DOI: 10.1534/genetics.109.104927

Transcriptional Regulation of Metabolism Associated With the Increased Desiccation Resistance of the Cactophilic *Drosophila mojavensis*

Luciano M. Matzkin[1] and Therese A. Markow[2]

Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721-0088

Manuscript received May 9, 2009
Accepted for publication May 19, 2009

ABSTRACT

In Drosophila, adaptation to xeric environments presents many challenges, greatest among them the maintenance of water balance. *Drosophila mojavensis*, a cactophilic species from the deserts of North America, is one of the most desiccation resistant in the genus, surviving low humidity primarily by reducing its metabolic rate. Genetic control of reduced metabolic rate, however, has yet to be elucidated. We utilized the recently sequenced genome of *D. mojavensis* to create an oligonucleotide microarray to pursue the identities of the genes involved in metabolic regulation during desiccation. We observed large differences in gene expression between male and female *D. mojavensis* as well as both quantitative and qualitative sex differences in their ability to survive xeric conditions. As expected, genes associated with metabolic regulation and carbohydrate metabolism were differentially regulated between stress treatments. Most importantly, we identified four points in central metabolism (*Glyceraldehyde 3-phosphate dehydrogenase*, *transaldolase*, *alcohol dehydrogenase*, and *phosphoenolpyruvate carboxykinase*) that indicate the potential mechanisms controlling metabolic rate reduction associated with desiccation resistance. Furthermore, a large number of genes associated with vision pathways also were differentially expressed between stress treatments, especially in females, that may underlie the initial detection of stressful environments and trigger subsequent metabolic changes.

AMONG the environmental challenges most stressful to insects are starvation, desiccation, and extreme heat or cold. As insects have radiated into different habitats such as the tropics and deserts, they have evolved metabolic adaptations to deal with their contrasting ecological conditions. We can take advantage of the diversity of habitats utilized by insects to address the ways in which metabolic pathways and the genes regulating them have evolved during the adaptation process. Nowhere is the opportunity to address these questions more promising than among the 12 species of Drosophila for which entire genome sequences are now available (Drosophila 12 Genomes Consortium 2007).

Understanding the metabolic basis of evolutionary responses to environmental change is unlikely to be achieved by simple experimental means, especially for higher eukaryotes such as Drosophila. For one thing, metabolism is complex and can vary with sex and reproductive status. At any given time, a proportion of female and male members of a population would have mated and thus their metabolic responses to environmental change are likely to vary relative to their virgin counterparts. To the extent to which selection acts on different individuals within a population sex and reproductive status represent critical components of the metabolic architecture of adaptation.

Metabolism, or the synthesis and breakdown of compounds in different biochemical pathways, can be influenced by abiotic variables such as temperature and relative humidity and by biotic factors such as diet (Hochachka and Somero 2002). Distinct but interconnected pathways underlie the metabolism of proteins, lipids, and carbohydrates, and furthermore, some gene products are expected to have more critical roles than others in controlling the flow or "flux" through these pathways (Kacser and Burns 1973; Fell 1997). What remains unclear, however, is precisely which steps in metabolism are modulated in response to environmental challenges.

For Drosophila, the ability to survive in dry habitats presents an especially formidable ecological and physiological challenge (Gibbs 2002). Xeric-adapted Drosophila species have responded to these challenges with high desiccation resistance (Gibbs and Matzkin 2001; Matzkin *et al.* 2007), lower water loss rates (Gibbs and Matzkin 2001), and lower metabolic rates under dry conditions (Gibbs *et al.* 2003b). Additionally, artificial selection experiments in *Drosophila melanogaster* suggest that increased desiccation resistance affects both meta-

Expression data from this article have been deposited in the Gene Expression Omnibus under series entry no. GSE16234.

Supporting information is available online at http://www.genetics.org/cgi/content/full/genetics.109.104927/DC1.

[1]*Corresponding author:* University of California, Division of Biological Sciences, 9500 Gilman Dr., No. 0116, La Jolla, CA 92093-0116.
E-mail: lmatzkin@ucsd.edu

[2]*Present address:* University of California, Division of Biological Sciences, 9500 Gilman Dr., No. 0116, La Jolla, CA 92093-0116.

bolic pool titer and utilization. For example, laboratory-selected desiccation-resistant *D. melanogaster* populations accumulate more glycogen (Djawdan *et al.* 1998) and differentially metabolize carbohydrates under desiccating conditions compared to unselected populations (Gibbs 2002). The physiological, behavioral, and morphological (*e.g.*, cuticle composition) adaptations associated with inhabiting a xeric habitat are likely to have resulted, in part, from the modulation of expression of the underlying genes and of flux through its associated metabolic pathways.

D. mojavensis offers an exceptional model system to study the genetics and genomics of adaptation, both because of our extensive knowledge of its desert ecology (Heed 1978) and because of the recent development of powerful species-specific genetic and genomic tools (Matzkin *et al.* 2006; Drosophila 12 Genomes Consortium 2007; Schaeffer *et al.* 2008). *D. mojavensis* is a cactophilic fly inhabiting the deserts of North America. Adaptation to its xeric habitat includes extreme desiccation resistance and the ability to reduce respiration rate or water loss rate under desiccating conditions (Gibbs and Matzkin 2001; Gibbs *et al.* 2003b; Matzkin *et al.* 2007). The relative humidity experienced by *D. mojavensis* in the field can be as low as 8%, although it can fluctuate depending on the time of day and location on the host (Gibbs *et al.* 2003c). Furthermore, *D. mojavensis* is similar to other Drosophila in which female desiccation resistance does not decrease postmating (Markow and O'Grady 2005); it actually has been shown to increase following mating (Knowles *et al.* 2004, 2005).

In this study we assessed transcriptional changes associated with the ability of *D. mojavensis* to survive in a xeric environment. Using the recently sequenced genome (Drosophila 12 Genomes Consortium 2007), we designed and created a complete genome oligonucleotide microarray. We exposed *D. mojavensis* to desiccating conditions and examined their transcriptional profile to begin to determine the genetic underpinnings of the known desiccation-dependent metabolic rate reduction in this species. To gain a more comprehensive and biologically representative picture of the desiccation response we examined transcriptional profiles of virgin and mated flies of both sexes, since mating status in the field is variable (see Figure 1).

MATERIALS AND METHODS

Microarray experimental design: The stock used in this study (15081-1352.22) was the same one utilized for the recently published *D. mojavensis* genome sequence (Drosophila 12 Genomes Consortium 2007). Flies were reared using standard Tucson Drosophila Stock Center banana/Opuntia media. The experimental design consisted of two mating status treatments (virgin and mated) and two stress treatments (desiccation and food) for both sexes (see Figure 1). Cultures were maintained in several banana/Opuntia bottle containers and virgin flies were collected and sexed within 24 hr of eclosion and placed in 8-dram banana/Opuntia vials (20 flies per vial). The virgin treatment consisted of keeping the flies in male-only or female-only vials for 8 days, transferring them into fresh food vials once at day 4. The mated treatment was identical to the virgin treatment for the first 7 days, and at the beginning of the eighth day male and female vials were combined. Flies remained in these mixed-sex vials for only 24 hr at which time they were separated by sex. At the end of the 8-day period half of the flies from the virgin and mated treatments were placed in new food vials and half in a desiccator. The desiccator was composed of a $30 \times 30 \times 30$-cm clear acrylic box. Ambient air was pumped through two columns filled with 500 cm^3 of Drierite desiccant into the chamber at a rate of 1.5 liters per minute. Additionally we placed inside the chamber a container filled with 200 cm^3 of Drierite desiccant. Empty 8-dram vials with either six males or six females were capped using a cotton ball and placed inside the desiccator. Relative humidity inside the chamber was sampled every minute, using a HOBO relative humidity data logger (ONSET Computer Corporation). The relative humidity throughout the experiment was maintained at $<2\%$. Prior to commencing the experiment the air in the chamber (with vials) was flushed for 15 min. All flies were left inside the desiccator for 40 hr, at which point $\sim 30\%$ of the flies have died (LT30). After this drying period, dead flies were aspirated and discarded while live flies were placed in groups of 15 in 1.5-ml tubes, snap frozen in liquid nitrogen, and placed in a $-80°$ freezer until processing.

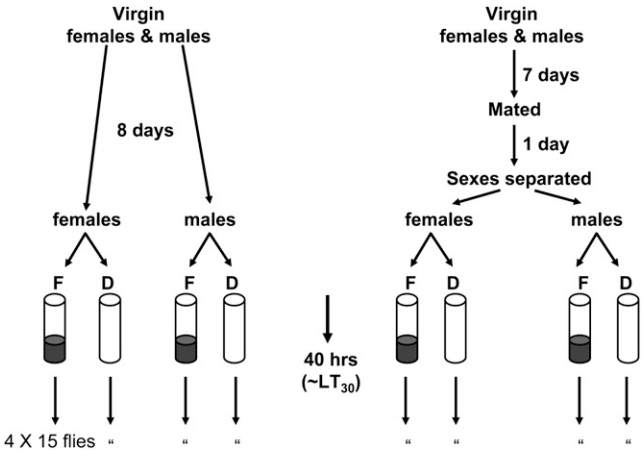

Figure 1.—Diagram of experimental setup.

Microarray design and processing: To access gene expression differences among treatments we design a complete *D. mojavensis* genome oligonucleotide microarray. We utilized custom NimbleGen's 4-plex microarrays. The design was based on the GLEANR gene predictions of the published *D. mojavensis* genome (Drosophila 12 Genomes Consortium 2007). The microarray consisted of 69,997 60-oligonucleotide probes representing 17,504 features (for all but 11 features there were 4 probes per feature). The bulk of these features, 17,477, were GLEANR gene predictions (2506 that contain transposable elements contaminants), 19 were *mojavensis*-specific genes (MSG) described in Matzkin *et al.* (2006), and 8 are from Kelleher *et al.* (2007).

Total RNA extraction was performed using Invitrogen's (Carlsbad, CA) PureLink Micro-to-Midi Total Purification System. The suggested manufacturer's instructions were followed, which included a DNase I digestion to remove any possible genomic DNA contamination. The quality and concentration of the RNA was determined using a NanoDrop spectrophotometer (NanoDrop Technologies). For all samples the 260/

280-nm ratio was >2.30 and the 260/230-nm ratio was >1.50. For each sex and mating status and stress treatment two replicates (of 15 flies each) were hybridized (16 total hybridizations). Samples were submitted (20 μg of total RNA per sample) to NimbleGen's Reykjavik, Iceland facility for processing. The cDNA synthesis, hybridization, and visualization were performed by NimbleGen (www.nimblegen.com).

Microarray statistical analysis: Gene expression intensities were quantile normalized (Bolstad *et al.* 2003) and gene calls generated using the robust multichip average (RMA) algorithm (Irizarry *et al.* 2003). Log_2 intensities were analyzed using a two-step ANOVA (Wolfinger *et al.* 2001). The two-step ANOVA consists of a global analysis in which random effects associated with hybridizations into different slides and the use of multiple probes were removed. The second step utilized the residuals from the global analysis to perform a gene-specific test. The statistical model used in this study was

Global:

$$Y_{ij} = \mu + \text{Slide}_i + \text{Probe}_j + \text{Residual}_{ij}$$

Gene specific:

$$\text{Residual}_{iklm} = \mu + \text{Slide}_i + \text{Sex}_k + \text{Mating}_l + \text{Stress}_m + \text{Sex} \times \text{Mating}_{kl} + \text{Sex} \times \text{Stress}_{km} + \text{Mating} \times \text{Stress}_{lm} + \text{Sex} \times \text{Mating} \times \text{Stress}_{klm} + \text{Error}_{iklm},$$

where $i = 1$–12, $j = 1$–4, $k = 1$–2, $l = 1$–2, and $m = 1$–2 and both the slide and the probe effects were random. The analysis was performed using the PROC MIXED model in SAS (ver. 9.0; SAS Institute, Cary, NC). Significance for each gene was determined using the false discovery rate (FDR) method (5% level) of Storey and Tibshirani (2003).

To examine the functional and biological characteristics of the genes that were differentially expressed in our study, we performed both a functional annotation and a functional clustering analysis. Both of these analyses were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) resource (http://niaid.abcc.ncifcrf.gov/) (Dennis *et al.* 2003). The functional annotation analysis determines the overrepresentation of Gene Ontology (GO) terms for all three categories (molecular function, biological process, and cellular component). Overrepresentation of GO terms was assessed using a conservative Fisher's exact test (see Hosack *et al.* 2003) and significance was determined both by examining the *P*-value and by the FDR method (set at 5%).

One issue with such analysis is that genes can have multiple GO terms and some of these are very general. For example, *physiological process* is a GO term, so one potentially could have an overrepresentation of this term due to many genes that have an effect on physiology, but that perform dissimilar functions. Alternatively, by clustering groups of genes using GO terms, protein domain information, pathway membership, and other such criteria, one can extract more relevant biological information. We utilized the heuristic fuzzy partition algorithm in DAVID, using medium stringency (Dennis *et al.* 2003) to determine and characterize functionally similar clusters of genes that were differentially regulated in response to the stress and/or mating status treatments.

Verification of microarray results: Given the possible false positive error associated with the microarray analysis, we validated the expression results, using quantitative PCR (QPCR), for a subset of the differentially expressed genes (see RESULTS). Furthermore, since desiccating flies are not given any food we repeated the experiment as described above (for virgin females) with the addition of a starvation treatment. Flies were either desiccated for 36.5 hr (LT 44) or maintained for the same amount of time in food vials or vials containing 0.5% agar (nonnutritive water source). After the 36.5 hr all survivors were placed in groups of five in 1.5-ml tubes, snap frozen in liquid nitrogen, and placed in a −80° freezer. Seven sets of five females were analyzed for each of the three treatments (control, desiccation, and starvation). Total RNA extraction was performed as above, using Invitrogen's PureLink Micro-to-Midi Total Purification System. Synthesis of the cDNA was performed using ABI's High Capacity RNA-to-cDNA kit with a starting total RNA template of 400 ng per reaction. Duplicate QPCRs were performed for each sample on an ABI 7000 Sequence Detection System machine, using ABI's Power SYBR Green PCR kit. Analysis of differential expression was calculated using the Relative Expression Software Tool v2.0.7 (Pfaffl *et al.* 2002), performing 10,000 bootstraps per gene.

RESULTS

Gene expression: Repeatability of signal intensity between replicates was very high. Correlation coefficients between replicates (r^2) ranged between 0.97 and 0.99 (data not shown). Table 1 shows the number of significant differentially expressed genes using the FDR method. Of the three single factors, sex had the greatest level of differentially expressed genes (9233), while the overall effect due to mating status affected the least number of genes (75). Exposure to a desiccating environment resulted in the modulation of 1679 genes, including 4 associated with central metabolism; glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) (GI20859), transaldolase (*Tal*) (GI18849), and alcohol dehydrogenase-2 (*Adh-2*) (GI17643) were downregulated and phosphoenolpyruvate carboxykinase (*Pepck*) (GI18450) was upregulated (Figure 2).

Of the genes differentially expressed between the sexes, 1.6 times more genes were upregulated in females relative to males. A greater number of genes (6.5 times more) were upregulated in mated flies relative to virgins. In addition to the single-factor analysis, there were three sets of two-factor interactions and one three-factor interaction. For each two-factor interaction there were 6 specific comparisons and 28 for the three-factor interaction. Since a large number of genes were differentially expressed between the sexes, Table 1 reports those two- and three-factor comparisons within females or males. The complete analysis is shown in supporting information, Table S1. A greater number of differentially expressed genes were observed for males than for females both in the comparison between mating status and in that between stress treatments (214 *vs.* 37 and 1927 *vs.* 809, respectively). Only 23 genes were differentially expressed between mated and virgin females in the food treatment and 36 genes while in the desiccator (Table 1). For males, 409 genes were differentially expressed between mating status treatments while in food, but only 47 genes after desiccation. All expression data have been placed in the Gene Expression Omnibus under series entry GSE16234.

Of the genes differentially expressed in response to desiccation in females (809) and males (1927), 344 were

TABLE 1

Number of significantly up- and downregulated genes in each specific comparison

Effect comparison							
Sex	Mating	Stress		Sex	Mating	Stress	Genes (up/down)
♀			/	♂			9233 (5740/3493)
	M		/		V		75 (65/10)[a]
		D	/			F	1679 (774/905)[a]
♀	M		/	♀	V		37 (27/10)[a]
♂	M		/	♂	V		214 (123/91)[a]
♀		D	/	♀		F	809 (384/425)[a]
♂		D	/	♂		F	1927 (875/1052)
♀	M	F	/	♀	V	F	23 (16/7)[a]
♀	M	F	/	♀	V	D	379 (255/124)
♀	M	D	/	♀	V	F	450 (242/208)
♀	V	D	/	♀	V	F	416 (226/190)
♀	M	D	/	♀	M	F	355 (122/233)
♀	M	D	/	♀	V	D	36 (23/13)[a]
♂	M	F	/	♂	V	F	409 (163/246)[a]
♂	M	F	/	♂	V	D	704 (482/222)
♂	V	D	/	♂	V	F	1261 (599/662)
♂	M	D	/	♂	M	F	1031 (448/583)
♂	M	D	/	♂	V	F	1977 (973/1004)
♂	M	D	/	♂	V	D	47 (30/17)[a]

Mating: M, mated; V, virgin. Stress: D, desiccation; F, food.
[a] Functional and clustering analyses were performed on these comparisons.

commonly expressed in both sexes. The majority of genes (95%) had a similar pattern of expression between the sexes ($r^2 = 0.79$, $t = 35.7$, $P < 0.001$). In other words, if one of these coexpressed genes was upregulated in response to desiccation in males, it likely was upregulated in females (Figure 3). Sixteen genes, however, were upregulated in males and downregulated in females under desiccation (Figure 3). Conversely, two genes exhibited downregulation in males and upregulation in females under desiccating conditions (Figure 3).

We performed both a functional annotation (*i.e.*, GO term overrepresentation) and functional clustering analysis on a select number of comparisons between treatment conditions. Using a FDR of 10% we observed 113 overrepresented GO terms in the desiccation *vs.* food comparison, 8 in the mated *vs.* virgin males, 89 in the desiccation *vs.* food females, 75 in the desiccation *vs.* food males, 2 in the mated-desiccation *vs.* virgin-desiccation females, and 23 in the mated-food *vs.* virgin-food males (Table S2). For all other comparisons, no overrepresented GO terms were observed. In the desiccation *vs.* food comparison a few of the overrepresented GO terms were amino acid metabolism, phototransduction, carbohydrate metabolism, rhodopsin metabolism, hydrolase activity, and proteolysis. The majority of the overrepresented terms in the mated *vs.* virgin comparison in males were associated with antibacterial response. When we analyzed the desiccation response in females and males separately, for both sexes there was an overrepresentation of GO terms associated with metabolism (lipid, amino acid, and carbohydrate), vision, phototransduction, and rhodopsin metabolism. Additionally, in females we detected an overrepresentation of chorion genes. Clustering of genes into functionally

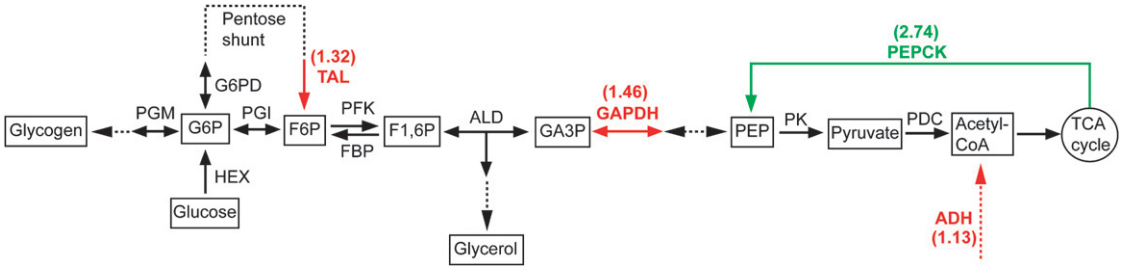

Figure 2.—Diagram of central metabolic pathway. Boxes indicate substrates, and enzymes are next to arrows. Single arrows indicate nonreversible reactions and double arrows are reversible reactions. Dashed arrows indicate multiple enzymatic steps. Enzymes in red are downregulated and green enzymes are upregulated under desiccating conditions. Parentheses indicate the fold expression difference associated with a desiccating environment.

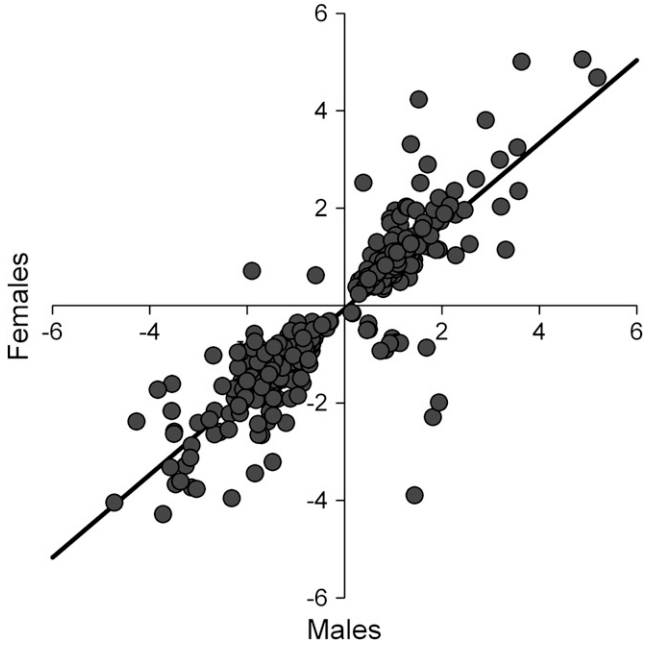

FIGURE 3.—Comparison of magnitude of female/male co-expression ($\log_2$ scale) of genes differentially expressed under desiccating conditions. Correlation is shown: $r = 0.88$, $P < 0.001$.

related groups allowed a more biologically meaningful examination of the expression changes. A total of 42 clusters were produced from the set of genes differentially expressed between the desiccation and food treatments (Table 2), 5 clusters in the male-mated *vs.* male-virgin comparison (Table S3), 23 clusters in the female-desiccation *vs.* female-food comparison (Table S4), 41 clusters in the male-desiccation *vs.* male-food comparison (Table S5), and 15 clusters in the mated-food *vs.* virgin-food comparison for males (Table S6).

Quantitative PCR validation: The four central metabolism genes (*Tal*, *Gapdh*, *Pepck*, and *Adh-2*) that were differentially expressed under desiccating conditions were verified using QPCR. Additionally, two ribosomal genes were used as controls, 16S (mitochondrial) and 18S (nuclear). The sequences of the QPCR primers used are shown in Table S7. The results obtained from the QPCR (downregulation of *Adh-2*, *Gapdh*, and *Tal* and the upregulation of *Pepck* under desiccation) were identical to those observed using the microarray (Figure 4). Furthermore, with the exception of *Pepck*, starvation did not significantly affect the expression of *Tal*, *Gapdh*, and *Adh-2*. Although *Pepck* expression is also affected by starvation, its induction under desiccation stress is 2.5 times greater ($P = 0.03$) than under starvation (Table S8).

DISCUSSION

While the main physiological changes accompanying desiccation in Drosophila are known (GIBBS and MATZKIN 2001; GIBBS *et al.* 2003a), the critical metabolic control points in the pathways underlying these changes have remained obscure. Here, rather than merely providing a list of genes by functional category whose expression changes with desiccation stress, we link phenotypic responses to particular metabolic pathway points and the genes that code for them.

Transcriptional adaptation to desiccation: Desert-adapted species face many challenges, among them the ability to survive in an environment of low water resources (HADLEY 1994). Nowhere is this challenge more serious than for terrestrial arthropods with unfavorable surface-to-volume ratios. In insects several mechanisms such as anhydrobiosis (WATANABE 2006), discontinuous gas exchange (LIGHTON 1996), and cuticle lipid composition (HADLEY 1979) have evolved as responses to inhabiting xeric environments. Within the genus Drosophila many species have adapted to the desert. One such example is *D. mojavensis*, a cactophile more resistant to desiccation than all of its congeners (VAN HERREWEGE and DAVID 1997; GIBBS and MATZKIN 2001; MATZKIN *et al.* 2007). *D. mojavensis* can survive for long periods in low humidity because of an overall reduction in metabolic rate and discontinuous gas exchange (GIBBS 2002; GIBBS *et al.* 2003b; MARRON *et al.* 2003). This physiological response to a desiccating environment appears to be unique to xeric and not to mesic species such as *D. melanogaster* (GIBBS *et al.* 2003b). A decreased metabolic rate effectively reduces the period during which the spiracles are open and water loss is maximized. For *D. mojavensis* humidity can drastically change temporally (monsoon *vs.* dry season) and spatially (inside *vs.* outside cactus necrosis) (GIBBS *et al.* 2003c). Comparison of the transcriptional profiles of desiccated and nondesiccated flies allows for the initial assessment of the metabolic modulations associated with the xeric-habitat adaptation of *D. mojavensis*.

A large number of genes (1679) were differentially expressed as a response to desiccating conditions. Furthermore, because these genes were differentially expressed in both sexes and mating status treatments, they can be assumed to underlie a general transcriptional response to desiccation in *D. mojavensis*. Gene ontology information is available for most (1018) of these genes. We performed a heuristic fuzzy partition analysis (DENNIS *et al.* 2003) that grouped differentially expressed genes into 42 functional clusters. The largest group was composed of 167 genes, all playing some role in protein metabolism (Table 2), which suggests, as predicted from previous work (GIBBS *et al.* 2003b; MARRON *et al.* 2003), that metabolism is affected as a response to desiccation. Of these 1679 genes, however, which ones play a major role in controlling metabolic flux?

Metabolic control analysis suggests that control of flux through a pathway lies at very few points (KACSER and BURNS 1973; FELL 1997). Control of flux of a particular enzyme in a pathway can be strongly dependent on its concentration and is measured as a control coefficient

TABLE 2

Results of functional clustering analysis between desiccation and food treatments

Cluster	No. of genes	Cluster function
1	50	Amino acid metabolism
2	43	Amino acid, carbohydrates, and ion transport
3	6	Cation transport
4	25	Lipid metabolism and catabolism
5	9	Secretion and vesicle-mediated transport
6	8	Fatty acid metabolism
7	28	Sensory transduction, signaling, neurophysiological process
8	10	Mitochondrial metabolism
9	13	Signal transduction, feeding behavior
10	8	Phototransduction, rhodopsin metabolism, detection of abiotic stimulus
11	7	Glucosidase activity
12	6	Innate immune response, peptidoglycan metabolism
13	7	Visual perception, detection of light stimulus, phototransduction
14	22	Nucleotide metabolism
15	17	Sensory perception of chemical stimulus, G-protein coupled receptor activity, olfaction
16	20	Glucosamine metabolism, biopolymer metabolism, polysaccharide metabolism
17	6	Short-chain dehydrogenase/reductase, oxidoreductase activity
18	15	Monooxygenase activity, toxin metabolism
19	12	Macromolecule metabolism
20	7	Phosphate metabolism
21	6	Endopeptidase inhibitor activity
22	15	Pyrimidine and purine nucleotide metabolism
23	6	Sensory perception of smell, response to pheromone
24	6	Hydrolase and transferase activity
25	10	Antibacterial humoral response, immune response, lysozyme activity
26	4	CoA-ligase activity
27	4	Carbohydrate metabolism, alcohol and monosaccharide biosynthesis
28	6	Alcohol, glucose, hexose, nucleotide, and acetyl-CoA catabolism
29	167	Protein metabolism, chymotrypsin activity, glycoprotein metabolism
30	5	Acyltransferase
31	12	RNA metabolism
32	10	Transferase, nucleotide binding
33	20	Protein transport, vesicle-mediated transport
34	6	Transmission of nerve impulse
35	6	Metal ion binding
36	6	Immunoglobulin
37	6	Calcium ion binding
38	41	Transcription regulation
39	5	Cell–cell adhesion
40	5	Induction of apoptosis
41	5	Oogenesis, vitelline membrane and chorion formation
42	6	Hydrolase activity, GTPase activity

that typically ranges from 0 (no effect on flux) to 1 (proportional effect on flux), but in certain cases can be negative and >1 (Kacser and Burns 1973). Pathways thus can buffer changes, be it via expression or amino acid substitutions, at points of low control of flux (Kacser and Burns 1981). Enzymes with high flux control coefficients tend to lie at the branch points of pathways and therefore are disproportionately affected by natural selection (Eanes 1999; Watt and Dean 2000). This is mostly due to the common substrate utilized by branch point enzymes, such as the enzymes that share glucose-6-phosphate as a substrate in *D. melanogaster* and *D. simulans* (Flowers *et al.* 2007). Additionally, the level of control of flux at any particular point is very much context dependent and is affected by the intracellular environment, such as activity changes at other points of the pathway (Dykhuizen and Hartl 1980; Dykhuizen *et al.* 1987). We can utilize the gene expression differences observed in this study to reveal the transcriptional changes responsible for the reduction in water loss rate, via a lower metabolic rate, in *D. mojavensis* exposed to a desiccating environment.

Our microarray analysis, subsequently verified by QPCR, showed the significant differential regulation

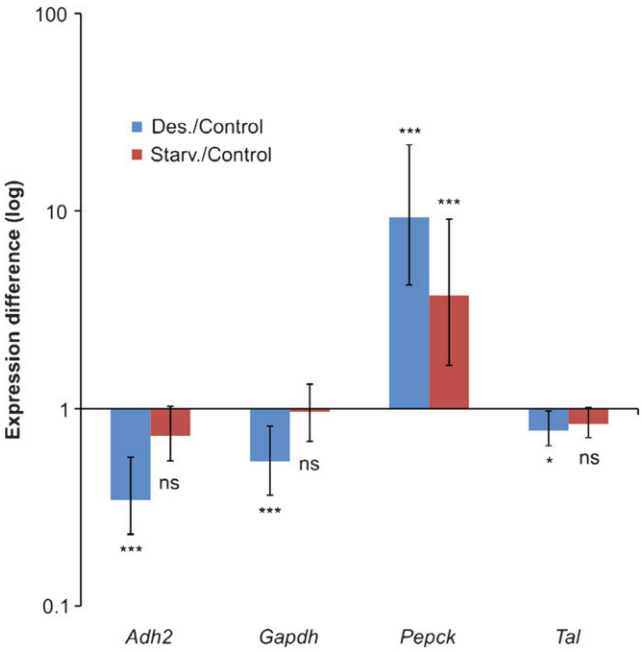

FIGURE 4.—Gene expression differences for desiccation/control and starvation/control treatments for four central metabolism genes. Standard error bars and Bonferroni-corrected *P*-values are shown. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; ns, not significant.

of four genes (*Gapdh*, *Tal*, *Adh*, and *Pepck*) associated with key points in central metabolism, which points to an overall reduction in metabolism in desiccated flies. These four enzymes are major components of the central metabolic pathway (Figure 2). The pentose shunt pathway, to which TAL belongs, is responsible for ~40% of the NADPH production (GEER *et al.* 1979). In *D. mojavensis*, as in many species, ADH metabolizes small alcohol molecules (MATZKIN 2005), but additionally, in *D. melanogaster* it has been shown to play a role in the fatty acid synthesis pathway (FRERIKSEN *et al.* 1991). As its placement in the pathways indicates (see Figure 2), GAPDH is important in both glycolysis and gluconeogenesis. PEPCK is crucial to the gluconeogenesis pathway, as it is the first step in that pathway (HERS and HUE 1983). In certain experimental conditions and tissues the flux control coefficients of GAPDH, ADH, and PEPCK have previously been observed to be low (<0.08) in *Saccharomyces cerevisiae*, *D. melanogaster*, and Rattus sp., respectively (GROEN *et al.* 1986; BRINDLE 1988; FRERIKSEN *et al.* 1994). But due to changes in the cellular environment, variations in flux control coefficients have been observed between different tissues or developmental stages within the same organism: for example, 0.03 in the kidney and 0.26 in the brain for the mitochondrial pyruvate carrier in Rattus (ROSSIGNOL *et al.* 2000) and 0.02 in adults and 1.0 in larvae for ADH in *D. melanogaster* (MIDDLETON and KACSER 1983; FRERIKSEN *et al.* 1991). Unfortunately control coefficients have never been measured in *D. mojavensis*, but given its distinct ecology it is reasonable to expect that the level of control of these metabolic enzymes might be different from that in other species. Taken together, our observations suggest that the coordinated concentration changes in these four enzymes could have a significant effect on flux.

Changes in gene expression seen in this study mirror those observed at *Gapdh* (ALEXANDER *et al.* 1988), *Tal* (HEINRICH *et al.* 1976), and *Pepck* (MAGNUSON *et al.* 1987) in response to decreased insulin titer. In Drosophila insulin signaling pathways have been known to be modulated under starvation conditions (RION and KAWECKI 2007). The changes at the four metabolic points observed in this study suggest that under desiccating conditions *D. mojavensis* might be increasing the rate of gluconeogenesis. Given our experimental design (flies had no food or water), a starvation-like response would not be unexpected. If so, the starvation response is likely not to be severe, since starvation resistance of *D. mojavensis* is significantly greater than desiccation resistance (MATZKIN *et al.* 2009). To try to tease out the effects of starvation we examined the gene expression of flies starved for the same amount of time that the flies were desiccated (36.5 hr). Although the changes in gene expression were mostly in the same direction for both stress treatments, only *Pepck* was significantly expressed under the starvation treatment, but not as greatly as for desiccation (Figure 4). This result suggests that at least in *D. mojavensis*, the modulation of *Pepck*, and most likely gluconeogenesis, is greatly affected under desiccating conditions and to a much lower extent under starvation.

In a recent study, GERSHMAN *et al.* (2007) examined the transcriptional profile of adult female *D. melanogaster* following a restoration of nutrition (yeast) from a nutritionally restricted diet. In that study they observed some transcriptional changes occurring within hours of nutrition restoration. Similarly to GERSHMAN *et al.* (2007) we observed the modulation *dilp* (*insulin-related peptides*) genes in the desiccation treatment, but unlike the current study they observed no expression differences in *Pepck*. Although in some aspects similar to starvation, this study indicates which points in the metabolic pathway (*Gapdh*, *Tal*, *Adh*, and *Pepck*) are specifically being modulated in response to desiccation. It is this reduction in metabolism that appears to be directly responsible for the decreased respiration rate and hence water loss rate in the xeric adapted *D. mojavensis*.

Metabolic responses to desiccation must first be triggered by perception of the stressful environment. Although no previous work exists regarding how *D. mojavensis* senses a desiccating environment, reports on *D. melanogaster* suggest involvement of phototransduction pathways. Members of phototransduction pathways have been shown to modulate in response to desiccation (SØRENSEN *et al.* 2007), starvation (HARBISON *et al.* 2005; SØRENSEN *et al.* 2007), feeding following a nutritional restriction treatment (GERSHMAN *et al.* 2007), insecti-

cide exposure (Pedra *et al.* 2004), heat (Nielsen *et al.* 2006; Sørensen *et al.* 2007), selection for longevity (Sørensen *et al.* 2007), and overall general metabolic stress (Minke and Parnas 2006). In our study we observed a significant ($P < 0.001$, after Bonferroni correction) overrepresentation of phototransduction genes within those differentially expressed under desiccating conditions. As previously suggested (Sørensen *et al.* 2007), our data further implicate phototransduction pathways playing a role in stress response.

Sex-specific gene expression and mating in *D. mojavensis*: The transcriptomes of females and males appear to be distinct and under different selective pressures (Ellegren and Parsch 2007; Zhang *et al.* 2007). In a previous analysis Zhang *et al.* (2007) observed that 25% of the transcriptome was sex biased, with roughly the same amount being expressed in either sex. In contrast, in this study we observed more than half (62%) of the transcriptome to be different among females and males, with the majority of those genes (62%) being upregulated in females. Also, comparisons of only mated or virgin flies still indicate a much greater level of sex-biased expression (54 and 46%, respectively; Table S1). Additionally, the fraction of female-biased genes is identical when examined experiment-wide (62%), for only mated flies (61%) or only virgin flies (59%). Selection at any particular gene is context dependent, be it in different tissues, developmental stages, or sex. Male-biased gene expression appears to be associated with higher levels of nucleotide divergence (Khaitovich *et al.* 2005; Zhang *et al.* 2007), suggesting that sexual selection can affect the evolution of the transcriptome. Our study suggests that sex-specific selection pressures might affect a much greater fraction of the *D. mojavensis* genome than previously thought.

Unlike *D. melanogaster*, *D. mojavensis* females are provided with energetic ejaculates by males (Markow and O'Grady 2005). Incorporation of male-derived substances after copulation may explain why *D. mojavensis* females experience no postmating reduction in stress performance (Markow and O'Grady 2005). Previous work revealed that both proteins and phosphorous derived from the male are incorporated into female somatic tissues (Pitnick *et al.* 1997; Markow *et al.* 2001). Given these different physiological outcomes of mating in *D. mojavensis* males and females, sex differences in transcription following mating are no surprise.

Glycogen functions as a long-term carbohydrate storage molecule that can affect many life history characteristics such as desiccation resistance and flight performance (Graves *et al.* 1992). Courting involves an intricate dance, composed of specific sets of wing beats and movements, which presumably is energetically costly (Ewing and Miyan 1986; Markow and Toolson 1990). Interestingly, virgin males have a slightly greater expression (9%) of *glycogen phosphorylase* (*GP*) than their mated counterparts. Glycogen phosphorylase is the enzyme responsible for breaking down glycogen to glucose-1-phosphate, which then can enter glycolysis. This appears contrary to our prediction that glycogen catabolism increases in response to courtship and mating. Recent work in *D. melanogaster*, however, suggests fitness effects of GP variation become apparent only when enzyme activity is roughly halved (Eanes *et al.* 2006). Therefore the changes we observed at GP are probably insufficient to negatively affect glycogen titer in males postmating.

Cuticular hydrocarbons long have been proposed to play a role in courting behavior and mating (Carlson *et al.* 1978; Markow and Toolson 1990). We observed a significant upregulation of *desaturase-2* (*desat2*) in *D. mojavensis* females following mating. The *desat2* gene, as well as its paralog *desat1*, codes for an enzyme with stearoyl-CoA 9-desaturase activity, which is responsible for the synthesis of cuticular hydrocarbons (Dallerac *et al.* 2000; Ueyama *et al.* 2005). Variation at *desat2* was previously implicated in premating isolation between cosmopolitan and Zimbabwean populations of *D. melanogaster* (Greenberg *et al.* 2003, 2006), although its role in behavioral isolation has recently been critically challenged (Coyne and Elwyn 2006a,b). Aside from its possible function in courtship, *desat2* could possibly affect the accumulation of triglycerides following mating in *D. mojavensis*. In *D. melanogaster*, *desat1* has been shown to be the rate-limiting step in the synthesis of fatty acids and to largely affect overall lipid pools (Ueyama *et al.* 2005). Interestingly *desat2* was not differentially modulated in males, suggesting the possible existence of sex-specific mechanisms of triglyceride allocation. Finally, it should be noted that all the changes observed in this study are at the transcriptional level. To further understand the gene modulation effects on protein function, analyses at the proteomic and metabolomic levels are necessary.

Conclusion: Survival in a xeric environment places many selective pressures on an organism. For arthropods, maintenance of adequate water balance is crucial. Cactophilic Drosophila have adapted to their desiccating environment by reducing their metabolic rate (Gibbs and Matzkin 2001; Gibbs *et al.* 2003b). Our results reveal that the relevant change in metabolic rate is modulated by the differential expression of four enzymatic points of the central metabolic pathway. Additionally, this study further implicates the role of phototransduction genes in stress sensing. As previously proposed (Lopez-Maury *et al.* 2008; Matzkin 2008), we have shown how gene expression modulation can play an important role in adaptation.

We thank National Science Foundation (NSF) Research Experiences for Teachers intern Georgina J. Matzkin for all her hard work setting up the desiccation experiments. We thank Walt Eanes, Jeremy Bono, Tamara Haselkorn, Erin Kelleher, Laura Reed, and two anonymous reviewers for helpful comments on the manuscript. This project was supported by a NSF Research Experiences for Teachers grant to T.A.M. and a grant from Science Foundation Arizona to T.A.M. and L.M.M.

LITERATURE CITED

Alexander, M. C., M. Lomanto, N. Nasrin and C. Ramaika, 1988 Insulin stimulates glyceraldehyde-3-phosphate dehydrogenase gene expression through cis-acting DNA sequences. Proc. Natl. Acad. Sci. USA **85:** 5092–5096.

Bolstad, B. M., R. A. Irizarry, M. Astrand and T. P. Speed, 2003 A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics **19:** 185–193.

Brindle, K. M., 1988 P-31 NMR magnetization transfer measurements of flux between inorganic phosphate and adenosine 5′-triphosphate in yeast cells genetically modified to overproduce phosphoglycerate kinase. Biochemistry **27:** 6187–6196.

Carlson, D. A., P. A. Langley and P. Huyton, 1978 Sex pheromone of tsetse fl: isolation, identification, and synthesis of contact aphrodisiacs. Science **201:** 750–753.

Coyne, J. A., and S. Elwyn, 2006a Desaturase-2, environmental adaptation, and sexual isolation in *Drosophila melanogaster*. Evolution **60:** 626–627.

Coyne, J. A., and S. Elwyn, 2006b Does the desaturase-2 locus in *Drosophila melanogaster* cause adaptation and sexual isolation? Evolution **60:** 279–291.

Dallerac, R., C. Labeur, J. M. Jallon, D. C. Knippie, W. L. Roelofs *et al.*, 2000 A delta 9 desaturase gene with a different substrate specificity is responsible for the cuticular diene hydrocarbon polymorphism in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **97:** 9449–9454.

Dennis, G., B. T. Sherman, D. A. Hosack, J. Yang, W. Gao *et al.*, 2003 David: database for annotation, visualization, and integrated discovery. Genome Biol. **4:** R60.

Djawdan, M., A. K. Chippindale, M. R. Rose and T. J. Bradley, 1998 Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. Physiol. Zool. **71:** 584–594.

Drosophila 12 Genomes Consortium, 2007 Evolution of genes and genomes on the Drosophila phylogeny. Nature **450:** 203–218.

Dykhuizen, D., and D. L. Hartl, 1980 Selective neutrality of 6pgd allozymes in *Escherichia coli* and the effects of genetic background. Genetics **96:** 801–817.

Dykhuizen, D. E., A. M. Dean and D. L. Hartl, 1987 Metabolic flux and fitness. Genetics **115:** 25–31.

Eanes, W. F., 1999 Analysis of selection on enzyme polymorphisms. Annu. Rev. Ecol. Syst. **30:** 301–326.

Eanes, W. F., T. J. S. Merritt, J. M. Flowers, S. Kumagai, E. Sezgin *et al.*, 2006 Flux control and excess capacity in the enzymes of glycolysis and their relationship to flight metabolism in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **103:** 19413–19418.

Ellegren, H., and J. Parsch, 2007 The evolution of sex-biased genes and sex-biased gene expression. Nat. Rev. Genet. **8:** 689–698.

Ewing, A. W., and J. A. Miyan, 1986 Sexual selection, sexual isolation and the evolution of song in the *Drosophila repleta* group of species. Anim. Behav. **34:** 421–429.

Fell, D., 1997 *Understanding the Control of Metabolism*. Portland Press, London.

Flowers, J. M., E. Sezgin, S. Kumagai, D. D. Duvernell, L. M. Matzkin *et al.*, 2007 Adaptive evolution of metabolic pathways in Drosophila. Mol. Biol. Evol. **24:** 1347–1354.

Freriksen, A., D. Seykens, W. Scharloo and P. W. H. Heinstra, 1991 Alcohol dehydrogenase controls the flux from ethanol into lipids in *Drosophila* larvae: a C-13 NMR-study. J. Biol. Chem. **266:** 21399–21403.

Freriksen, A., D. Seykens and P. W. H. Heinstra, 1994 Differences between larval and adult *Drosophila* in metabolic degradation of ethanol. Evolution **48:** 504–508.

Geer, B. W., D. L. Lindel and D. M. Lindel, 1979 Relationship of the oxidative pentose shunt pathway to lipid synthesis in *Drosophila melanogaster*. Biochem. Genet. **17:** 881–895.

Gershman, B., O. Puig, L. Hang, R. M. Peitzsch, M. Tatar *et al.*, 2007 High-resolution dynamics of the transcriptional response to nutrition in *Drosophila*: a key role for dFOXO. Physiol. Genomics **29:** 24–34.

Gibbs, A. G., 2002 Water balance in desert Drosophila: lessons from non-charismatic microfauna. Comp. Biochem. Physiol. A-Mol. Integr. Physiol. **133:** 781–789.

Gibbs, A. G., and L. M. Matzkin, 2001 Evolution of water balance in the genus *Drosophila*. J. Exp. Biol. **204:** 2331–2338.

Gibbs, A., F. Fukuzato and L. Matzkin, 2003a Evolution of water conservation mechanisms in Drosophila. J. Exp. Biol. **206:** 1183–1192.

Gibbs, A. G., F. Fukuzato and L. M. Matzkin, 2003b Evolution of water conservation mechanisms in *Drosophila*. J. Exp. Biol. **206:** 1183–1192.

Gibbs, A. G., M. C. Perkins and T. A. Markow, 2003c No place to hide: microclimates of Sonoran desert *Drosophila*. J. Therm. Biol. **28:** 353–362.

Graves, J. L., E. C. Toolson, C. Jeong, L. N. Vu and M. R. Rose, 1992 Desiccation, flight, glycogen, and postponed senescence in *Drosophila melanogaster*. Physiol. Zool. **65:** 268–286.

Greenberg, A. J., J. R. Moran, J. A. Coyne and C. I. Wu, 2003 Ecological adaptation during incipient speciation revealed by precise gene replacement. Science **302:** 1754–1757.

Greenberg, A. J., J. R. Moran and C. I. Wu, 2006 Proper control of genetic background with precise allele substitution: a comment on coyne and elwyn. Evolution **60:** 623–625.

Groen, A. K., C. W. T. Van roermund, R. C. Vervoorn and J. M. Tager, 1986 Control of gluconeogenesis in rat liver cells: flux control coefficients of the enzymes in the gluconeogenic pathway in the absence and presence of glucagon. Biochem. J. **237:** 379–389.

Hadley, N. F., 1979 Wax secretion and color phases of the desert Tenebrionid beetle *Cryptoglossa verrucosa* (LeConte). Science **203:** 367–369.

Hadley, N. F., 1994 *Water Relations of Terrestrial Arthropods*. Academic Press, San Diego.

Harbison, S. T., S. Chang, K. P. Kamdar and T. F. C. Mackay, 2005 Quantitative genomics of starvation stress resistance in *Drosophila*. Genome Biol. **6:** R36.

Heed, W. B., 1978 Ecology and genetics of Sonoran desert Drosophila, pp. 109–126 in *Ecological Genetics: The Interface*, edited by P. F. Brussard. Springer-Verlag, Berlin/Heidelberg, Germany/New York.

Heinrich, P. C., H. P. Morris and G. Weber, 1976 Behavior of transaldolase (EC 2.2.1.2) and transketolase (EC 2.2.1.1) activities in normal, neoplastic, differentiating, and regenerating liver. Cancer Res. **36:** 3189–3197.

Hers, H. G., and L. Hue, 1983 Gluconeogenesis and related aspects of glycolysis. Annu. Rev. Biochem. **52:** 617–653.

Hochachka, P. W., and G. N. Somero, 2002 *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. Oxford University Press, New York.

Hosack, D. A., G. Dennis, B. T. Sherman, H. C. Lane and R. A. Lempicki, 2003 Identifying biological themes within lists of genes with ease. Genome Biol. **4:** R70.

Irizarry, R. A., B. Hobbs, F. Collin, Y. D. Beazer-Barclay, K. J. Antonellis *et al.*, 2003 Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics **4:** 249–264.

Kacser, H., and J. A. Burns, 1973 The control of flux. Symp. Soc. Exp. Biol. **27:** 65–104.

Kacser, H., and J. A. Burns, 1981 The molecular basis of dominance. Genetics **97:** 639–666.

Kelleher, E. S., W. J. Swanson and T. A. Markow, 2007 Gene duplication and adaptive evolution of digestive proteases in *Drosophila arizonae* female reproductive tracts. PLoS Genet. **3:** 1541–1549.

Khaitovich, P., I. Hellmann, W. Enard, K. Nowick, M. Leinweber *et al.*, 2005 Parallel patterns of evolution in the genomes and transcriptomes of humans and chimpanzees. Science **309:** 1850–1854.

Knowles, L. L., B. B. Hernandez and T. A. Markow, 2004 Exploring the consequences of postmating-prezygotic interactions between the sexes. Proc. R. Soc. Lond. Ser. B Biol. Sci. **271:** S357–S359.

Knowles, L. L., B. B. Hernandez and T. A. Markow, 2005 Nonantagonistic interactions between the sexes revealed by the ecological consequences of reproductive traits. J. Evol. Biol. **18:** 156–161.

Lighton, J. R. B., 1996 Discontinuous gas exchange in insects. Annu. Rev. Entomol. **41:** 309–324.

Lopez-Maury, L., S. Marguerat and J. Bahler, 2008 Tuning gene expression to changing environments: from rapid responses to evolutionary adaptation. Nat. Rev. Genet. **9:** 583–593.

Magnuson, M. A., P. G. Quinn and D. K. Granner, 1987 Multihormonal regulation of phosphoenolpyruvate carboxykinase-chloramphenicol acetyltransferase fusion genes: insulin's effects oppose those of cAMP and dexamethasone. J. Biol. Chem. **262:** 14917–14920.

Markow, T. A., and P. M. O'Grady, 2005 Evolutionary genetics of reproductive behavior in *Drosophila*: connecting the dots. Annu. Rev. Genet. **39:** 263–291.

Markow, T. A., and E. C. Toolson, 1990 Temperature effects on epicuticular hydrocarbons and sexual isolation in *Drosophila mojavensis*, pp. 315–331 in *Ecological and Evolutionary Genetics of Drosophila*, edited by J. S. F. Barker, W. T. Starmer and R. J. MacIntyre. Plenum Press, New York.

Markow, T. A., A. Coppola and T. D. Watts, 2001 How *Drosophila* males make eggs: it is elemental. Proc. R. Soc. Lond. Ser. B Biol. Sci. **268:** 1527–1532.

Marron, M. T., T. A. Markow, K. J. Kain and A. G. Gibbs, 2003 Effects of starvation and desiccation on energy metabolism in desert and mesic Drosophila. J. Insect Physiol. **49:** 261–270.

Matzkin, L. M., 2005 Activity variation in alcohol dehydrogenase paralogs is associated with adaptation to cactus host use in cactophilic *Drosophila*. Mol. Ecol. **14:** 2223–2231.

Matzkin, L. M., 2008 The molecular basis of host adaptation in cactophilic Drosophila: molecular evolution of a glutathione s-transferase gene (*GstD1*) in *Drosophila mojavensis*. Genetics **178:** 1073–1083.

Matzkin, L. M., T. D. Watts, B. G. Bitler, C. A. Machado and T. A. Markow, 2006 Functional genomics of cactus host shifts in *Drosophila mojavensis*. Mol. Ecol. **15:** 4635–4643.

Matzkin, L. M., T. D. Watts and T. A. Markow, 2007 Desiccation resistance in four Drosophila species: sex and population effects. Fly **1:** 268–273.

Matzkin, L. M., T. D. Watts and T. A. Markow, 2009 Evolution of stress resistance in Drosophila: interspecific variation in tolerance to desiccation and starvation. Funct. Ecol. **23:** 521–527.

Middleton, R. J., and H. Kacser, 1983 Enzyme variation, metabolic flux and fitness: alcohol dehydrogenase in *Drosophila melanogaster*. Genetics **105:** 633–650.

Minke, B., and M. Parnas, 2006 Insights on trp channels from in vivo studies in *Drosophila*. Annu. Rev. Physiol. **68:** 649–684.

Nielsen, M. M., J. G. Sorensen, M. Kruhoffer, J. Justesen and V. Loeschcke, 2006 Phototransduction genes are up-regulated in a global gene expression study of *Drosophila melanogaster* selected for heat resistance. Cell Stress Chaperones **11:** 325–333.

Pedra, J. H. F., L. M. McIntyre, M. E. Scharf and B. R. Pittendrigh, 2004 Genome wide transcription profile of field- and laboratory-selected dichlorodiphenyltrichloroethane (DDT)-resistant *Drosophila*. Proc. Natl. Acad. Sci. USA **101:** 7034–7039.

Pfaffl, M. W., G. W. Horgan and L. Dempfle, 2002 Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time pcr. Nucleic Acids Res. **30:** e36.

Pitnick, S., G. S. Spicer and T. Markow, 1997 Phylogenetic examination of female incorporation of ejaculate in Drosophila. Evolution **51:** 833–845.

Rion, S., and T. J. Kawecki, 2007 Evolutionary biology of starvation resistance: what we have learned from Drosophila. J. Evol. Biol. **20:** 1655–1664.

Rossignol, R., T. Letellier, M. Malgat, C. Rocher and J. P. Mazat, 2000 Tissue variation in the control of oxidative phosphorylation: implication for mitochondrial diseases. Biochem. J. **347:** 45–53.

Schaeffer, S. W., A. Bhutkar, B. F. McAllister, M. Matsuda, L. M. Matzkin *et al.*, 2008 Polytene chromosomal maps of 11 Drosophila species: the order of genomic scaffolds inferred from genetic and physical maps. Genetics **179:** 1601–1655.

Sørensen, J. G., M. M. Nielsen and V. Loeschcke, 2007 Gene expression profile analysis of *Drosophila melanogaster* selected for resistance to environmental stressors. J. Evol. Biol. **20:** 1624–1636.

Storey, J., and R. Tibshirani, 2003 Statistical significance for genomewide studies. Proc. Natl. Acad. Sci. USA **100:** 9440–9445.

Ueyama, M., T. Chertemps, C. Labeur and C. Wicker-Thomas, 2005 Mutations in the *desat1* gene reduce the production of courtship stimulatory pheromones through a marked effect on fatty acids in *Drosophila melanogaster*. Insect Biochem. Mol. Biol. **35:** 911–920.

van Herrewege, J., and J. R. David, 1997 Starvation and desiccation tolerances in *Drosophila*: comparison of species from different climatic origins. Ecoscience **4:** 151–157.

Watanabe, M., 2006 Anhydrobiosis in invertebrates. Appl. Entomol. Zool. **41:** 15–31.

Watt, W. B., and A. M. Dean, 2000 Molecular functional studies of adaptive genetic variation in prokaryotes and eukaryotes. Annu. Rev. Genet. **34:** 593–622.

Wolfinger, R. D., G. Gibson, E. D. Wolfinger, L. Bennett, H. Hamadeh *et al.*, 2001 Assessing gene significance from cDNA microarray expression data via mixed models. J. Comput. Biol. **8:** 625–637.

Zhang, Y., D. Sturgill, M. Parisi, S. Kumar and B. Oliver, 2007 Constraint and turnover in sex-biased gene expression in the genus *Drosophila*. Nature **450:** 233–237.

Communicating editor: G. Gibson

GENETICS

Supporting Information
http://www.genetics.org/cgi/content/full/genetics.109.104927/DC1

Transcriptional regulation of metabolism associated with the increased desiccation resistance of the cactophilic *Drosophila mojavensis*

Luciano M. Matzkin and Therese A. Markow

DOI: 10.1534/genetics.109.104927

TABLE S1

Number of significantly up and down regulated genes in each specific comparison

Effect comparison						Genes (up/down)
Sex	Mating	Stress	Sex	Mating	Stress	
♀			♂			9233 (5740/3493)
	M			V		75 (65/10)
		D			F	1679 (774/905)
♀	M		♀	V		37 (27/10)
♂	M		♂	V		214 (123/91)
♀	M		♂	M		8052 (4943/3109)
♀	V		♂	M		8148 (4939/3209)
♀	M		♂	V		6766 (4050/2716)
♀	V		♂	V		6977 (4115/2862)
♀		D	♀		F	809 (384/425)
♂		D	♂		F	1927 (875/1052)
♀		F	♂		D	8588 (5206/3382)
♀		D	♂		D	8333 (5123/3210)
♀		F	♂		F	6769 (4012/2757)
♀		D	♂		F	7185 (4231/2954)
	M	D		M	F	958 (410/548)
	M	D		V	D	33 (29/4)
	V	D		V	F	851 (374/477)
	M	D		V	F	1033 (536/497)
	M	F		V	F	85 (64/21)
	M	F		V	D	859 (552/307)
♀	M	F	♀	V	F	23 (16/7)
♀	M	F	♀	V	D	379 (255/124)
♀	M	D	♀	V	F	450 (242/208)
♀	V	D	♀	V	F	416 (226/190)
♀	M	D	♀	M	F	355 (122/233)
♀	M	D	♀	V	D	36 (23/13)
♂	M	F	♂	V	F	409 (163/246)
♂	M	F	♂	V	D	704 (482/222)
♂	V	D	♂	V	F	1261 (599/662)
♂	M	D	♂	M	F	1031 (448/583)
♂	M	D	♂	V	F	1977 (973/1004)
♂	M	D	♂	V	D	47 (30/17)
♀	M	F	♂	M	D	6351 (3569/2782)
♀	V	F	♂	M	D	6761 (3813/2948)
♀	M	D	♂	M	D	6420 (3667/2753)
♀	V	D	♂	M	D	6413 (3665/2748)

♀	M	F	♂	V	D	6363 (3690/2673)
♀	V	F	♂	V	D	6793 (3933/2860)
♀	M	D	♂	V	D	6398 (3750/2648)
♀	V	D	♂	V	D	6414 (3763/2651)
♀	M	F	♂	V	F	997 (455/542)
♀	M	F	♂	M	F	5985 (3506/2479)
♀	V	F	♂	M	F	6403 (3713/2690)
♀	V	F	♂	V	F	1408 (632/776)
♀	V	D	♂	M	F	6678 (3869/2809)
♀	M	D	♂	M	F	6631 (3840/2791)
♀	M	D	♂	V	F	684 (344/340)
♀	V	D	♂	V	F	769 (389/380)

Mating (M = Mated, V = Virgin), Stress (D = Desiccation, F = Food).

TABLE S2

Table S2 is available for download as an Excel file at http://www.genetics.org/cgi/content/full/genetics.109.104927/DC1.

TABLE S3

Males mated vs. Males virgins

Cluster	Number of genes	Cluster function
1	12	Regulation of transcription
2	5	Glycoprotein and protein metabolism
3	4	Protein localization and transport
4	4	RNA processing
5	4	Translation, ribosomal proteins

TABLE S4

Female desiccation vs. Female food treatments

Cluster	Number of genes	Cluster function
1	27	Porter activity
2	18	Synaptic transmission, ionic transport
3	18	Circadian behavior, serotonin, dopamine and other hormones metabolism
4	8	Neuropeptide signaling pathway, feeding behavior
5	5	visual perception, phototransduction, rhabdomere
6	7	Mitochondrial metabolism
7	7	Lipid and fatty acid metabolism
8	5	peptidoglycan metabolism, defense response
9	11	Lipase and triacylglycerol lipase activity
10	11	G-protein coupled receptor activity, sensory perception of chemical stimulus
11	12	chitin catabolism, amino sugar metabolism
12	5	antibacterial humoral response
13	12	Purine and pyrimidine nucleotide metabolism
14	4	neurophysiological process, leucine-rich repeat
15	6	carbohydrate metabolism, response to chemical stimulus
16	6	Oogenesis
17	7	Cytochrome P450 activity
18	7	Kinase, transferase activity, nucleotide binding
19	77	protein processing and modification
20	4	serine-type endopeptidase inhibitor activity
21	5	synaptic vesicle transport, transmission of nerve impulse
22	17	regulation of transcription
23	8	metal ion binding

TABLE S5

Male desiccation vs. Male food treatments

Cluster	Number of genes	Cluster function
1	23	Amino acid (Val, Leu, Ile, Gly, Ser, Thr, Glu), acyl-CoA and fatty acid metabolism
2	13	Lipid and fatty acid metabolism
3	28	Amino acid (Phe, Tyr, Trp, Arg, Pro, Cys, Met, Gly, Ser, Thr, Ala, Asp, Glu) metabolism
4	5	Peroxisome
5	28	Mitochondrial metabolism
6	59	Cation transport, electrochemical potential-driven transporter activity
7	202	Protein biosynthesis
8	21	Phospholipid metabolism, triacylglycerol lipase activity
9	4	Polysaccharide metabolism, glucosidase activity
10	4	Phosphate transport
11	56	Protein transport and localization, secretion
12	39	RNA binding, processing and metabolism
13	10	Response to toxin, UDP-glycosyltransferase activity
14	6	DNA metabolism
15	5	DNA binding and repair
16	36	ATPase activity
17	7	Serine-type endopeptidase inhibitor activity
18	5	Phosphorus metabolism
19	16	Glucosamine and chitin metabolism
20	5	Rhodopsin biosynthesis, phototransduction
21	10	Glucose, alcohol, acetyl-CoA, TCA metabolism
22	5	Acyltransferase
23	6	Innate immune response, peptidoglycan metabolism
24	4	Hexose (fucose) metabolism
25	4	Response to toxin, Glutathione S-transferase
26	6	Acetyltransferase activity
27	8	Neuropeptide signaling pathway, amidation
28	6	Methyltransferase activity
29	59	regulation of transcription, regulation of development
30	9	Cytochrome P450, monooxygenase activity
31	5	serine-type endopeptidase inhibitor activity
32	18	G-protein coupled receptor activity, sensory perception of light and chemical stimulus
33	6	Pheromone/general odorant binding protein
34	6	purine nucleotide metabolism
35	6	methyltransferase
36	4	Glucose/ribitol dehydrogenase
37	6	antibacterial humoral response
38	4	neuron development
39	7	calcium ion binding
40	7	Transferase, Kinase
41	5	Immunoglobulin

TABLE S6

Male mated food vs. Male virgin food treatments

Cluster	Number of genes	Cluster function
1	37	Regulation of transcription
2	9	DNA metabolism, helicase activity
3	7	Fatty acid metabolism
4	12	Protein modification and metabolism
5	5	DNA metabolism, chromatin assembly
6	6	RNA processing and splicing
7	13	Serine-type endopeptidase activity
8	8	Phosphate metabolism
9	8	Protein localization and transport
10	4	RNA metabolism, exonuclease activity
11	18	Macromolecule metabolism, ribosomes
12	4	Neurogenesis
13	4	RNA metabolism, mRNA binding
14	4	Neurotransmitter secretion, synaptic vesicle transport
15	4	Antibacterial humoral response

TABLE S7

QPCR Primers

Gene	RT Primers	Cds start/stop	Amplicon Size	Temp
16S	CTCGTCCAACCATTCATTCC / GAAATTTTAAATGGCCGCAGT	39/129	90	54
18S	GGTCTGTGATGCCTTTAGATGTCC / GACCTCTCGGTCTAGGAAATACAC	1614/1695	82	56
Gapdh	CCGTAATCCCGAATTGTGTGTGAG / GTCGTCGAGTTGCAGTAGAT	7785/7883	99	54
Tal	GTACTGGAGACCGCTTTATCTG / CGGCTCTCGTCAATGGTAATCTTC	772/845	74	54
Adh-2	TTGAAGACAATCTTCGACAAGC / ACGCTCGATCTGGTAGTCGT	277/361	85	54
Pepck	CTACAACTTCGGCGACTATGTG / CCAGTTGACATGGAAGATCTTGGG	1539/1620	82	54

TABLE S8

Pairwise comparisons for the four metabolic genes

Adh-2		Numerator		
		Control	Starvation	Desiccation
Denominator	Control	-	0.727	0.344
	Starvation	0.12	-	0.473
	Desiccation	< 0.001	< 0.001	-

Gapdh		Numerator		
		Control	Starvation	Desiccation
Denominator	Control	-	0.965	0.541
	Starvation	0.99	-	0.561
	Desiccation	< 0.001	< 0.001	-

Pepck		Numerator		
		Control	Starvation	Desiccation
Denominator	Control	-	3.722	9.261
	Starvation	< 0.001	-	2.489
	Desiccation	< 0.001	0.03	-

Tal		Numerator		
		Control	Starvation	Desiccation
Denominator	Control	-	0.838	0.775
	Starvation	0.12	-	0.926
	Desiccation	0.03	0.99	-

Expression differences between treatments are shown in the upper diagonal and Bonferroni corrected P-values are shown in the lower diagonal.