphogenesis	12	3.92%	4.17E-02
			cellular macromolecule metabolic process	60	19.61%	1.77E-02
			RNA metabolic process	36	11.76%	2.49E-02
			biopolymer modification	28	9.15%	4.06E-02
			biopolymer metabolic process	71	23.20%	1.85E-03
			nervous system development	19	6.21%	3.44E-02
			protein modification process	28	9.15%	2.37E-02
			embryonic development via the syncytial blastoderm	8	2.61%	2.80E-02

			cellular component organization and biogenesis	57	18.63%	1.68E-02
			gene expression	45	14.71%	1.35E-02
			morphogenesis of an epithelium	9	2.94%	2.13E-02
			post-translational protein modification	24	7.84%	1.71E-02
			cell projection organization and biogenesis	12	3.92%	4.17E-02
			embryonic development ending in birth or egg hatching	8	2.61%	3.26E-02
			macromolecule metabolic process	97	31.70%	8.70E-03
			cellular protein metabolic process	59	19.28%	9.01E-03
			protein metabolic process	60	19.61%	1.03E-02
		GO Cellular Component	organelle lumen	25	8.17%	9.89E-04
			intracellular non-membrane-bound organelle	28	9.15%	1.44E-02
			nucleolar part	3	0.98%	4.08E-02
			nuclear part	24	7.84%	3.27E-02
			membrane-enclosed lumen	25	8.17%	9.89E-04
			non-membrane-bound organelle	28	9.15%	1.44E-02
			intracellular part	92	30.07%	2.21E-02
			intracellular organelle part	46	15.03%	4.33E-02
			organelle part	46	15.03%	4.52E-02
			chromosome	14	4.58%	1.20E-02
			intracellular	101	33.01%	1.01E-02
			mitochondrial matrix	9	2.94%	3.78E-02
			nuclear lumen	16	5.23%	1.47E-02
			mitochondrial lumen	9	2.94%	3.78E-02
		GO MF	RNA polymerase II transcription factor activity	12	3.92%	1.86E-02
			transcription regulator activity	22	7.19%	3.66E-02
			structural constituent of ribosome	7	2.29%	4.94E-02
		Keywords	nucleus	27	8.82%	8.61E-03
			metalloprotease	6	1.96%	2.21E-02
			ribonucleoprotein	6	1.96%	3.94E-02
			Transcription regulation	14	4.58%	2.01E-02
			Transcription	14	4.58%	2.39E-02
	Module 7	**GO BP**	cell projection morphogenesis	8	4.91%	2.96E-02
			cell part morphogenesis	8	4.91%	2.96E-02

			regulation of programmed cell death	5	3.07%	3.55E-02
			neuromuscular junction development	3	1.84%	2.88E-02
			biopolymer metabolic process	35	21.47%	2.99E-02
			reproduction	13	7.98%	3.23E-02
			nervous system development	13	7.98%	8.94E-03
			neuron morphogenesis during differentiation	8	4.91%	1.15E-02
			muscle development	7	4.29%	8.86E-03
			neuron development	8	4.91%	1.35E-02
			cell development	16	9.82%	2.67E-02
			cell death	7	4.29%	3.88E-02
			death	7	4.29%	3.99E-02
			cellular morphogenesis during differentiation	8	4.91%	3.34E-02
			system development	19	11.66%	4.67E-02
			cellular component organization and biogenesis	31	19.02%	2.11E-02
			programmed cell death	7	4.29%	3.49E-02
			neurite development	8	4.91%	1.15E-02
			regulation of gene expression	15	9.20%	4.37E-02
			gene silencing	4	2.45%	4.83E-02
			dendrite morphogenesis	5	3.07%	2.41E-02
			neurite morphogenesis	8	4.91%	1.15E-02
			neuron differentiation	8	4.91%	1.82E-02
			dendrite development	5	3.07%	2.41E-02
			cell projection organization and biogenesis	8	4.91%	2.96E-02
			neurogenesis	8	4.91%	4.40E-02
			generation of neurons	8	4.91%	3.50E-02
			cellular protein metabolic process	30	18.40%	4.27E-02
		GO CC	organelle lumen	13	7.98%	1.89E-02
			chromosomal part	7	4.29%	4.00E-02
			membrane-enclosed lumen	13	7.98%	1.89E-02
			intracellular part	49	30.06%	2.64E-02
			chromosome	10	6.13%	4.72E-03
			cell part	64	39.26%	1.02E-02
			cell	64	39.26%	1.02E-02
			intracellular	55	33.74%	3.85E-03
			mitochondrial matrix	6	3.68%	4.36E-02
			mitochondrial lumen	6	3.68%	4.36E-02
		GO MF	protein binding	65	39.88%	2.85E-02
			transcription regulator activity	14	8.59%	2.35E-02

TAG storage	**Regression List**	**GO BP**	response to stress	8	6.20%	1.84E-02
			response to heat	4	3.10%	1.01E-02
			response to temperature stimulus	4	3.10%	1.40E-02
			lipid metabolic process	10	7.75%	3.59E-02
		GO CC	membrane part	21	16.28%	5.97E-03
			integral to membrane	17	13.18%	1.13E-02
			integral to plasma membrane	7	5.43%	6.32E-03
			intrinsic to plasma membrane	7	5.43%	6.77E-03
			intrinsic to membrane	17	13.18%	1.16E-02
		GO MF	eye pigment precursor transporter activity	2	1.60%	4.20E-02
		Keywords	transmembrane	16	12.40%	6.80E-03
			glycoprotein	9	7.00%	3.70E-02
	Module 4	**GO BP**	negative regulation of cellular metabolic process	4	8.33%	4.62E-02
			regulation of translation	3	6.25%	1.45E-02
			negative regulation of biological process	6	12.50%	2.08E-02
			regulation of oskar mRNA translation	2	4.17%	3.43E-02
			response to temperature stimulus	3	6.25%	1.81E-02
			response to heat	3	6.25%	1.45E-02
			regulation of biosynthetic process	3	6.25%	1.72E-02
			chromatin assembly	3	6.25%	4.26E-02
			cellular component assembly	6	12.50%	2.58E-02
			regulation of cellular biosynthetic process	3	6.25%	1.72E-02
			negative regulation of oskar mRNA translation	2	4.17%	2.87E-02
		KEGG	alpha-linolenic acid metabolism	2	4.20%	3.80E-02
Total glycerol	**Regression List**	**GO BP**	M phase	18	4.51%	3.21E-02
			DNA metabolic process	21	5.26%	4.25E-02
			apoptosis	11	2.76%	1.14E-02
			death	13	3.26%	3.35E-02
			RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	11	2.76%	1.95E-02

			adult locomotory behavior	4	1.00%	4.21E-02
			biopolymer metabolic process	82	20.55%	2.52E-02
			RNA splicing, via transesterification reactions	11	2.76%	1.95E-02
			cell cycle phase	19	4.76%	2.62E-02
			cell death	13	3.26%	3.22E-02
			RNA splicing	12	3.01%	1.14E-02
			mRNA processing	15	3.76%	1.63E-02
			mRNA metabolic process	15	3.76%	2.39E-02
			nuclear mRNA splicing, via spliceosome	11	2.76%	1.95E-02
			macromolecule metabolic process	118	29.57%	4.12E-02
		GO CC	intracellular part	121	30.30%	2.40E-03
			intracellular	129	32.30%	5.70E-03
			intracellular organelle part	60	15.00%	1.60E-02
			organelle part	60	15.00%	1.70E-02
			nucleus	62	15.50%	1.80E-02
			chromosome	16	4.00%	1.90E-02
			polytene chromosome	7	1.80%	2.00E-02
			spliceosome	8	2.00%	4.10E-02
			snRNP U6	3	0.80%	4.20E-02
			cytoplasm	66	16.50%	4.30E-02
			ribonucleoprotein complex	15	3.80%	4.60E-02
		GO MF	RNA polymerase II transcription factor activity	16	4.00%	6.80E-03
			general RNA polymerase II transcription factor activity	8	2.00%	4.00E-02
			structure-specific DNA binding	4	1.00%	4.60E-02
		Keywords	Transcription regulation	22	5.50%	1.30E-04
			Transcription	21	5.30%	4.90E-04
			nucleus	35	8.80%	1.50E-03
			zinc-finger	22	5.50%	1.60E-03
			alternative splicing	25	6.30%	7.60E-03
			cytoplasm	17	4.30%	1.80E-02
			dna-binding	16	4.00%	4.30E-02
			rna-binding	8	2.00%	4.30E-02
Metabolic Rate	**Regression List**	**GO BP**	pigmentation during development	4	4.10%	1.50E-02
			pigmentation	4	4.10%	1.50E-02
			proteolysis	11	11.30%	2.10E-02
			nucleobase catabolic process	2	2.10%	2.20E-02
			uracil metabolic process	2	2.10%	2.20E-02

			beta-alanine biosynthetic process	2	2.10%	2.20E-02
			uracil catabolic process	2	2.10%	2.20E-02
			beta-alanine metabolic process	2	2.10%	2.20E-02
			pyrimidine base catabolic process	2	2.10%	2.20E-02
			nonprotein amino acid biosynthetic process	2	2.10%	2.20E-02
			carbohydrate metabolic process	9	9.30%	2.40E-02
			amine metabolic process	8	8.20%	2.90E-02
			metabolic process	45	46.40%	3.00E-02
			nitrogen compound metabolic process	8	8.20%	3.20E-02
			secondary metabolic process	4	4.10%	4.70E-02
		GO CC	membrane	17	17.50%	2.00E-02
		GO MF	catalytic activity	48	49.50%	1.30E-04
			hydrolase activity	25	25.80%	5.10E-03
			alpha-glucosidase activity	3	3.10%	6.90E-03
			hydrolase activity,acting on ester bonds	10	10.30%	1.70E-02
			glucosidase activity	3	3.10%	1.90E-02
			peptidase activity	11	11.30%	2.10E-02
			aspartate 1-decarboxylase activity	2	2.10%	2.10E-02
			carboxy-lyase activity	3	3.10%	2.20E-02
			cysteine-type peptidase activity	4	4.10%	2.50E-02
			lyase activity	5	5.20%	2.80E-02
			glutamate decarboxylase activity	2	2.10%	3.20E-02
			phosphoric ester hydrolase activity	6	6.20%	3.30E-02
			carbon-carbon lyase activity	3	3.10%	3.80E-02
			glutamyl aminopeptidase activity	2	2.10%	4.20E-02
		Keywords	hydrolase	19	19.60%	3.50E-03

APPENDIX D

SUPPLEMENTARY TABLE 4

Analysis of modules of correlated transcripts associated with each of the mitochondrial bioenergetic traits.The P-values statistics are from the regression analyses. Degree = the average correlation of a transcript with all other transcripts in its module. Average Degree = the average correlation of all transcripts in the module.

Trait	Probe Set ID	Gene Name	*P*-Value	Module	Average degree	Degree
Mitochondrial state 3 respiration rate	1625287_at	CG40178	9.50E-03	1	0.94456	0.94456
	1625862_x_at	CG40178	7.96E-03	1	0.94456	0.94456
	1626022_at	Cyp12e1	5.78E-03	2	0.88202	0.88202
	1637309_a_at	Cyp12e1	7.47E-04	2	0.88202	0.88202
	1623431_at	CG13423	4.81E-03	3	0.65344	0.65344
	1640942_at	CG3251	8.02E-03	3	0.65344	0.65344
	1622906_at	CG13200	3.91E-03	4	0.59399	0.59399
	1636961_a_at	CG9027	4.95E-03	4	0.59399	0.59399
	1626745_at	Sucb	3.48E-03	5	0.4957	0.48832
	1637251_a_at	Sucb	7.76E-03	5	0.4957	0.61153
	1639001_a_at	Esterase-9	8.99E-03	5	0.4957	0.38726
	1633021_s_at	Spinophilin /// CG32295	4.28E-03	6	0.47133	0.41618
	1633491_s_at	CG31866 /// CG31865	1.78E-03	6	0.47133	0.57733
	1637473_s_at	CG18789 /// CG18787	6.33E-03	6	0.47133	0.48019
	1638190_at	CG9667	4.54E-04	6	0.47133	0.41163
	1623291_at	CG31673	3.11E-04	7	0.46977	0.5153
	1625334_at	Niemann-Pick Type C-2	6.11E-03	7	0.46977	0.45874
	1625901_s_at	---	3.51E-03	7	0.46977	0.55241
	1628272_a_at	CG40486	5.12E-03	7	0.46977	0.32555
	1630320_at	Trypsin	3.18E-03	7	0.46977	0.51922
	1635498_at	CG9259	3.21E-03	7	0.46977	0.4272
	1637767_at	CG3305	8.64E-03	7	0.46977	0.59684
	1639286_s_at	CG31689	3.53E-03	7	0.46977	0.36294
	1626234_at	---	6.80E-03	8	0.45645	0.57529
	1630095_a_at	Derailed 2	2.16E-03	8	0.45645	0.37408

	1632960_at	Eclosion hormone	3.96E-03	8	0.45645	0.54295
	1637815_s_at	Connector of kinase to AP-1	4.34E-04	8	0.45645	0.33347
	1631680_a_at	CG31878	7.33E-03	9	0.43196	0.51831
	1633738_at	Gustatory receptor 64f	3.04E-03	9	0.43196	0.51418
	1636524_at	---	5.89E-03	9	0.43196	0.40423
	1639971_at	CG12224	1.81E-03	9	0.43196	0.32496
	1640598_s_at	---	6.18E-03	9	0.43196	0.39812
	1625338_at	Cyp4d20	6.39E-03	10	0.41735	0.3123
	1626251_at	Rhodopsin 4	3.44E-03	10	0.41735	0.55864
	1638021_at	CG4757	2.87E-04	10	0.41735	0.26842
	1640642_at	Rhodopsin 6	5.34E-05	10	0.41735	0.53006
	1623791_s_at	CG32673 /// Rab9D /// CG9807 /// CG32671	2.29E-03	11	0.40166	0.54079
	1623834_at	CG18641	9.09E-03	11	0.40166	0.226
	1623868_a_at	CG30275	9.72E-03	11	0.40166	0.50726
	1624212_at	CG6784	6.23E-03	11	0.40166	0.17934
	1624514_at	Vha16-3	2.92E-03	11	0.40166	0.50481
	1625135_at	Chibby	8.89E-03	11	0.40166	0.33967
	1625248_at	Ribosomal protein S29	8.63E-03	11	0.40166	0.40229
	1625339_at	CG31677	6.08E-03	11	0.40166	0.3218
	1625630_at	---	9.99E-04	11	0.40166	0.38621
	1625635_at	CG9316	9.62E-03	11	0.40166	0.46233
	1625933_at	CG14731	5.19E-03	11	0.40166	0.48958
	1626462_at	CG7691	8.58E-03	11	0.40166	0.34101
	1626511_at	CG32305	1.39E-04	11	0.40166	0.27407
	1626800_at	CG11630	8.75E-03	11	0.40166	0.40105
	1627033_at	CG11048	2.98E-03	11	0.40166	0.13819
	1627166_at	CG17287	7.04E-03	11	0.40166	0.42103
	1627437_at	ref2	6.71E-03	11	0.40166	0.52743
	1627457_at	CG10793	3.23E-04	11	0.40166	0.48749
	1627891_at	CG12725	8.57E-03	11	0.40166	0.52456
	1628225_at	CG32391	1.77E-03	11	0.40166	0.47204
	1629253_at	RabX2	7.30E-03	11	0.40166	0.28062
	1629698_at	CG3515	8.87E-03	11	0.40166	0.36898
	1630834_at	Stromalin-2	5.22E-03	11	0.40166	0.26256
	1631032_at	CG14659	2.13E-03	11	0.40166	0.4914
	1631171_at	CheB93a	6.10E-03	11	0.40166	0.27437
	1631270_at	no hitter	7.58E-03	11	0.40166	0.51473
	1631293_at	CG31600	7.83E-03	11	0.40166	0.34408
	1631598_at	CG3184	3.11E-03	11	0.40166	0.31598

	1632696_at	CG15323	1.63E-03	11	0.40166	0.49479
	1633211_a_at	CG7795	2.71E-03	11	0.40166	0.25389
	1634214_a_at	Copper transporter 1C	8.17E-04	11	0.40166	0.52202
	1634422_at	CG31213	6.79E-03	11	0.40166	0.45462
	1635108_at	CG5964	2.87E-03	11	0.40166	0.36253
	1635201_at	CG15824	5.62E-03	11	0.40166	0.3793
	1635345_at	---	7.96E-03	11	0.40166	0.42701
	1635607_at	CG31742	7.69E-03	11	0.40166	0.3763
	1636862_at	CG11322	8.29E-05	11	0.40166	0.4357
	1636871_at	CG12943	8.63E-03	11	0.40166	0.50943
	1637906_at	---	2.49E-03	11	0.40166	0.50249
	1637919_at	CG15233	5.09E-03	11	0.40166	0.40026
	1638793_at	CG31406	6.35E-03	11	0.40166	0.37389
	1639201_at	---	5.39E-03	11	0.40166	0.41267
	1639670_at	cannonball	2.64E-03	11	0.40166	0.37157
	1639781_at	CG13402	5.10E-03	11	0.40166	0.50527
	1639848_at	matotopetli	3.98E-03	11	0.40166	0.54725
	1639996_at	tombola	1.12E-04	11	0.40166	0.41411
	1641690_a_at	CG14929	9.50E-03	11	0.40166	0.33534
	1625325_s_at	simjang	7.64E-03	12	0.38024	0.35729
	1629914_at	CG14328	1.13E-03	12	0.38024	0.39577
	1631843_at	CG6749	7.13E-03	12	0.38024	0.45758
	1632648_at	---	7.08E-03	12	0.38024	0.44952
	1634370_a_at	pannier	3.28E-03	12	0.38024	0.24107
	1630486_at	Bteb2	1.16E-03	13	0.37362	0.46285
	1633379_s_at	cap-n-collar	5.31E-03	13	0.37362	0.40461
	1635544_s_at	---	2.37E-06	13	0.37362	0.31058
	1638090_at	CG4592	1.15E-03	13	0.37362	0.4798
	1638381_s_at	CG32506 /// CG1695	4.49E-03	13	0.37362	0.48301
	1638756_at	CG17279	7.81E-03	13	0.37362	0.27366
	1639295_at	CG4734	5.50E-03	13	0.37362	0.20084
	1623602_a_at	Protein tyrosine phosphatase 69D	4.65E-03	14	0.3409	0.34263
	1625147_at	CG7866	7.63E-03	14	0.3409	0.38545
	1627452_a_at	lethal (3) malignant brain tumor	2.33E-03	14	0.3409	0.44453
	1629950_at	CG1785	6.02E-03	14	0.3409	0.39044
	1634412_at	CG2135	4.74E-03	14	0.3409	0.40863
	1638892_at	CG5397	8.93E-04	14	0.3409	0.16295
	1640281_s_at	CG17129	3.41E-03	14	0.3409	0.25166
	1624939_at	Cyp6a21	8.70E-03	15	0.32538	0.10746

	1625486_a_at	Peptidoglycan recognition protein LA	2.06E-03	15	0.32538	0.4523
	1626600_at	CG30016	7.45E-03	15	0.32538	0.36162
	1628469_a_at	CG32529	2.84E-05	15	0.32538	0.19989
	1628547_at	CG5167	3.80E-03	15	0.32538	0.48116
	1629476_at	CG7542	7.92E-04	15	0.32538	0.38777
	1630528_at	CG17751	2.57E-03	15	0.32538	0.20058
	1632870_at	CG32364	7.71E-03	15	0.32538	0.30596
	1633499_at	retinin /// CG33060 /// CG33061	3.67E-05	15	0.32538	0.34354
	1634455_at	CG14406	1.86E-03	15	0.32538	0.38561
	1635757_at	CG11440	1.33E-03	15	0.32538	0.4606
	1635975_s_at	---	1.87E-03	15	0.32538	0.1295
	1638485_s_at	SP2637	7.59E-03	15	0.32538	0.38902
	1639594_at	CG40485	1.46E-03	15	0.32538	0.40043
	1640515_s_at	liquid facets	5.17E-03	15	0.32538	0.49865
	1640755_at	Cytochrome P450-6a8	2.47E-03	15	0.32538	0.2129
	1641239_at	---	5.67E-03	15	0.32538	0.21447
	1623035_at	Na<up>+</up>-dependent inorganic phosphate cotransporter	8.19E-04	16	0.32512	0.25807
	1623697_s_at	CG33465	5.54E-03	16	0.32512	0.32222
	1624233_at	Gustatory receptor 8a	7.77E-03	16	0.32512	0.46252
	1624384_at	CG13749	4.84E-03	16	0.32512	0.23201
	1624402_at	CG17906	1.34E-03	16	0.32512	0.36363
	1624442_at	CG12586	2.88E-03	16	0.32512	0.26553
	1624626_at	---	1.63E-04	16	0.32512	0.3524
	1624964_at	CG17105	5.87E-03	16	0.32512	0.35923
	1625092_at	methuselah-like 6	7.58E-03	16	0.32512	0.31483
	1625445_s_at	schnurri	7.76E-05	16	0.32512	0.33617
	1625577_at	CCK-like receptor at 17D3	2.13E-03	16	0.32512	0.39578
	1625832_at	---	5.59E-03	16	0.32512	0.23622
	1626009_s_at	CG30427	5.42E-03	16	0.32512	0.31351
	1626243_at	CG11626	6.89E-03	16	0.32512	0.22473
	1626255_at	Gustatory receptor 61a	1.19E-03	16	0.32512	0.10225
	1626311_at	CG15088	6.39E-03	16	0.32512	0.40671
	1626362_at	missing oocyte	9.48E-03	16	0.32512	0.29741
	1626753_at	CG12947	1.31E-03	16	0.32512	0.30143
	1627011_at	---	7.99E-03	16	0.32512	0.35658

	1627225_at	Tetraspanin 26A	9.46E-04	16	0.32512	0.25483
	1627548_at	CG12541	7.39E-03	16	0.32512	0.32187
	1627935_a_at	Odorant-binding protein 59a	1.04E-03	16	0.32512	0.25974
	1628086_at	CG5369	3.03E-03	16	0.32512	0.26478
	1628436_at	CG13203	5.94E-03	16	0.32512	0.26581
	1628950_at	CG10178	8.51E-03	16	0.32512	0.13046
	1629024_at	pickpocket 25	3.07E-03	16	0.32512	0.43774
	1629105_at	CG16735	1.97E-03	16	0.32512	0.49859
	1629174_at	CG30264	8.55E-03	16	0.32512	0.35726
	1630037_at	Odorant receptor 59b	4.32E-03	16	0.32512	0.412
	1630061_at	---	6.92E-03	16	0.32512	0.35639
	1630762_at	CG9918	2.66E-03	16	0.32512	0.38247
	1631748_at	---	8.42E-04	16	0.32512	0.45922
	1632183_at	roughoid	7.37E-05	16	0.32512	0.41796
	1632808_at	CG6012	5.17E-03	16	0.32512	0.22544
	1632823_at	CG17788	9.33E-03	16	0.32512	0.32793
	1632910_at	CG7512	4.57E-03	16	0.32512	0.39764
	1632920_at	CG10700	4.81E-03	16	0.32512	0.26901
	1633155_at	---	5.49E-03	16	0.32512	0.23875
	1633167_s_at	CG32496 /// CG6788	6.52E-03	16	0.32512	0.29375
	1633275_at	CG31562	1.51E-03	16	0.32512	0.37855
	1633393_at	CG6499	4.62E-03	16	0.32512	0.3425
	1634062_at	CG14218	5.04E-03	16	0.32512	0.33093
	1634125_at	CG30440	3.55E-03	16	0.32512	0.13811
	1634530_at	CG13040	4.52E-03	16	0.32512	0.28337
	1634816_at	CG8401	7.97E-03	16	0.32512	0.36804
	1635243_at	CG31371	1.91E-03	16	0.32512	0.41249
	1635251_at	CG32553	4.59E-04	16	0.32512	0.3036
	1635287_at	CG15366	8.72E-03	16	0.32512	0.31293
	1635562_at	---	6.83E-03	16	0.32512	0.21895
	1636272_at	CG8780	5.43E-03	16	0.32512	0.41484
	1636283_at	CG14826	8.81E-03	16	0.32512	0.19177
	1636495_at	---	5.33E-03	16	0.32512	0.22285
	1636532_at	CG13958	5.85E-05	16	0.32512	0.43606
	1636542_at	CG15277	1.99E-04	16	0.32512	0.39852
	1637310_at	---	1.51E-03	16	0.32512	0.3417
	1637358_at	CG10232	7.75E-03	16	0.32512	0.46666
	1637886_at	CG10339	3.04E-03	16	0.32512	0.38018
	1637889_at	CG5883	8.67E-03	16	0.32512	0.1646
	1638309_at	CG16733	2.89E-03	16	0.32512	0.48036

	1638492_at	CG13913	8.36E-04	16	0.32512	0.35929
	1638696_a_at	CG3552	6.08E-03	16	0.32512	0.306
	1638911_at	CG14551	1.92E-03	16	0.32512	0.40109
	1639044_at	Cad96Cb	4.32E-03	16	0.32512	0.43692
	1639748_at	CG15922	9.63E-03	16	0.32512	0.28048
	1640670_at	breathless	5.78E-03	16	0.32512	0.42896
	1640890_at	CG2973	2.72E-03	16	0.32512	0.20875
	1641564_a_at	Ribonuclear protein at 97D	4.27E-04	16	0.32512	0.22571
	1641712_at	CG8784	5.37E-04	16	0.32512	0.40087
	1625511_at	CG9322	2.30E-03	17	0.31436	0.38123
	1626554_at	CG16777	6.38E-03	17	0.31436	0.2747
	1627173_at	CG4040 /// CG33224	4.83E-03	17	0.31436	0.2006
	1628190_at	---	9.94E-03	17	0.31436	0.24188
	1629573_at	CG33975	2.06E-03	17	0.31436	0.45572
	1632409_a_at	cAMP-dependent protein kinase 1	4.11E-03	17	0.31436	0.33785
	1632541_at	Blimp-1	2.27E-03	17	0.31436	0.34433
	1634083_at	Neurofibromin 1	9.06E-03	17	0.31436	0.33999
	1634440_s_at	Ecdysone-induced protein 74EF	8.03E-04	17	0.31436	0.36569
	1638040_at	shade	1.02E-04	17	0.31436	0.1621
	1638383_at	CG34014	3.37E-03	17	0.31436	0.3408
	1639114_at	---	4.13E-04	17	0.31436	0.32483
	1640817_at	zormin	5.42E-03	17	0.31436	0.31694
	1624244_at	RPS6-p70-protein kinase	8.53E-03	18	0.28272	0.39173
	1624411_at	CG18437	3.27E-03	18	0.28272	0.36775
	1624957_a_at	Tequila	2.21E-03	18	0.28272	0.32892
	1626064_at	CG4648	2.49E-03	18	0.28272	0.30802
	1626577_at	CG15556	8.37E-03	18	0.28272	0.1484
	1627836_at	CG13801	1.19E-04	18	0.28272	0.29287
	1628363_at	innexin 3	1.83E-03	18	0.28272	0.38257
	1629081_at	orientation disrupter	3.86E-04	18	0.28272	0.25772
	1630515_s_at	Glutactin	5.22E-03	18	0.28272	0.2705
	1634486_at	CG30047	2.56E-05	18	0.28272	0.12484
	1634834_at	CG4053	8.18E-03	18	0.28272	0.16201
	1635135_at	---	8.10E-04	18	0.28272	0.34363
	1635583_s_at	frizzled 2	2.09E-03	18	0.28272	0.32023
	1635862_at	CG33120	2.11E-03	18	0.28272	0.35318
	1637843_at	Retinoid- and fatty-acid binding protein	5.52E-03	18	0.28272	0.33912
	1640231_a_at	CG8908	2.57E-03	18	0.28272	0.14514

	1640451_at	CG10863	4.73E-03	18	0.28272	0.26955
	1623695_a_at	CG11255	4.01E-03	19	0.25697	0.17372
	1623991_s_at	coro	9.63E-03	19	0.25697	0.36902
	1624015_at	CG13732	4.36E-03	19	0.25697	0.13324
	1624755_a_at	flap wing	2.34E-04	19	0.25697	0.37024
	1625466_at	CG2656	2.08E-03	19	0.25697	0.30167
	1625997_s_at	---	3.82E-03	19	0.25697	0.13152
	1626109_a_at	Drip	6.82E-03	19	0.25697	0.27297
	1626740_at	CG4784	6.40E-03	19	0.25697	0.10076
	1626801_at	CG10638	3.03E-03	19	0.25697	0.24289
	1626988_at	CG6805	7.32E-03	19	0.25697	0.28372
	1627167_a_at	CG40485	6.26E-03	19	0.25697	0.17889
	1627656_at	gurken	3.98E-03	19	0.25697	0.32467
	1628565_at	CG11454	7.28E-03	19	0.25697	0.32084
	1628915_s_at	CG3799	7.17E-03	19	0.25697	0.17943
	1629191_s_at	CG6154	7.30E-03	19	0.25697	0.24743
	1629515_at	Pyruvate dehydrogenase kinase	8.67E-05	19	0.25697	0.29577
	1629525_at	CG10404 /// GA15681	5.71E-03	19	0.25697	0.3694
	1629532_at	CG18157	6.02E-03	19	0.25697	0.16233
	1629875_a_at	Cytochrome P450-4d1	3.07E-03	19	0.25697	0.10027
	1631059_at	Protein C kinase 98E	8.49E-03	19	0.25697	0.18184
	1631291_at	---	5.70E-03	19	0.25697	0.14078
	1631783_at	CG4267	3.25E-03	19	0.25697	0.19236
	1631834_at	CG31098	5.61E-03	19	0.25697	0.21813
	1631931_s_at	Syndecan	8.93E-03	19	0.25697	0.32042
	1632098_at	CG4364	9.06E-03	19	0.25697	0.38681
	1632377_at	CG8399	6.40E-04	19	0.25697	0.29791
	1632417_a_at	CG5021	9.34E-03	19	0.25697	0.33913
	1632540_at	Trypsin	4.00E-03	19	0.25697	0.25333
	1632818_at	CG32281	6.37E-03	19	0.25697	0.21677
	1633483_a_at	CG14207	2.67E-03	19	0.25697	0.33028
	1633559_at	CG8331	9.68E-03	19	0.25697	0.3727
	1633716_at	CG10747	3.52E-03	19	0.25697	0.22954
	1633743_at	RNA polymerase II 15kD subunit	1.06E-03	19	0.25697	0.35978
	1634036_at	CG8788	8.33E-04	19	0.25697	0.23741
	1634250_at	Actin 57B	2.40E-03	19	0.25697	0.33239
	1634972_at	CG4802	4.74E-03	19	0.25697	0.38006
	1635321_at	CG14688	2.31E-03	19	0.25697	0.22052

	1635658_at	CG3592	7.72E-04	19	0.25697	0.28216
	1635661_at	CG14777	9.55E-03	19	0.25697	0.35031
	1637491_s_at	CG3835	1.84E-03	19	0.25697	0.23722
	1637526_s_at	G protein 30A	2.98E-03	19	0.25697	0.31046
	1638181_at	CG14743	3.07E-04	19	0.25697	0.11757
	1638301_at	CG8157	5.16E-03	19	0.25697	0.15354
	1638321_s_at	CG4847	2.11E-04	19	0.25697	0.15653
	1638528_at	CG15531	6.26E-05	19	0.25697	0.23302
	1638878_at	CG17302	6.15E-04	19	0.25697	0.16526
	1639429_at	Organic anion transporting polypeptide 58Da	6.62E-03	19	0.25697	0.2115
	1639439_at	CG2471	7.68E-03	19	0.25697	0.37901
	1640989_at	CG12811	8.10E-03	19	0.25697	0.37311
	1641320_s_at	CG3168	6.80E-03	19	0.25697	0.29939
	1641492_at	---	9.94E-03	19	0.25697	0.33691
	1641508_s_at	Tetraspanin 42Eh	1.97E-03	19	0.25697	0.1876
	1622924_at	CG18540	1.04E-03	20	0.21086	0.25677
	1623037_a_at	CG5594	1.46E-03	20	0.21086	0.35033
	1624033_at	amyloid protein precursor-like	7.71E-03	20	0.21086	0.33396
	1624542_at	CG31757	9.35E-03	20	0.21086	0.24671
	1627024_s_at	CG9164	1.95E-03	20	0.21086	0.27246
	1628265_a_at	CG32245	7.15E-03	20	0.21086	0.21152
	1628406_s_at	---	2.94E-04	20	0.21086	0.16893
	1628644_at	CG17350	5.06E-03	20	0.21086	0.13865
	1629091_at	CG5177	4.01E-04	20	0.21086	0.18728
	1629688_at	Oseg4	4.80E-03	20	0.21086	0.09243
	1630169_a_at	Synapse-associated protein 47kD	3.68E-03	20	0.21086	0.30147
	1630761_at	CG11854	5.38E-03	20	0.21086	0.16574
	1631417_s_at	CG6282	1.33E-03	20	0.21086	0.24736
	1631606_at	CG14285	5.39E-03	20	0.21086	0.14996
	1634344_at	lethal (2) 35Bg	2.12E-03	20	0.21086	0.10498
	1635338_a_at	CG10233	2.24E-03	20	0.21086	0.26295
	1635500_a_at	prospero	3.32E-03	20	0.21086	0.20817
	1635870_at	CG1628	9.04E-03	20	0.21086	0.15501
	1636345_s_at	synaptotagmin	5.77E-03	20	0.21086	0.30568
	1636566_at	CG32368	2.54E-03	20	0.21086	0.15331
	1638424_at	CG10514	5.24E-03	20	0.21086	0.16743
	1639017_at	---	4.69E-03	20	0.21086	0.14901
	1639292_at	Frequenin 1	9.92E-03	20	0.21086	0.21884
	1641131_at	CG5621	5.21E-04	20	0.21086	0.21174

Mitochondrial state 4 respiration rate	1629380_at	Chorion protein 16	7.96E-04	1	0.71498	0.69344
	1633465_at	Chorion protein a at 7F	2.58E-03	1	0.71498	0.70775
	1634091_at	Chorion protein 19	2.18E-03	1	0.71498	0.78244
	1636427_a_at	DNA methyltransferase 2	9.04E-04	1	0.71498	0.50244
	1637395_at	CG13084	1.41E-03	1	0.71498	0.79308
	1641113_at	CG13083	2.19E-03	1	0.71498	0.81072
	1634287_at	CG13737	8.81E-03	2	0.70023	0.70023
	1636750_s_at	CG13737	7.51E-03	2	0.70023	0.70023
	1624400_a_at	cdc2-related-kinase	2.69E-04	3	0.60965	0.4329
	1624806_at	yellow-g	4.54E-03	3	0.60965	0.70035
	1625731_at	CG13114	3.49E-03	3	0.60965	0.69047
	1632992_at	CG11381	4.31E-03	3	0.60965	0.7287
	1634154_at	CG15570	8.16E-03	3	0.60965	0.67722
	1634395_at	yellow-g2	9.26E-03	3	0.60965	0.71238
	1636215_at	---	4.17E-03	3	0.60965	0.25129
	1637707_at	CG4009	7.34E-03	3	0.60965	0.72502
	1641158_a_at	defective chorion 1	4.42E-03	3	0.60965	0.56854
	1624058_at	female sterile (1) Nasrat	2.08E-03	4	0.35842	0.50018
	1624573_at	CG4998	8.07E-03	4	0.35842	0.07109
	1624613_at	CG5986	3.65E-03	4	0.35842	0.21215
	1625323_at	CG32397	5.35E-04	4	0.35842	0.19153
	1625768_s_at	longitudinals lacking	8.69E-03	4	0.35842	0.53198
	1626223_at	CG2543	8.37E-03	4	0.35842	0.30146
	1626375_a_at	Usf	8.23E-03	4	0.35842	0.24693
	1626647_at	Minichromosome maintenance 5	7.21E-03	4	0.35842	0.542
	1626665_at	CG7408	5.62E-03	4	0.35842	0.12209
	1627348_at	CG5285	1.70E-03	4	0.35842	0.38243
	1629746_at	CG4041	7.52E-04	4	0.35842	0.43978
	1630239_at	Tubulin at 67C	7.57E-04	4	0.35842	0.47945
	1630555_at	CG33346	8.90E-03	4	0.35842	0.11654
	1630950_at	nop5	9.49E-04	4	0.35842	0.49263
	1630957_s_at	slimfast	8.34E-03	4	0.35842	0.37455
	1631007_at	Ribonucleoside diphosphate reductase large subunit	9.20E-03	4	0.35842	0.54281
	1631078_at	zero population growth	7.70E-03	4	0.35842	0.50093
	1631125_at	CG15737	5.58E-03	4	0.35842	0.51857

	1631331_a_at	pxt	4.12E-03	4	0.35842	0.52597
	1632177_at	homothorax	8.67E-05	4	0.35842	0.17051
	1632283_at	CG8950	1.07E-03	4	0.35842	0.48909
	1632516_at	CG8506	3.80E-03	4	0.35842	0.19878
	1632669_at	Minichromosome maintenance 2	2.05E-03	4	0.35842	0.55285
	1632713_at	nanos	6.32E-03	4	0.35842	0.49815
	1633417_at	giant nuclei	3.80E-03	4	0.35842	0.48251
	1633760_at	CG10399	6.55E-03	4	0.35842	0.2647
	1637133_x_at	CG11350	8.65E-03	4	0.35842	0.22404
	1637240_a_at	Autophagy-specific gene 7	8.02E-03	4	0.35842	0.39718
	1639054_s_at	---	5.16E-03	4	0.35842	0.144
	1639097_at	female sterile (1) Young arrest	1.85E-03	4	0.35842	0.50168
	1639983_at	---	8.21E-03	4	0.35842	0.14339
	1640052_at	CG15434	8.85E-04	4	0.35842	0.23534
	1640877_at	tRNA-guanine transglycosylase	7.99E-03	4	0.35842	0.24332
	1640972_at	Cyclin J	6.86E-03	4	0.35842	0.54781
	1623675_at	Odorant-binding protein 99b	7.68E-03	5	0.31653	0.28353
	1625120_at	CG14630 /// CG33082	3.96E-03	5	0.31653	0.35707
	1626718_at	CG33127	9.55E-03	5	0.31653	0.39717
	1628608_at	CG17475	3.99E-03	5	0.31653	0.30791
	1635239_at	CG13901	6.69E-03	5	0.31653	0.23496
	1635524_at	CG30427	1.21E-03	5	0.31653	0.19657
	1638246_at	CG5804	8.03E-03	5	0.31653	0.43845
	1623004_a_at	CG18449	8.69E-03	6	0.29182	0.4189
	1624924_at	CG31182	3.09E-03	6	0.29182	0.11778
	1625657_at	CG32335	4.77E-03	6	0.29182	0.2346
	1627084_s_at	Syntaxin Interacting Protein 2	5.20E-03	6	0.29182	0.36844
	1627093_at	CG9284	7.89E-03	6	0.29182	0.33444
	1627129_at	---	3.91E-03	6	0.29182	0.28731
	1628261_at	CG13067	7.02E-03	6	0.29182	0.14063
	1631548_at	CG30196	6.08E-03	6	0.29182	0.07312
	1632823_at	CG17788	2.94E-03	6	0.29182	0.32783
	1634401_at	fos intronic gene	6.32E-03	6	0.29182	0.40584
	1635315_at	---	3.06E-03	6	0.29182	0.33594
	1636121_at	CG12924	7.23E-03	6	0.29182	0.2893
	1637969_at	CG16984	8.28E-03	6	0.29182	0.41769
	1638494_at	CG3124	7.76E-03	6	0.29182	0.43513
	1638629_at	CG15918	2.44E-03	6	0.29182	0.15749

	1639147_s_at	CG30438	1.00E-02	6	0.29182	0.2495
	1639155_at	---	4.00E-03	6	0.29182	0.35617
	1639885_at	brachyenteron	7.95E-03	6	0.29182	0.28505
	1640335_at	CG31304	6.66E-03	6	0.29182	0.15233
	1641237_a_at	SP555	8.80E-03	6	0.29182	0.44892
	1623522_at	CG11668	2.83E-03	7	0.21231	0.13585
	1625082_a_at	CG7882	3.12E-03	7	0.21231	0.20681
	1626837_a_at	CG32245	7.89E-03	7	0.21231	0.26962
	1629106_at	CG2233	6.31E-03	7	0.21231	0.19631
	1630375_at	Mec2	1.37E-03	7	0.21231	0.19907
	1633329_at	yellow-h	4.60E-03	7	0.21231	0.26897
	1633553_s_at	SP1173	3.12E-03	7	0.21231	0.28504
	1634529_at	CG3290	1.93E-03	7	0.21231	0.15703
	1636576_s_at	no receptor potential A	1.70E-03	7	0.21231	0.18826
	1636954_at	Netrin-B	4.22E-03	7	0.21231	0.14124
	1640284_at	Choline acetyltransferase	7.16E-03	7	0.21231	0.28038
	1640465_at	CG17124	3.59E-03	7	0.21231	0.21909
	1623103_at	CG14946	1.22E-03	8	0.18844	0.09179
	1623864_at	CG6321	1.74E-03	8	0.18844	0.22518
	1624023_at	Protein phosphatase 1 at 87B	1.36E-03	8	0.18844	0.33197
	1626734_at	CG6484	1.38E-03	8	0.18844	0.13621
	1630084_at	CG10205	6.23E-03	8	0.18844	0.12143
	1632063_s_at	CG11357	1.27E-03	8	0.18844	0.27372
	1632573_at	Mitf	8.70E-03	8	0.18844	0.20459
	1634666_at	---	1.24E-03	8	0.18844	0.14975
	1635070_at	CG9953	6.98E-04	8	0.18844	0.28491
	1637516_at	CG15449	2.63E-03	8	0.18844	0.11621
	1638612_at	CG11142	8.17E-03	8	0.18844	0.12422
	1638809_at	---	1.65E-03	8	0.18844	0.2767
	1639170_at	Cyp4d21	5.97E-03	8	0.18844	0.11179
	1639203_at	CG32556	5.21E-03	8	0.18844	0.19223
	1641422_at	huntingtin	8.09E-03	8	0.18844	0.18589
ADP/O ratio	1628310_at	---	7.50E-03	1	0.62002	0.50101
	1632854_s_at	Paramyosin	6.49E-03	1	0.62002	0.61175
	1637483_at	CG8736	9.83E-03	1	0.62002	0.70615
	1641474_s_at	CG31904 /// Adult cuticle protein 1	8.53E-03	1	0.62002	0.66115
	1624578_at	CG9675	3.95E-03	2	0.13291	0.16641
	1624893_at	CG8852	9.56E-03	2	0.13291	0.13499

	1625087_a_at	CG32137	2.23E-03	2	0.13291	0.19586
	1626709_at	CG30179	9.15E-03	2	0.13291	0.08597
	1629066_at	GRHRII	2.68E-03	2	0.13291	0.17148
	1631168_at	CG5687	8.93E-03	2	0.13291	0.13806
	1631682_at	twist	7.49E-03	2	0.13291	0.0825
	1632170_a_at	CG14685	6.42E-03	2	0.13291	0.18057
	1632796_s_at	Ecdysone receptor	8.30E-03	2	0.13291	0.105
	1633481_at	CG14394	7.82E-03	2	0.13291	0.11127
	1635005_a_at	Resistant to dieldrin	9.92E-03	2	0.13291	0.12872
	1635006_at	CG7077	5.17E-03	2	0.13291	0.14892
	1638033_at	CG6055	8.03E-03	2	0.13291	0.09661
	1639469_a_at	Punch	4.12E-03	2	0.13291	0.1315
	1640732_s_at	---	5.30E-03	2	0.13291	0.11577

APPENDIX E

SUPPLEMENTARY TABLE 5

Over-representation of Gene Ontology (GO) Categories (BP=Biological Processes; CC= Cellular Component; MF= Molecular Function), KEGG Pathways and Keywords for transcripts associated with mitochondrial quantitative traits. Count = the number of genes in the annotation category. % = the number of genes in the annotation category/total number of significant genes. The *P* value is from a modified Fisher exact test for enrichment of genes in an annotation category.

Trait	Analysis	Category	Term	Count	%	P-Value
Mitochondrial state 3 respiration rate	**Module 10**	**GO BP**	GO:0009314~response to radiation	2	50.00%	2.54E-02
			GO:0009416~response to light stimulus	2	50.00%	2.23E-02
			GO:0007602~phototransduction	2	50.00%	1.36E-02
			GO:0009605~response to external stimulus	2	50.00%	4.20E-02
			GO:0007601~visual perception	2	50.00%	2.62E-02
			GO:0007600~sensory perception	2	50.00%	4.70E-02
			GO:0050962~detection of light stimulus during sensory perception	2	50.00%	1.51E-02
			GO:0018298~protein-chromophore linkage	2	50.00%	2.00E-03
			GO:0050953~sensory perception of light stimulus	2	50.00%	2.62E-02
			GO:0009628~response to abiotic stimulus	2	50.00%	4.35E-02
			GO:0051606~detection of stimulus	2	50.00%	1.99E-02
			GO:0009584~detection of visible light	2	50.00%	1.55E-02
			GO:0050908~detection of light stimulus during visual perception	2	50.00%	1.51E-02
			GO:0009583~detection of light stimulus	2	50.00%	1.55E-02
			GO:0009582~detection of abiotic stimulus	2	50.00%	1.67E-02
			GO:0050906~detection of stimulus during sensory perception	2	50.00%	1.71E-02
			GO:0009581~detection of external stimulus	2	50.00%	1.87E-02

		GO MF	GO:0004930~G-protein coupled receptor activity	2	50.00%	3.42E-02
			GO:0009881~photoreceptor activity	2	50.00%	2.66E-03
			GO:0001584~rhodopsin-like receptor activity	2	50.00%	2.07E-02
			GO:0008020~G-protein coupled photoreceptor activity	2	50.00%	2.66E-03
		Keywords	G-protein coupled receptor	2	50.00%	3.87E-02
			transducer	2	50.00%	4.63E-02
			retinal protein	2	50.00%	3.92E-03
			chromophore	2	50.00%	3.92E-03
			Vision	2	50.00%	2.95E-02
			sensory transduction	2	50.00%	4.25E-02
			photoreceptor protein	2	50.00%	3.92E-03
	Module 17	**GO BP**	GO:0048856~anatomical structure development	4	28.57%	2.05E-02
			GO:0000003~reproduction	3	21.43%	3.82E-02
			GO:0045475~locomotor rhythm	2	14.29%	1.43E-02
			GO:0048477~oogenesis	3	21.43%	1.42E-02
			GO:0007276~gamete generation	3	21.43%	2.75E-02
			GO:0042048~olfactory behavior	2	14.29%	4.79E-02
			GO:0019953~sexual reproduction	3	21.43%	2.90E-02
			GO:0019935~cyclic-nucleotide-mediated signaling	2	14.29%	5.59E-03
			GO:0019933~cAMP-mediated signaling	2	14.29%	5.59E-03
			GO:0019932~second-messenger-mediated signaling	2	14.29%	2.69E-02
			GO:0007623~circadian rhythm	2	14.29%	2.77E-02
			GO:0008355~olfactory learning	2	14.29%	2.69E-02
			GO:0007622~rhythmic behavior	2	14.29%	2.14E-02
			GO:0048512~circadian behavior	2	14.29%	1.99E-02
			GO:0048511~rhythmic process	2	14.29%	2.85E-02
			GO:0007242~intracellular signaling cascade	3	21.43%	2.38E-02
			GO:0007613~memory	2	14.29%	1.67E-02
			GO:0007612~learning	2	14.29%	2.93E-02
			GO:0007611~learning and/or memory	2	14.29%	3.86E-02
			GO:0007292~female gamete generation	3	21.43%	1.50E-02
			GO:0007166~cell surface receptor linked signal transduction	3	21.43%	3.49E-02

Mitochondrial state 4 respiration rate	**Module 1**	**GO BP**	GO:0030707~ovarian follicle cell development	2	33.33%	4.08E-02
			GO:0007275~multicellular organismal development	3	50.00%	4.63E-02
			GO:0030703~eggshell formation	2	33.33%	1.95E-02
			GO:0007306~eggshell chorion formation	2	33.33%	1.59E-02
			GO:0007304~chorion-containing eggshell formation	2	33.33%	1.95E-02
		GO CC	GO:0030312~external encapsulating structure	4	66.67%	1.26E-07
			GO:0042600~chorion	4	66.67%	9.67E-08
		GO MF	GO:0005213~structural constituent of chorion	2	33.33%	2.95E-03
	Module 4	**GO BP**	GO:0007350~blastoderm segmentation	3	8.82%	4.47E-02
			GO:0000280~nuclear division	2	5.88%	1.83E-02
			GO:0000279~M phase	6	17.65%	4.98E-03
			GO:0000278~mitotic cell cycle	5	14.71%	1.91E-02
			GO:0000003~reproduction	8	23.53%	2.19E-03
			GO:0000087~M phase of mitotic cell cycle	5	14.71%	1.01E-02
			GO:0007338~single fertilization	3	8.82%	1.53E-03
			GO:0007067~mitosis	5	14.71%	9.78E-03
			GO:0019953~sexual reproduction	7	20.59%	5.11E-03
			GO:0006267~pre-replicative complex assembly	2	5.88%	3.18E-02
			GO:0022414~reproductive process	4	11.76%	1.27E-02
			GO:0006261~DNA-dependent DNA replication	3	8.82%	3.53E-02
			GO:0006260~DNA replication	5	14.71%	1.28E-03
			GO:0006259~DNA metabolic process	6	17.65%	1.34E-02
			GO:0009566~fertilization	3	8.82%	1.53E-03
			GO:0022403~cell cycle phase	6	17.65%	6.16E-03
			GO:0022402~cell cycle process	6	17.65%	1.80E-02
			GO:0007049~cell cycle	6	17.65%	2.44E-02
			GO:0030261~chromosome condensation	3	8.82%	6.15E-03
			GO:0006139~nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	11	32.35%	2.94E-02

			GO:0051301~cell division	4	11.76%	3.62E-02
			GO:0009790~embryonic development	5	14.71%	3.31E-02
		GO CC	GO:0005656~pre-replicative complex	2	5.88%	4.65E-02
		GO MF	GO:0043138~3'-5' DNA helicase activity	2	5.88%	4.15E-02
			GO:0003676~nucleic acid binding	10	29.41%	1.68E-02
			GO:0003688~DNA replication origin binding	2	5.88%	4.52E-02
		Keywords	DNA-binding	5	14.71%	2.15E-02
ADP/O ratio	**Module 2**	**GO BP**	GO:0007517~muscle development	3	17.65%	2.48E-02
		GO MF	GO:0030976~thiamin pyrophosphate binding	2	11.76%	8.25E-03
			GO:0016903~oxidoreductase activity, acting on the aldehyde or oxo group of donors	2	11.76%	3.66E-02
			GO:0016624~oxidoreductase activity, acting on the aldehyde or oxo group of donors, disulfide as acceptor	2	11.76%	1.24E-02
			GO:0004591~oxoglutarate dehydrogenase (succinyl-transferring) activity	2	11.76%	8.25E-03
		Keywords	receptor	3	17.65%	3.19E-02