MOLECULAR ECOLOGY

Molecular Ecology (2012) **21**, 2428–2439 doi: 10.1111/j.1365-294X.2012.05549.x

Population transcriptomics of cactus host shifts in *Drosophila mojavensis*

LUCIANO M. MATZKIN*†

*Department of Biological Sciences, Huntsville, University of Alabama in Huntsville, AL 35899, USA, †Section of Ecology, Behaviour and Evolution La Jolla, University of California, San Diego, CA 92093-0116, USA

Abstract

In the presence of environmental change, natural selection can shape the transcriptome. Under a scenario of environmental change, genotypes that are better able to modulate gene expression to maximize fitness will tend to be favoured. Therefore, it is important to examine gene expression at the population level to distinguish random or neutral gene expression variation from the pattern produced by natural selection. This study investigates the natural variation in transcriptional response to a cactus host shift utilizing the mainland Sonora population of *Drosophila mojavensis*. *Drosophila mojavensis* is a cactophilic species composed of four cactus host populations endemic to the deserts of North America. Overall, the change in cactus host was associated with a significant reduction in larval viability as well as the differential expression of 21% of the genome (3109 genes). Among the genes identified were a set of genes previously known to be involved in xenobiotic metabolism, as well as genes involved in cellular energy production, oxidoreductase/carbohydrate metabolism, structural components and mRNA binding. Interestingly, of the 3109 genes whose expression was affected by host use, there was a significant overrepresentation of genes that lacked an orthologous call to the *D. melanogaster* genome, suggesting the possibility of an accelerated rate of evolution in these genes. Of the genes with a significant cactus effect, the majority, 2264 genes, did not exhibit a significant cactus-by-line interaction. This population-level approach facilitated the identification of genes involved in past cactus host shifts.

Keywords: Adaptation, transcriptional variation, host shifts, cactophilic *Drosophila*, genotype-by-environment interactions.

Received 15 June 2010; revision received 5 February 2012; accepted 8 February 2012

Introduction

Transcriptional-level changes have long been implicated in playing a large role in creating phenotypic variation (Shapiro *et al*. 2004; Abzhanov *et al*. 2006; Werner *et al*. 2010). Examination of variants within a coding region traditionally has been more tractable given the structure of the genetic code. Although recent work suggests a significant role for *cis*-regulatory changes in the transcriptional divergence between species (Wittkopp *et al*. 2004, 2008), elucidating the distribution of sequence variants affecting transcription and determining how natural selection could shape this distribution has been more difficult. Factors underlying the maintenance of transcriptional variation in natural populations, while not well understood, are likely to reflect an organism's ecology and evolutionary history (Whitehead & Crawford 2006; Wittkopp *et al*. 2008; Meyer *et al*. 2011; Williams & Oleksiak 2011).

The role of selection can best be captured by examining genetic variation in the ecological context of the focal species (Feder & Mitchell-Olds 2003). In this study, the transcriptional variation of *Drosophila mojavensis*, a species for which large amount of ecological data exist, was examined. A cactophilic fly endemic to the deserts of northwestern Mexico and southwestern United States, *D. mojavensis*, is composed of four, recently diverged (0.5 Ma) populations or subspecies

Correspondence: Luciano M. Matzkin, Fax: +1 256 824 6305; E-mail: lmm0015@uah.edu

Present address: Department of Biological Sciences, University of Alabama in Huntsville, Huntsville, Alabama 35899, USA.

that each utilizes a distinct cactus host (Heed 1978; Machado *et al.* 2007; Matzkin 2008; Pfeiler *et al.* 2009). The different cactus hosts offer a unique set of chemical compounds, some toxic, to which resident *Drosophila* must adapt (Vacek 1979; Kircher 1982). The chemical composition of the cactus necroses as well as the performance of the *D. mojavensis* is largely dependent on the yeast and bacterial communities that are known to inhabit the necroses in the field (Starmer 1982a,b; Fogleman & Starmer 1985; Starmer *et al.* 1990). Previous studies revealed that both coding sequence and functional variation in detoxification genes such as *Alcohol dehydrogenase* and *Glutathione S-transferase D1* have been shaped by host usage (Starmer *et al.* 1977; Heed 1978; Matzkin 2004, 2005, 2008). Several detoxification genes appear to be differentially expressed in response to a cactus host shift (Matzkin *et al.* 2006), although no population-level response could be assessed as only one isofemale line was utilized in that study. Furthermore, in considering host shifts, genes associated with host preference might be involved in addition to those related to detoxification and overall survival. Hence, it is likely that variation at sensory pathway genes has also been shaped by host shifts, as previously observed for *D. sechellia* and its natural host *Morinda citrifolia* (McBride 2007; Dworkin & Jones 2009). In addition to being crucial for host recognition in adults, gustatory and odorant receptors are important for the microhabitat selection of larvae (Vosshall & Stocker 2007). A subset of the adult-expressed odorant and gustatory receptors are expressed in larvae. *Drosophila mojavensis* larvae preferentially feed on specific species of yeasts, while avoiding others (Fogleman *et al.* 1981). Thus, the ecologically distinct and recently isolated *D. mojavensis* populations are well suited to examine the transcriptional variation and its evolution across the genome as it relates to host adaptation (Starmer *et al.* 1977; Lofdahl 1985; Etges 1990; Matzkin 2005).

As proposed by Matzkin (2008), the pattern and level of neutral genetic variation for transcriptional modulation can be changed in different environmental contexts. Under certain ecological scenarios, genetic backgrounds (genotypes) that are transcriptionally plastic will be favoured (Baldwin 1896; Waddington 1953; West-Eberhard 2003), ultimately reducing the numbers of significant genotype × environment (G × E) interactions observed (Matzkin 2008). The prediction would be that given standing genetic transcriptional variation in a population, the alleles of genes whose modulation in expression is advantageous in an alternative environment are more likely to get fixed. Once fixed, the pattern of transcriptional variation observed would be one of a reduced overall G × E, but a persistence of an environmental response. If the environment change consistently continues, it is possible that over time the plasticity might be lost. Therefore, to be able to observe such a pattern of transcriptional variation associated with environmental change, recently diverged species or populations must be examined (e.g. *D. mojavensis*). Alternatively, it is possible that such a pattern of transcriptional variation (environmental effect and no G × E) would be observed in general stress genes. Knowledge of gene ontology and of the ecology of this species will be used to try to differentiate between these two groups of genes. The aim of this study is to assess the transcriptional response to cactus host use, as well as to identify genes whose pattern of regulation have possibly been shaped by past host shifts. This was done by assessing the transcriptional variation of nine isofemale lines of *D. mojavensis* from the organ pipe cactus (*Stenocereus thurberi*) population when reared in its native host as well as in the chemically distinct ancestral host, agria cactus (*S. gummosus*).

Materials and methods

Experimental design

A total of nine *D. mojavensis* isofemale lines, each established from a gravid female from Organ Pipe National Monument, Arizona, USA, was used in this study. The experiment was conducted approximately four generations after the isofemale lines were established in the laboratory. Prior to the commencement of the experiment, isofemale lines were maintained in standard banana/*Opuntia* media in half-pint bottles. One day prior to collecting embryos, adults were transferred to embryo collection cages (Genesee Scientific) each fitted with a 100-mm petri dish containing banana/*Opuntia* media. Embryos were then removed from the petri dishes and dechorionated to eliminate any surface contaminants with a bleach solution and washed with sterile water (Starmer & Gilbert 1982). For each isofemale line, approximately 400 embryos were placed in either 250 mL of necrotic organ pipe or 250 mL of necrotic agria cactus. These necrotic cactus containers were placed in a 25 °C incubator with a 12/12 hour light/dark cycle. Third-instar larvae were collected approximately midday and placed in groups of 20 in 1.5-mL microcentrifuge tubes and snap frozen in liquid nitrogen. Samples were then stored at −80 °C until processing.

The microflora of the cactus necrosis is essential for the formation of the larval resource in the wild. In the laboratory, cactus necroses were recreated by incorporating the known microflora (Starmer 1982a; Fogleman & Starmer 1985) following procedures outlined in Matzkin *et al.* (2006). The native agria and organ pipe microflora used was composed of five yeast species,

Pichia cactophila, *P. amethionina*, *Candida sonorensis*, *C. ingens* and *Sporopachydermia cereana* (provided by W. T. Starmer, Syracuse University) and one bacteria, *Pectobacterium cacticida* (American Type Culture Collection #49482). The organ pipe cactus was provided by the Arizona-Sonora Desert Museum in Tucson, Arizona, and agria was collected near the Desemboque region in Sonora, Mexico under CITES permit MX35032. The cactus tissues were inoculated with the above microflora after sterilization via autoclave. Cacti were maintained at 25 °C in an incubator with a 12/12 hour light/dark cycle for 5 weeks (as in Lofdahl 1985) and then approximately 250 mL of each necrosis was placed in one-litre sterile plastic containers to which the embryos were added.

Microarray design and processing

To assess gene expression differences among treatments, a complete *D. mojavensis* genome oligonucleotide microarray was designed using NimbleGen's custom 4-plex platform. The design was based on the GLEANR gene predictions of the published *D. mojavensis* genome (Drosophila 12 Genomes Consortium 2007) and consisted of 69 997 60-oligonucleotide probes representing 17 504 features (for all but 11 features there were four probes per feature). The bulk of these features, 17 477, were GLEANR gene predictions (2506 of which contain transposable elements contaminants and were not used in the analysis), 19 were mojavensis-specific genes (MSG) described in Matzkin *et al.* (2006) and eight were unannotated *D. mojavensis* transcripts from an expressed sequence tag library of the lower reproductive tract from Kelleher *et al.* (2007).

A minimum of 40 larvae were collected for all samples except for isofemale line OPNM25 on agria cactus, from which only 27 larvae were recovered (these were divided into two samples of 13 and 14 larvae each). All third-instar larvae were collected until no more could be seen or the total number collected per isofemale line and treatment reached 220. Because of the differences in developmental time (see Results) between flies reared in the two cacti, cultures were vigilantly monitored to select larvae that were at the same developmental landmark (third instar) from each cactus type. RNA extraction was performed by first a TRIzol reagent extraction (Invitrogen) followed by processing through PureLink Micro-to-Midi Total RNA Columns (Invitrogen) with an inserted DNase I digestion step to remove genomic DNA. Quantification of the total RNA was calculated using a NanoDrop spectrophotometer (NanoDrop Technologies). For each isofemale line and cactus treatment, two independent RNA extractions and hybridizations were performed (36 total hybridizations). Total RNA samples were then submitted to NimbleGen's Reykjavik, Iceland microarray facility (http://www.nimblegen.com) for cDNA synthesis, hybridization and scanning.

Microarray statistical analysis

The analysis of gene expression was performed on Robust Multichip Average (RMA) normalized intensities (Bolstad *et al.* 2003; Irizarry *et al.* 2003). The $\log_2$ of these normalized intensities was analysed using a two-step mixed model ANOVA (Wolfinger *et al.* 2001). The first step is a global ANOVA to remove any random effects produced by having different microarray slides per RNA pool as well as having multiple 60-oligonucleotide probes per gene. The residuals from the first step are used for subsequent gene-specific analysis. The statistical model used in this study was:

Global

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

Gene specific

$$\text{Residual}_{ikl} = \mu + \text{Slide}_i + \text{Line}_k + \text{Cactus}_l + \text{Line} \times \text{Cactus}_{kl} + \text{Error}_{ikl}$$

where i = 1–36, j = 1–4, k = 1–9 and l = 1–2, and both the slide and probe effects were random. The analysis was performed using the PROC MIXED model in SAS (ver. 9.0; SAS Institute Inc.). Significance for each gene was determined using the False Discovery Rate (FDR) method (5% level) of Storey & Tibshirani (2003).

Genes whose expression was significantly modified by cactus host use and for which functional knowledge was available were subjected to a Gene Ontology (GO) term analysis. The *D. mojavensis* FlyBase (Tweedie *et al.* 2009) orthologous calls to *D. melanogaster* genes were used in the analysis. This orthologous gene list was supplemented with additional gene annotations of detoxification and chemosensory genes. GO terms, from the *D. melanogaster* orthologs, for two categories (Molecular Function and Biological Process) were utilized. This functional annotation analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) resource (http://niaid.abcc.ncifcrf.gov/) (Dennis *et al.* 2003), which determines the overrepresentation of GO terms. Significant overrepresentation was determined using a Fisher's exact test (Hosack *et al.* 2003), and multiple test corrections were performed using the FDR method (set at 5%).

Given that the annotation was semi-complete in the published genome, the *D. mojavensis* genome was scanned for previously missing paralogs of *odorant*

receptors (*Or*), *gustatory receptors* (*Gr*), *cytochrome P450* (*P450*), *glutathione s-transferases* (*Gst*) and *UDP-glycosyltransferases* (*UGT*). Since the publication of the *D. mojavensis* genome, some further annotations of *Or* (Guo & Kim 2007) and *Gst* (Low *et al.* 2007) have been published. *Drosophila melanogaster* gene family members for which no orthologous call had been originally made to *D. mojavensis* were used to rescan the *D. mojavensis* genome using BLAST (Altschul *et al.* 1990). E-value cutoff was set to less than 1×10^{-10}. The gene list is shown in supportive information, Table S1 (Supporting information).

Results

The viability of *D. mojavensis* larvae from Organ Pipe National Monument (Sonora population) reared in the alternative host, agria, was 40% that of the viability observed in its native host, organ pipe (*t*-test, $P < 0.001$) (Fig. S1, Supporting information). While larval developmental time was not recorded, the time to first adult emergence differed between the cactus treatments. Flies reared on agria had a significantly (*t*-test, $P < 0.01$) faster eclosion time (12.1 days; SE ±0.21) than when reared on organ pipe (13.2 days; SE ±0.24).

Two of the 14 998 genes in the array had only one probe per gene and therefore were not included in the analysis. All expression data have been placed in the Gene Expression Omnibus under series entry #GSE35937. The number of genes with significant Line, Cactus and Cactus × Line effects using different FDR levels is shown in Table 1. Differences in expression between the *D. mojavensis* isofemale lines used in this study was observed in 1578 genes, 10.5% of the genome. Of the 926 genes with *D. melanogaster* orthologs, overrepresented GO terms involved metabolism and the structural components of the cuticle (see Table S2, Supporting information). A total of 1122 genes had a significant Cactus × Line effect, in which *D. melanogaster* ontology information was known for 655. The GO terms observed to be overrepresented in this set of genes were oxidation–reduction and metabolic process (Table S3, Supporting information).

Table 1 Number of genes with significant Cactus, Line or Line×Cactus effects using different FDR cut-offs

	False discovery rate (FDR)		
Effect	5%	1%	0.1%
Cactus	3109	2066	1303
Line	1578	1070	640
Line×Cactus	1122	667	304

The number of genes whose expression was modulated in response to cactus host utilization was approximately double to those that differ between isofemale lines. This pattern of twice the number of genes with a significant cactus effect relative to line effects was observed at multiple FDR levels (Table 1). Twenty-one per cent of the genes (3109) exhibited a significant cactus effect at a FDR of 5%. The majority of these (2032) had orthologous calls to a *D. melanogaster* gene. The overrepresentation of GO terms was tested to examine the functional characteristics of the differentially expressed genes (Table 2). Sixteen Molecular Function or Biological Processes GO terms were overrepresented at a FDR less than 5%. Metabolism-related pathways and genes associated with the response to abiotic factors and/or toxins were the major components of the overrepresented GO terms (Table 2). The overrepresented GO terms could be grouped in into five categories: (i) abiotic/toxin response, (ii) energy, (iii) oxidoreductase/carbohydrate metabolism, (iv) structural and (v) mRNA binding.

A smaller fraction of genes (1077) that exhibited a significant cactus effect had no orthologous call back to the *D. melanogaster* genome; these genes will be referred as ORFans. When splitting the cactus effect genes into those that are ORFans and those which have calls to *D. melanogaster*, a different distribution of genes was observed than expected (Fig. 1). Using the total fraction of genes exhibiting a cactus effect (0.21) as the expectation, there is a significant departure of results for both data sets: the *D. melanogaster* orthologs ($\chi^2 = 19.8$, $P < 0.001$) and the ORFan genes ($\chi^2 = 49.5$, $P < 0.001$) (Fig. 1). A greater number of ORFan genes appear to be differentially affected by cactus use (Fig. 1).

Given the ecological importance of performance (detoxification) and preference (sensory pathways) genes in cactus host adaptation, their expression was examined separately. When pooling the detoxification gene families (*P450*, *Gst* and *UGT*), it was observed that they were significantly overrepresented in the cactus treatment effect ($\chi^2 = 5.6$, $P = 0.02$) (Fig. S2, Supporting information). No significant deviation from expected was observed, however, for *Or* and *Gr* genes combined (Fig. S2, Supporting information).

Overall, the fraction of genes that increased in expression in the organ pipe (45%) and agria (55%) treatment was similar (Fig. 2). A slightly greater proportion of genes with a *D. melanogaster* call were upregulated in the agria treatment (60%), while fewer were observed for the ORFans (46%). The pattern of expression differs across the sets of genes that make up the five categories of overrepresented GO terms (Table 2). Genes that play a role in the abiotic/toxin response (66%) and mRNA binding (94%) appear to be largely upregulated in the

Table 2 Analysis of Molecular Function (MF) and Biological Process (BP) Gene Ontology terms of genes that exhibit a significant cactus effect

Category (ID)	Description	Count	Fold Enrichment	Group[d]
BP (09628)	Response to abiotic stimulus	37	1.8[b]	Abiotic/toxin response
BP (42221)	Response to chemical stimulus	66	1.5[b]	
BP (09636)	Response to toxin	21	2.1[c]	
BP (06118)	Electron transport	80	1.8[a]	Energy
BP (42775)	Organelle ATP synthesis coupled electron transport	28	3.0[a]	
BP (42773)	ATP synthesis coupled electron transport	28	2.9[a]	
BP (06091)	Generation of precursor metabolites and energy	95	1.7[a]	
BP (06120)	Mitochondrial electron transport, NADH to ubiquinone	15	2.6[c]	
MF (16491)	Oxidoreductase activity	141	1.3[b]	Oxidoreductase/ carbohydrate metabolism
BP (06119)	Oxidative phosphorylation	35	1.9[b]	
MF (16679)	Oxidoreductase activity, acting on diphenols and related substances as donors	8	3.9[c]	
BP (05975)	Carbohydrate metabolic process	82	1.4[c]	
MF (05214)	Structural constituent of chitin-based cuticle	32	2.2[b]	Structural
MF (42302)	Structural constituent of cuticle	34	2.0[b]	
MF (05198)	Structural molecule activity	114	1.3[c]	
MF (03729)	mRNA binding	49	1.6[c]	mRNA binding

[a]False Discovery Rate (FDR) < 0.05.
[b]FDR < 0.01.
[c]FDR < 0.001.
[d]See text for description of groups.

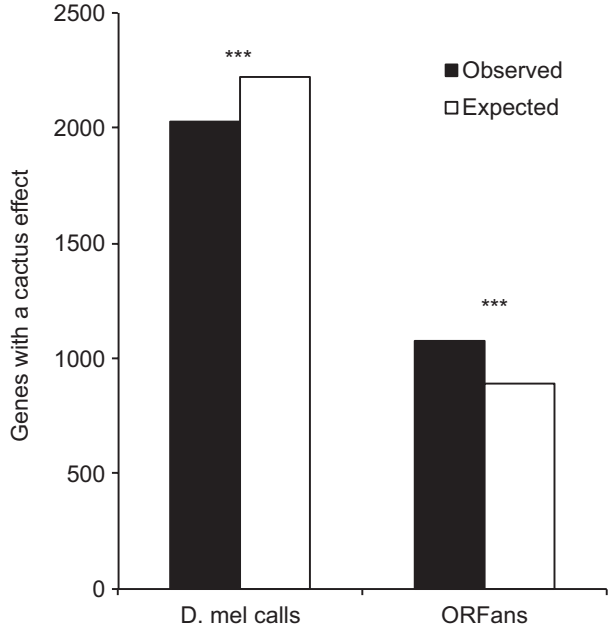

Fig. 1 Numbers of genes that have orthologous calls to *Drosophila melanogaster* and ORFans that have a significant cactus effect (black) and the expected numbers (white). Both observations significantly depart from the expected numbers (***$P < 0.01$).

agria treatment (Fig. 2). Conversely, fewer of the genes associated with energy (32%), carbohydrate metabolism (38%) and structural components (33%) are upregulated in agria.

Analysis of the effects of the ecological shifts depends on the analysis of G × E interactions. The majority of the genes (72.8% or 2264 genes) with a significant Cactus effect did not exhibit a significant Cactus × Line (G × E) effect. An example of two detoxification genes, *Cyp6g1* and *Ugt86Dd*, that exhibit a significant Cactus effect and no G × E is shown in Fig. 3 (an example of a gene with a significant Cactus and Cactus × Line effect is shown in Fig. S3, Supporting information). Similar to what was observed for Cactus effect genes, there was a significant overrepresentation of ORFan ($\chi^2 = 19.3$, $P < 0.001$) within the set of genes that have a Cactus effect and no G × E effect.

Discussion

Fitness consequences of host shifts

A large literature exists on the resource ecology of the different *Drosophila mojavensis* cactus host populations (Fellows & Heed 1972; Heed 1978; Ruiz & Heed 1988;

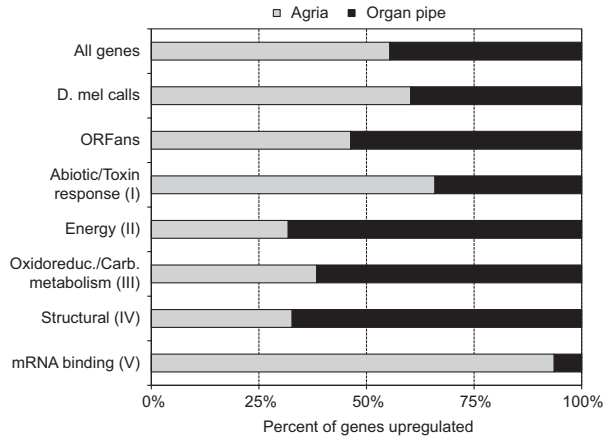

Fig. 2 Per cent of genes that were upregulated in the agria and organ pipe treatments. Data for all genes, genes with *Drosophila melanogaster* orthologs, ORFans and those corresponding to the five functional categories (see Table 2) are shown.

Ruiz *et al.* 1990). Host shift experiments conducted in the laboratory with these flies typically produce changes in viability and life history characters, such as developmental time and thorax length (Etges & Heed 1987; Etges 1989, 1990; Bono & Markow 2009). Thus, the reduced survival of organ pipe breeding *D. mojavensis* when larvae are forced to develop in agria cactus was not unexpected.

The magnitude and nature of genetic changes that underlie adaptation to different hosts can be addressed by examining the transcriptional responses in an ecological context. It has been proposed that *D. mojavensis* originated in Baja California utilizing agria and later colonized other regions and cactus species (Johnson 1980; Heed 1982; Ruiz *et al.* 1990). Genetic analysis suggests that *D. mojavensis* and its sister species *D. arizonae* diverged approximately 0.5 MYA (Reed *et al.* 2007; Matzkin 2008). Therefore, it can be inferred that the four *D. mojavensis* populations have recently (< 0.5 MYA) shifted or specialized onto their respective cactus hosts. Interestingly, as agria is assumed to be the ancestral host, the large reduction in survival of Sonoran flies on agria was surprising and strongly supports the interpretation that genes, and their modulation, underlying host use can evolve rapidly (McBride 2007).

Transcriptional response to cactus host shifts

The two cactus host plants present different ecological and developmental challenges to the larvae feeding in them. *Drosophila mojavensis* larvae from OPNM (Sonora) appear more stressed in agria, as evidenced by their low survival (Fig. S1, Supporting information). As the larvae attempt to overcome the challenges presented by the different chemical environment of the necrotic agria, differential expression of certain types of genes, especially those involved in detoxification, development and sensory perception, is expected.

Analysis of Gene Ontology terms can enhance our understanding of the transcriptional effects of cactus host shifts, although several *D. mojavensis* genes do not have orthologous call to *D. melanogaster* and hence no GO information. The overrepresentation of certain GO terms (see Table 2) that are differentially expressed suggest major functional groups are significantly affected. Among those are members of known detoxification families, such as *Gst*, *P450* and *UGT* (Fig. S2, Supporting information). These specific sets of genes were found to be significantly overrepresented among those modulated by the cactus environment. It should also be noted that membership to either the *Gst*, *P450* or *UGT* gene families does not necessarily imply that the genes are directly involved in detoxification, because some members of these families have other

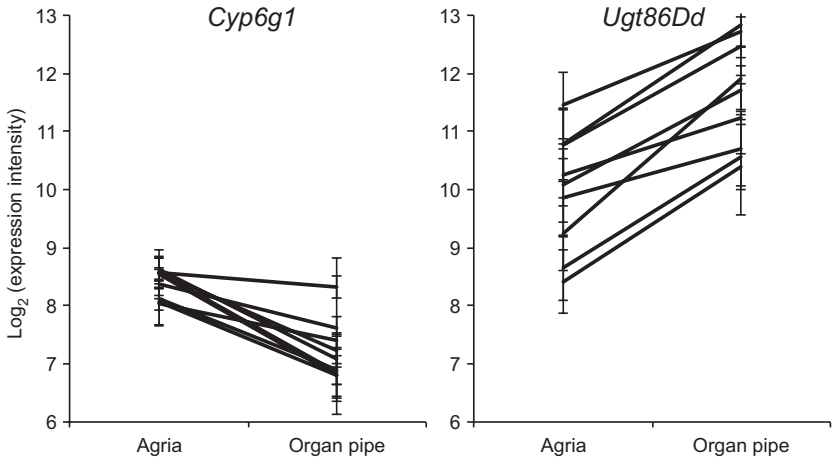

Fig. 3 Transcriptional modulation of two detoxification genes, *Cyp6g1* and *Ugt86Dd*, in response to cactus host. The mean expression intensity (log_2) and standard error for each isofemale line are shown.

nondetoxification-related functions and for others, their function is unknown (Feyereisen 2005; Ranson & Hemingway 2005). While 14 *Or* and *Gr* were significantly differentially expressed in third-instar larvae in response to host use, GO terms associated with their functions were not overrepresented in the set of cactus-related genes. This does not negate the possible role of some, if not all, of the observed receptors in host adaptation or in earlier developmental stages. The connection between host usage and genes involved in structural components and mRNA binding is less clear and warrants further investigation.

Although there are many chemical similarities between organ pipe and agria (both are members of the genus *Stenocereus*), there also are substantial chemical differences (Vacek 1979; Kircher 1982; Fogleman & Abril 1990). For example, agria has a greater concentration of unhydrolysed and partially hydrolysed glycosides. More specifically, agria has twice the amount of glucose, while organ pipe has five times the amount of arabinose, which could influence the fermentation by the microflora (Fogleman & Abril 1990). Prior analyses indicate the existence of distinctly different concentrations of volatiles between necrotic organ pipe and agria (Vacek 1979). These and the other distinguishing chemical and physical characteristics between the cacti are likely responsible for the transcriptional and life history responses observed. What is yet to be determined is linking the specific differences both within and across cactus species with specific transcriptional and life history responses in *D. mojavensis*.

Among the five general groups of genes observed to be differentially expressed, the majority (Fig. 2), associated with mRNA binding and abiotic/toxic response, are expressed at higher levels in the alternative cactus treatment (agria). At the same time, the majority of genes associated with energy and carbohydrate metabolism and structural components were expressed at lower levels on agria. The upregulation of detoxification genes on agria suggests that the larvae are responding to a challenging chemical/toxic substrate. These compounds also appear to have an affect on the metabolic state and development of the *D. mojavensis* larvae as demonstrated by the differences in survival rate (see Fig. S1, Supporting information).

An earlier transcriptional study in *D. mojavensis* using a cDNA array on one isofemale line from the Baja California population showed the differential expression of 154 genes with *D. melanogaster* orthologs (Matzkin *et al.* 2006). Approximately 23% of the significant genes in the earlier study were also differentially expressed in this study. Similar to the previous study, *Adh-2* experienced an approximately twofold expression increase when flies were reared in the organ pipe cactus. Interestingly, *GstD1* did not exhibit a significantly change in expression. Using quantitative PCR, Matzkin *et al.* (2006) found that the change in expression of *GstD1* when reared in organ pipe was 1.3-fold. The failure to observe a significant transcriptional difference in *GstD1* in this study could be due to increased variance (multiple lines used in this study) making significance testing of a small expression difference difficult. Alternatively, this difference in the *GstD1* results suggests the possibility of population-specific mechanisms of detoxification.

Genetic variation of transcriptional response

Although the analysis of all genes that are differentially modulated in response to host use (i.e., cactus effect) is important, it does not take into account within-population variation in transcriptional response. A certain level of segregating variation exists within populations, which is a function of mutation, selection and random forces as well as the demographic properties of the population (Ohta 1992). Transcriptional variation is no exception to this rule. Multiple studies have illustrated the vast extent of transcriptional variation in experimental systems, both within and between species (Rifkin *et al.* 2003; Wittkopp *et al.* 2004; Gilad *et al.* 2005). In this study, 1578 genes exhibited significant within-population variation, but double that number of genes (3109) appear to be affected by cactus host utilization. An important question is, how the pattern of transcriptional variation is shaped by a change in the environmental context in which a population resides?

In a constant environment, a certain level of transcriptional variation is expected to exist (as observed in this study by the genes with a significant Line effect). Furthermore, given the ease in which DNA binding sequences can arise via the mutational process (Stone & Wray 2001; Wray *et al.* 2003), this can lead to silent variation for gene modulation (i.e. transcriptional plasticity). In a new environmental context, those genetic backgrounds or genotypes able to modulate genes of ecological importance will persist in the population (Pigliucci & Murren 2003; West-Eberhard 2005; Badyaev 2009). Therefore, under strong enough selection, these genotypes will become fixed in the population, resulting in a uniform transcriptional or plastic response to the environmental perturbation within the population (i.e. no G × E interaction).

If the new environmental conditions persist, over time, subsequent mutations could canalize the alternate expression pattern (Baldwin 1896; Waddington 1953; West-Eberhard 2003). If captured sufficiently early in the environmental shift, it may be possible to identify some of the genes whose variation has been shaped by

the adaptation. Such genes are expected to differentially modulate between the ancestral (i.e. agria cactus) and new environment (i.e. organ pipe cactus), but show no G×E interaction. In this study 2264 genes were found to fit this criterion. As previously stated, this transcriptional pattern could alternatively be observed in general stress genes and their respective downstream targets. Although this could be the case for a great number of the loci observed in this study, information relating to the ecology of this species can narrow the list of candidate genes.

Given the known cactophilic ecology of *D. mojavensis*, certain gene families, such as detoxification and sensory pathways, are presumed to play a role in cactus host

Table 3 Summary of known functions of the detoxification gene family members with a significant cactus and no Cactus × Line effect

Drosophila mojavensis annotation	*D. melanogaster* ortholog	Gene name	Gene family	Function and/or modulation[A]	References
GI16623	CG17523	GstE2	GST	Insecticide resistance in mosquito.	1–3
GI16624		GstE2b	GST	No available data.	
GI19388	CG17522	GstE10	GST	Transcriptional response to insecticides and oxidative stress.	2–4
GI20124	CG17534	GstE9	GST	Transcriptional response to insecticides and oxidative stress.	2–4
GI23193		GstD1b	GST	No available data.	
GI23196	CG17639	CG17639	GST	Gst of no known function or modulation.	2, 3
GI10234	CG9716	Cyp313b1	P450	P450 of no known function or modulation.	5
GI13002	CG33503	Cyp12d1-d	P450	Insecticide resistance; Transcriptional response to insecticides, phenobarbitol and stress.	4–12
GI16117	CG3656	Cyp4d1	P450	Transcriptional response to cactus-derived compounds (*D. mettleri*).	5, 7, 13, 14
GI16990	CG9964	Cyp309a1	P450	P450 of no known function or modulation.	5, 7
GI18674	CG3540	Cyp4d14	P450	Transcriptional response to phenobarbitol.	7, 11
GI18951	CG8859	Cyp6g2	P450	Insecticide resistance; Transcriptional response to insecticides.	5, 7, 8, 12, 15
GI20221		Cyp9h1b	P450	No available data.	
GI20230	CG13977	Cyp6a18	P450	P450 of no known function or modulation.	5, 7, 15
GI20590	CG8453	Cyp6g1	P450	Insecticide resistance; transcriptional response to insecticides and *Morinda citrifolia* (host of *D. sechellia*).	4, 7, 8, 12, 16-19
GI24047	CG14680	Cyp12e1	P450	P450 of no known function or modulation.	5
GI10119	CG4739	Ugt86Dc	UGT	UGT of no known function or modulation.	20, 21
GI10120	CG18578	Ugt86Da	UGT	UGT of no known function or modulation; expressed in olfactory organs.	20–22
GI10122	CG4772	Ugt86Dh	UGT	Transcriptional response to insecticides.	4, 20, 21
GI14390	CG11289	CG11289	UGT	UGT of no known function or modulation.	20
GI17058	CG13271	Ugt36Bb	UGT	UGT of no known function or modulation; expressed in olfactory organs.	20, 21, 23
GI17522	CG11012	Ugt37a1	UGT	UGT of no known function or modulation; expressed in olfactory organs.	20, 22
GI22627	CG6644	Ugt35a	UGT	UGT of no known function or modulation; expressed in olfactory organs.	20, 22
GI22628	CG6649	Ugt35b	UGT	Transcriptional response to insecticides; expressed in olfactory organs.	4, 20–22
GI22630	CG6633	Ugt86Dd	UGT	Transcriptional response to insecticides, phenobarbitol.	4, 11, 20, 21

[A]Unless otherwise stated, studies were based on *D. melanogaster*.

1. (Lumjuan *et al.* 2005); 2. (Li *et al.* 2008); 3. (Ranson *et al.* 2001); 4. (Pedra *et al.* 2004); 5. (Tijet *et al.* 2001); 6. (Telonis-Scott *et al.* 2009); 7. (Chung *et al.* 2009), 8. (Daborn *et al.* 2007), 9. (Le Goff *et al.* 2006); 10. (Willoughby *et al.* 2006); 11. (Sun *et al.* 2006); 12. (Festucci-Buselli *et al.* 2005); 13. (Danielson *et al.* 1998); 14. (Danielson *et al.* 1997); 15. (Daborn *et al.* 2001); 16. (Dworkin & Jones 2009); 17. (Bogwitz *et al.* 2005); 18. (Pyke *et al.* 2004); 19. (Daborn *et al.* 2002); 20. (Luque & O'Reilly 2002); 21. (Theopold *et al.* 1999); 22. (Wang *et al.* 1999); 23. (Sambandan *et al.* 2008).

adaptation. Detoxification genes make up a small fraction of those differentially expressed. Of the 2264 genes, 25 are either *Gst*, *P450* or *UGT* (Table 3). Approximately half have been known to either be transcriptionally modulated or to metabolize xenobiotics (Table 3). Several of these genes, such as *GstE2*, *Cyp12d1-d*, *Cyp6g2* and *Cyp6g1*, are known to directly confer resistance to insecticides such as dichlorodiphenyltrichloroethane (DDT) when over expressed using transgenes (Daborn *et al.* 2001, 2002, 2007; Ranson *et al.* 2001; Festucci-Buselli *et al.* 2005; Lumjuan *et al.* 2005). Similar insecticide detoxification mechanisms demonstrated in *D. melanogaster* and other insects may have actively participated in the adaptation to detoxify cactus-derived compounds.

The transcriptional response of *P450s* to cactus-derived compounds was illustrated previously in *D. mettleri* (Danielson *et al.* 1997, 1998), a Sonoran Desert endemic in the same repleta species group (Wasserman 1982) as *D. mojavensis*. In *D. mettleri*, *Cyp4d1* has been implicated in the detoxification of the toxic isoquinoline alkaloids that are found in the necrotic tissues of its host, the saguaro cactus (*Carnegiea gigantea*). Although alkaloids are not present in either agria or organ pipe necroses (Kircher 1982), the data suggest that *Cyp4d1* may have contributed to adaptation of *D. mojavensis* to its respective hosts.

Although coding (Matzkin 2004, 2008), transcriptional (this study, Matzkin *et al.* 2006) and functional (Matzkin 2005) changes in detoxification genes appear to be associated with cactus host shifts in *D. mojavensis*, it is likely that the adaptation to the distinct cactus hosts that occurred in this species involved more that just selection at detoxification genes (e.g. nutritional composition). This could largely explain the differential expression of other nondetoxification genes observed in this study. To better assess this, and to provide further evidence of the role of the candidate detoxification genes, future studies should examine the transcriptional variation in all four cactus host populations.

Evolutionary rate of cactus host-associated transcripts

A disproportionally large number of the genes modulated by cactus had no orthologous call to *D. melanogaster*. The ORFan genes could be due to either a gain or a loss of genes in either lineage, or reflect a decreased efficacy of algorithms to properly make the orthologous calls where there have been large numbers of substitutions (Drosophila 12 Genomes Consortium 2007). Recent genomic analysis suggests a high degree of gene gain and losses in the *Drosophila* genomes, which is likely contributing to the numbers of ORFan genes between *D. mojavensis* and *D. melanogaster* (Hahn *et al.* 2007). Although given the divergence time and vast ecological differences between *D. melanogaster* and *D. mojavensis*, there has been ample opportunity for molecular changes associated with their respective ecologies to accumulate.

An accelerated rate of evolution does not necessarily imply selection, but could in some instances. The inability to make an orthologous call can serve, therefore, as a general metric for evolutionary change. An overrepresentation of these 'fast-evolving' genes was present within the set of genes affected by cactus host. It appears, therefore, that these transcriptionally responsive genes tend to have an accelerated rate of evolution. The findings support the notion that both transcriptional and sequence changes are involved in adaptation and that both of these categories of changes can occur at a given locus (King & Wilson 1975; Nuzhdin *et al.* 2004). Host shifts are common in evolution and require adaptation in both adult and larval traits, particularly those associated with preference and performance. The large number of genes revealed here from olfactory, gustatory and detoxification gene families provide good candidates for further studies of the genetic underpinnings of host shifts during evolution.

Acknowledgements

I thank Therese Ann Markow for her support and collaboration in this project. I thank Jasmin Uribe and NSF Research Experiences for Teachers intern Georgina J. Matzkin for their assistance setting up the experiment. I thank the Arizona-Sonora Desert Museum for providing the cactus material. I also thank Jeremy Bono, Tamara Haselkorn, Maxi Polihronakis, Sarah Johnson and anonymous reviewers for providing helpful comments on the manuscript. This project was supported by a NSF Research Experiences for Teachers grant to TAM, a grant from Science Foundation Arizona to TAM and LMM and a NSF award (DEB-1020009) to LMM.

References

Abzhanov A, Kuo WP, Hartmann C *et al.* (2006) The calmodulin pathway and evolution of elongated beak morphology in Darwin's finches. *Nature*, **442**, 563–567.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology*, **215**, 403–410.

Badyaev AV (2009) Evolutionary significance of phenotypic accommodation in novel environments: an empirical test of the baldwin effect. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **364**, 1125–1141.

Baldwin JM (1896) A new factor in evolution. *American Naturalist*, **30**, 441–451, 536–553.

Bogwitz MR, Chung H, Magoc L *et al.* (2005) *Cyp12a4* confers lufenuron resistance in a natural population of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA*, **102**, 12807–12812.

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

Bono JM, Markow TA (2009) Post-zygotic isolation in cactophilic *Drosophila*: larval viability and adult life-history traits of *D. mojavensis/D. arizonae* hybrids. *Journal of Evolutionary Biology*, **22**, 1387–1395.

Chung H, Sztal T, Pasricha S *et al.* (2009) Characterization of *Drosophila melanogaster* cytochrome P450 genes. *Proceedings of the National Academy of Sciences, USA*, **106**, 5731–5736.

Consortium DG (2007) Evolution of genes and genomes on the *Drosophila* phylogeny. *Nature*, **450**, 203–218.

Daborn P, Boundy S, Yen J, Pittendrigh B, ffrench-Constant R (2001) DDT resistance in *Drosophila* correlates with *cyp6g1* over-expression and confers cross-resistance to the neonicotinoid imidacloprid. *Molecular Genetics and Genomics*, **266**, 556–563.

Daborn PJ, Yen JL, Bogwitz MR *et al.* (2002) A single P450 allele associated with insecticide resistance in *Drosophila*. *Science*, **297**, 2253–2256.

Daborn PJ, Lumb C, Boey A *et al.* (2007) Evaluating the insecticide resistance potential of eight *Drosophila melanogaster* cytochrome P450 genes by transgenic over-expression. *Insect Biochemistry and Molecular Biology*, **37**, 512–519.

Danielson P, MacIntyre R, Fogleman J (1997) Molecular cloning of a family of xenobiotic-inducible drosophilid cytochrome p450s: evidence for involvement in host-plant allelochemical resistance. *Proceedings of the National Academy of Sciences, USA*, **94**, 10797–10802.

Danielson PB, Foster JLM, McMahill MM, Smith MK, Fogleman JC (1998) Induction by alkaloids and phenobarbital of family 4 cytochrome p450s in *Drosophila*: evidence for involvement in host plant utilization. *Molecular and General Genetics*, **259**, 54–59.

Dennis G, Sherman BT, Hosack DA *et al.* (2003) David: database for annotation, visualization, and integrated discovery. *Genome Biology*, **4**, R60.

Dworkin I, Jones CD (2009) Genetic changes accompanying the evolution of host specialization in *Drosophila sechellia*. *Genetics*, **181**, 721–736.

Etges WJ (1989) Evolution of developmental homeostasis in *Drosophila mojavensis*. *Evolutionary Ecology*, **3**, 189–201.

Etges WJ (1990) Direction of life history evolution in *Drosophila mojavensis*. In: *Ecological and Evolutionary Genetics of Drosophila* (eds Barker JSF, Starmer WT and MacIntyre RJ), pp. 37–56. Plenum Press, New York, NY.

Etges WJ, Heed WB (1987) Sensitivity to larval density in populations of *Drosophila mojavensis*: influences of host plant variation on components fitness. *Oecologia*, **71**, 375–381.

Feder ME, Mitchell-Olds T (2003) Evolutionary and ecological functional genomics. *Nature Review Genetics*, **4**, 649–655.

Fellows DF, Heed WB (1972) Factors affecting host plant selection in desert-adapted cactiphilic *Drosophila*. *Ecology*, **53**, 850–858.

Festucci-Buselli RA, Carvalho-Dias AS, de Oliveira-Andrade M *et al.* (2005) Expression of *cyp6g1* and *cyp12d1* in DDT resistant and susceptible strains of *Drosophila melanogaster*. *Insect Molecular Biology*, **14**, 69–77.

Feyereisen R (2005) Insect cytochrome P450. In: *Comprehensive Molecular Insect Science* (eds Gilbert LI, Iatrou K and Gill SS), pp. 1–77. Elsevier, Amsterdam.

Fogleman JC, Abril JR (1990) Ecological and evolutionary importance of host plant chemistry. In: *Ecological and Evolutionary Genetics of Drosophila* (eds Barker JSF, Starmer WT and MacIntyre RJ), pp. 121–143. Plenum Press, New York, NY.

Fogleman JC, Starmer WT (1985) Analysis of the community structure of yeasts associated with the decaying stems of cactus. III. *Stenocereus thurberi*. *Microbial Ecology*, **11**, 165–173.

Fogleman JC, Starmer WT, Heed WB (1981) Larval selectivity for yeast species by *Drosophila mojavensis* in natural substrates. *Proceedings of the National Academy of Sciences, USA*, **78**, 4435–4439.

Gilad Y, Rifkin SA, Bertone P, Gerstein M, White KP (2005) Multi-species microarrays reveal the effect of sequence divergence on gene expression profiles. *Genome Research*, **15**, 674–680.

Guo S, Kim J (2007) Molecular evolution of *Drosophila* odorant receptor genes. *Molecular Biology and Evolution*, **24**, 1198–1207.

Hahn MW, Han MV, Han S-G (2007) Gene family evolution across 12 *Drosophila* genomes. *Plos Genetics*, **3**, e197.

Heed WB (1978) Ecology and genetics of Sonoran desert *Drosophila*. In: *Ecological Genetics: The interface* (ed Brussard PF), pp. 109–126. Springer-Verlag, New York.

Heed WB (1982) The origin of Drosophila in the sonoran desert. In: *Ecological Genetics and Evolution: The Cactus-yeast-Drosophila Model System* (eds Barker JSF and Starmer WT), pp. 65–80. Academic Press, New York.

Hosack DA, Dennis G, Sherman BT, Lane HC, Lempicki RA (2003) Identifying biological themes within lists of genes with ease. *Genome Biology*, **4**, R70.

Irizarry RA, Hobbs B, Collin F *et al.* (2003) Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*, **4**, 249–264.

Johnson WR (1980) *Chromosomal Polymorphism in Natural Populations of the Desert Adapted Species,* Drosophila mojavensis. University of Arizona, Tucson, AZ.

Kelleher ES, Swanson WJ, Markow TA (2007) Gene duplication and adaptive evolution of digestive proteases in *Drosophila arizonae* female reproductive tracts. *Plos Genetics*, **3**, 1541–1549.

King M-C, Wilson AC (1975) Evolution at two levels in humans and chimpanzees. *Science*, **188**, 107–116.

Kircher HW (1982) Chemical composition of cacti and its relationship to Sonoran desert Drosophila. In: *Ecological Genetics and Evolution: The Cactus-yeast-Drosophila Model System* (eds Barker JSF and Starmer WT), pp. 143–158. Academic Press, New York.

Le Goff G, Hilliou F, Siegfried BD *et al.* (2006) Xenobiotic response in *Drosophila melanogaster*: sex dependence of P450 and gst gene induction. *Insect Biochemistry and Molecular Biology*, **36**, 674–682.

Li HM, Buczkowski G, Mittapalli O *et al.* (2008) Transcriptomic profiles of *Drosophila melanogaster* third instar larval midgut and responses to oxidative stress. *Insect Molecular Biology*, **17**, 325–339.

Lofdahl KL (1985) *A Quantitative Genetic Analysis of Habitat Selection Behavior in the Cactus-breeding Species* Drosophila mojavensis. Ph.D. Thesis, University of Chicago, Chicago.

Low WY, Ng HL, Morton CJ *et al.* (2007) Molecular evolution of glutathione s-transferases in the genus Drosophila. *Genetics*, **177**, 1363–1375.

Lumjuan N, McCarroll L, Prapanthadara LA, Hemingway J, Ranson H (2005) Elevated activity of an epsilon class glutathione transferase confers DDT resistance in the dengue vector, *Aedes aegypti*. *Insect Biochemistry and Molecular Biology*, **35**, 861–871.

Luque T, O'Reilly DR (2002) Functional and phylogenetic analyses of a putative *Drosophila melanogaster* UDP-glycosyltransferase gene. *Insect Biochemistry and Molecular Biology*, **32**, 1597–1604.

Machado CA, Matzkin LM, Reed LK, Markow TA (2007) Multilocus nuclear sequences reveal intra- and interspecific relationships among chromosomally polymorphic species of cactophilic *Drosophila*. *Molecular Ecology*, **16**, 3009–3024.

Matzkin LM (2004) Population genetics and geographic variation of alcohol dehydrogenase (*Adh*) paralogs and glucose-6-phosphate dehydrogenase (*G6pd*) in *Drosophila mojavensis*. *Molecular Biology and Evolution*, **21**, 276–285.

Matzkin LM (2005) Activity variation in alcohol dehydrogenase paralogs is associated with adaptation to cactus host use in cactophilic. *Drosophila Molecular Ecology*, **14**, 2223–2231.

Matzkin LM (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 LM, Watts TD, Bitler BG, Machado CA, Markow TA (2006) Functional genomics of cactus host shifts in *Drosophila mojavensis*. *Molecular Ecology*, **15**, 4635–4643.

McBride CS (2007) Rapid evolution of smell and taste receptor genes during host specialization in *Drosophila sechellia*. *Proceedings of the National Academy of Sciences, USA*, **104**, 4996–5001.

Meyer E, Aglyamova GV, Matz MV (2011) Profiling gene expression responses of coral larvae (*Acropora millepora*) to elevated temperature and settlement inducers using a novel RNA-seq procedure. *Molecular Ecology*, **20**, 3599–3616.

Nuzhdin S, Wayne M, Harmon K, McIntyre L (2004) Common pattern of evolution of gene expression level and protein sequence in Drosophila. *Molecular Biology and Evolution*, **21**, 1308–1317.

Ohta T (1992) The nearly neutral theory of molecular evolution. *Annual Review of Ecology and Systematics*, **23**, 263–286.

Pedra JHF, McIntyre LM, Scharf ME, Pittendrigh BR (2004) Genome wide transcription profile of field- and laboratory-selected dichlorodiphenyltrichloroethane (DDT)-resistant *Drosophila*. *Proceedings of the National Academy of Sciences, USA*, **101**, 7034–7039.

Pfeiler E, Castrezana S, Reed LK (2009) Genetic, ecological and morphological differences among populations of the cactophilic *Drosophila mojavensis* from southwestern USA and northwestern Mexico, with descriptions of two new subspecies. *Journal of Natural History*, **43**, 923–938.

Pigliucci M, Murren CJ (2003) Perspective: genetic assimilation and a possible evolutionary paradox: can macroevolution sometimes be so fast as to pass us by? *Evolution*, **57**, 1455–1464.

Pyke FM, Bogwitz MR, Perry T *et al.* (2004) The genetic basis of resistance to diazinon in natural populations of *Drosophila melanogaster*. *Genetica*, **121**, 13–24.

Ranson H, Hemingway J (2005) Glutathione transferases. In: *Comprehensive Molecular Insect Science* (eds Gilbert LI, Iatrou K and Gill SS), pp. 383–402. Elsevier, Amsterdam, the Netherland.

Ranson H, Rossiter L, Ortelli F *et al.* (2001) Identification of a novel class of insect glutathione s-transferases involved in resistance to DDT in the malaria vector *Anopheles gambiae*. *Biochemical Journal*, **359**, 295–304.

Reed LK, Nyboer M, Markow TA (2007) Evolutionary relationships of *Drosophila mojavensis* geographic host races and their sister species *Drosophila arizonae*. *Molecular Ecology*, **16**, 1007–1022.

Rifkin SA, Kim J, White KP (2003) Evolution of gene expression in the *Drosophila melanogaster* subgroup. *Nature Genetics*, **33**, 138–144.

Ruiz A, Heed WB (1988) Host-plant specificity in the cactophilic *Drosophila mulleri* species complex. *Journal of Animal Ecology*, **57**, 237–249.

Ruiz A, Heed WB, Wasserman M (1990) Evolution of the mojavensis cluster of cactophilic *Drosophila* with descriptions of two new species. *Journal of Heredity*, **81**, 30–42.

Sambandan D, Carbone MA, Anholt RRH, Mackay TEC (2008) Phenotypic plasticity and genotype by environment interaction for olfactory behavior in *Drosophila melanogaster*. *Genetics*, **179**, 1079–1088.

Shapiro MD, Marks ME, Peichel CL *et al.* (2004) Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature*, **428**, 717–723.

Starmer WT (1982a) Analysis of the community structure of yeasts associated with the decaying stems of cactus. I. *Stenocereus gummosus*. *Microbial Ecology*, **8**, 71–81.

Starmer WT (1982b) Associations and interactions among yeasts, Drosophila and their habitats. In: *Ecological Genetics and Evolution: The Cactus-yeast-Drosophila Model System* (eds Barker JSF and Starmer WT), pp. 159–174. Academic Press, New York, NY.

Starmer WT, Gilbert DG (1982) A quick and reliable method for sterilizing eggs. *Drosophila Information Service*, **58**, 170–171.

Starmer WT, Heed WB, Rockwood-Sluss ES (1977) Extension of longevity in *Drosophila mojavensis* by environmental ethanol: differences between subraces. *Proceedings of the National Academy of Sciences, USA*, **74**, 387–391.

Starmer WT, Lachance MA, Phaff HJ, Heed WB (1990) The biogeography of yeasts associated with decaying cactus tissue in North America, the Caribbean, and northern Venezuela. In: *Evolutionary Biology* (eds Hecht MK, Wallace B and Macintyre RJ), pp. 253–296. Plenum Publishing Corporation, New York.

Stone J, Wray G (2001) Rapid evolution of *cis*-regulatory sequences via local point mutations. *Molecular Biology and Evolution*, **18**, 1764–1770.

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

Sun W, Margam VM, Sun L *et al.* (2006) Genome-wide analysis of phenobarbital-inducible genes in *Drosophila melanogaster*. *Insect Molecular Biology*, **15**, 455–464.

Telonis-Scott M, Hallas R, McKechnie SW, Wee CW, Hoffmann AA (2009) Selection for cold resistance alters gene transcript levels in *Drosophila melanogaster*. *Journal of Insect Physiology*, **55**, 549–555.

Theopold U, Rissler M, Fabbri M, Schmidt O, Natori S (1999) Insect glycobiology: a lectin multigene family in *Drosophila melanogaster*. *Biochemical and Biophysical Research Communications*, **261**, 923–927.

Tijet N, Helvig C, Feyereisen R (2001) The cytochrome P450 gene superfamily in *Drosophila melanogaster*: annotation, intron-exon organization and phylogeny. *Gene*, **262**, 189–198.

Tweedie S, Ashburner M, Falls K *et al.* (2009) FlyBase: enhancing *Drosophila* gene ontology annotations. *Nucleic Acids Research*, **37**, D555–D559.

Vacek DC (1979) *The Microbial Ecology of the Host Plants of* Drosophila mojavensis. Ph.D. Thesis, University of Arizona, Tucson, AZ.

Vosshall LB, Stocker RE (2007) Molecular architecture of smell and taste in *Drosophila*. *Annual Review of Neuroscience*, **30**, 505–533.

Waddington CH (1953) Genetic assimilation of an acquired character. *Evolution*, **7**, 118–126.

Wang Q, Hasan G, Pikielny CW (1999) Preferential expression of biotransformation enzymes in the olfactory organs of *Drosophila melanogaster*, the antennae. *Journal of Biological Chemistry*, **274**, 10309–10315.

Wasserman M (1982) Evolution in the repleta group. In: *The Genetics and Biology of* Drosophila (eds Ashburner M, Carson HL and Thompson JN), pp. 61–139. Academic Press, London.

Werner T, Koshikawa S, Williams TM, Carroll SB (2010) Generation of a novel wing colour pattern by the Wingless morphogen. *Nature*, **464**, 1143–1148.

West-Eberhard MJ (2003) *Developmental Plasticity and Evolution*. Oxford University Press, New York.

West-Eberhard MJ (2005) Phenotypic accommodation: adaptive innovation due to developmental plasticity. *Journal of Experimental Zoology Part B-Molecular and Developmental Evolution*, **304B**, 610–618.

Whitehead A, Crawford DL (2006) Neutral and adaptive variation in gene expression. *Proceedings of the National Academy of Sciences, USA*, **103**, 5425–5430.

Williams LM, Oleksiak MF (2011) Evolutionary and functional analyses of cytochrome P4501A promoter polymorphisms in natural populations. *Molecular Ecology*, **20**, 5236–5247.

Willoughby L, Chung H, Lumb C *et al.* (2006) A comparison of *Drosophila melanogaster* detoxification gene induction responses for six insecticides, caffeine and phenobarbital. *Insect Biochemistry and Molecular Biology*, **36**, 934–942.

Wittkopp PJ, Haerum BK, Clark AG (2004) Evolutionary changes in *cis* and *trans* gene regulation. *Nature*, **430**, 85–88.

Wittkopp PJ, Haerum BK, Clark AG (2008) Regulatory changes underlying expression differences within and between Drosophila species. *Nature Genetics*, **40**, 346–350.

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

Wray GA, Hahn MW, Abouheif E *et al.* (2003) The evolution of transcriptional regulation in eukaryotes. *Molecular Biology and Evolution*, **20**, 1377–1419.

Data accessibility

All expression data have been placed in the Gene Expression Omnibus under series entry #GSE35937.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Gene list of newly annotated genes.

Table S2 Analysis of Molecular Function (MF) and Biological Process (BP) Gene Ontology Terms of genes that exhibit a significant Line effect.

Table S3 Analysis of Molecular Function (MF) and Biological Process (BP) Gene Ontology Terms of genes that exhibit a significant Line×Cactus effect.

Fig. S1 Numbers of third instar larvae collected from agria and organpipe necroses for each of the nine isofemale lines used.

Fig. S2 The numbers of expected and observed genes that are significantly modulated by cactus host for A) detoxification genes (Gst, P450, UGT) and B) odorant and gustatory receptors.

Fig. S3 Transcriptional modulation of GI22738 a gene with significant Cactus and Cactus×Line effect. The mean expression intensity (log2) and standard error for each isofemale line is shown. Note, for clarity the axis range is different from Fig. 3 in the text.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.