Deciphering life history transcriptomes in different environments

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Abstract

We compared whole transcriptome variation in six preadult stages and seven adult female ages in two populations of cactophilic *Drosophila mojavensis* reared on two host plants in order to understand how differences in gene expression influence standing life history variation. We used Singular Value Decomposition (SVD) to identify dominant trajectories of life cycle gene expression variation, performed pair-wise comparisons of stage and age differences in gene expression across the life cycle, identified when genes exhibited maximum levels of life cycle gene expression, and assessed population and host cactus effects on gene expression. Life cycle SVD analysis returned four significant components of transcriptional variation, revealing functional enrichment of genes responsible for growth, metabolic function, sensory perception, neural function, translation and aging. Host cactus effects on female gene expression revealed population and stage specific differences, including significant host plant effects on larval metabolism and development, as well as adult neurotransmitter binding and courtship behavior gene expression levels. In 3 - 6 day old virgin females, significant up-regulation of genes associated with meiosis and oogenesis was accompanied by down-regulation of genes associated with somatic maintenance, evidence for a life history tradeoff. The transcriptome of *D. mojavensis* reared in natural environments throughout its life cycle revealed core developmental transitions and genome wide influences on life history variation in natural populations.

Keywords

life history; development; gene expression; microarray; aging; *Drosophila*

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Introduction

Understanding life history evolution requires knowledge of the forces shaping correlated suites of fitness characters in response to patterns of age-specific mortality (Hamilton 1966; Reznick 1982; Reznick *et al.* 2004; Roff 2002; Stearns 1977; Williams 1957). Therefore, it is necessary to integrate how life history traits are expressed across environments (Caswell 1983; Etges 1993; Gupta & Lewontin 1982; Scheiner 1993) and standing levels of genetic variation in fitness components (Gustafsson 1986; Istock *et al.* 1976; Price & Schluter 1991; Walsh & Blows 2009) with patterns of demographic and environmental variability (Caswell 2009; Orzack & Tuljapurkar 1989; Steiner & Tuljapurkar 2012; Tuljapurkar 1989). In order to predict life history patterns, we must also examine the genetic architecture of life history variation and the unfolding of organismal developmental programs over the life cycle (Levitis 2011). In particular, we need to understand the number and kind of genes responsible for life history differences, how coordinated groups of genes are expressed at different life cycle stages, and how environmental effects on genes influence internal and external buffering and genotype by environment (GxE) interactions (Arbeitman *et al.* 2002; Fiedler *et al.* 2010; Koutsos *et al.* 2007; Stolc *et al.* 2004).

Currently, large gaps remain in our understanding of how genomic expression throughout the life cycle is influenced by relevant ecological variables. In organisms where expression of genetic differences in life histories depends upon local ecological variation, examination of the sensitivity of gene expression, as well as gene expression-environment interactions, is necessary to evaluate the adaptive significance of life history variation in response to ecological variability. Environmental variation can maintain genetic polymorphism in populations, directly influence gene expression leading to GxE interactions (Gillespie & Turelli 1989), and be limited in its selective effects if alleles are neutral in some environments but not others (Anderson *et al.* 2013). Limits to plasticity of genome expression (Zhou *et al.* 2012) are of direct concern to organismal persistence in changing environments unless standing levels of genetic variability are high enough to allow short-term microevolutionary change. Although levels of genetic variation in components of fitness, as well as fitness itself, are sometimes low (Gustafsson 1986), it is essential to understand the nature of genome expression throughout the life history (Gibson 2008; Hodgins-Davis & Townsend 2009).

Here, we examine transcriptional profiles throughout the life cycle in *Drosophila mojavensis*, a cactophilic species endemic to the deserts of northwestern Mexico and southwestern USA, using whole transcriptome microarrays in order to document patterns and sensitivity of gene expression in populations characterized by genetically differentiated life history differences. We assessed transcriptional variation from embryogenesis to four week-old female adults to characterize the range of variation in gene expression and gene function in interrelated groups of genes. We focused on pre-adult stages and revealed expression shifts related to development, while analyses across female adult life stages revealed expression changes underlying maturation, senescence, and tradeoffs between reproduction and somatic maintenance in different environments.

Ecology and evolution of *D. mojavensis*

Throughout the arid lands of the New World, over half of the ca 100 species in the large *D. repleta* group use fermenting cactus tissues to carry out their life cycles (Filchak *et al.* 2005; Heed 1982; Oliveira *et al.* 2012; Wasserman 1992). Within the *mulleri* species complex, *D. mojavensis* and its two closest relatives, *D. arizonae* and *D. navojoa*, form a monophyletic group endemic to Mexico and the southwestern United States (Ruiz *et al.* 1990). *Drosophila mojavensis* became isolated in present-day peninsular Baja California from its closest relative, *D. arizonae*, on the mainland due to tectonic drift and changing sea levels (Gastil *et al.* 1975). Natural populations of *D. mojavensis* from the Sonoran and Mojave Deserts and adjacent arid lands use different host cacti across their range, i.e. pitaya agria cactus, *Stenocereus gummosus*, on the peninsula and organ pipe, *S. thurberi*, and sina cactus, *S. alamosensis* in mainland Mexico and Arizona (Etges *et al.* 1999; Heed & Mangan 1986). In the Mojave Desert in southern California and central Arizona, barrel cactus, *Ferocactus cylindraceus*, is a major host and populations of *D. mojavensis* on Santa Catalina Island near Los Angeles, California use *Opuntia* cactus. Southern California populations likely split from mainland Sonora-southern Arizona populations ca. 117–135 kya with little recurring gene flow (Smith *et al.* 2012).

Natural populations of *D. mojavensis* show considerable genetic variation in life histories, including host plant-influenced differences in adult mortality rates (Jaureguy & Etges 2007). Baja California populations express shorter egg to adult development times, higher viabilities, and smaller thorax sizes than mainland populations when reared on fermenting agria vs. organ pipe cactus in common garden experiments suggesting adaptation to these hosts in nature (Etges 1990; Etges *et al.* 2010; Etges & Heed 1987). Mainland Sonoran Desert *D. mojavensis* are characterized by larger body sizes (Etges 1992; Etges & Ahrens 2001), higher metabolic rates, more ovarioles (Heed, unpubl. data) and higher lifetime fecundities, but earlier ages at first reproduction than Baja populations (Etges & Klassen 1989). Genetic variation in development time and thorax size in both Baja and mainland populations, as well as significant GxE interactions when reared on different host plants, and positive across-host genetic correlations suggested ongoing life history evolution and evidence for ecological generalism (Etges 1993). Baja California and mainland populations also harbor significant genetic variation for adult longevity and average numbers of eggs laid per day, as well as a genetic tradeoff between early and late-life fecundity (Etges & Heed 1992). Together, these data suggest that as *D. mojavensis* colonized mainland Mexico and Arizona by switching host cacti, new life histories evolved in these derived populations (Etges 1993), with correlated shifts in reproductive isolation (Etges 1998; Etges *et al.* 2010).

We measured whole genome transcriptional responses of *D. mojavensis* from two populations exposed to fermenting tissues of two host cacti, i.e. agria, *S. gummosus*, and organ pipe cactus, *S. thurberi*, in pre-adult stages and adult of increasing age in order to reveal whole transcriptome responses to different host plants over the life cycle. We approached the analysis of our data with two distinct goals in mind. First, we assessed effects of stage/age independent of population and diet by generating a pooled dataset composed of mean expression levels for all genes (averaged across populations, diets, and biological replicates) at each stage/age. We used this averaged dataset to investigate highly

conserved trajectories of gene expression across the *D. mojavensis* life cycle, independent of diet or population effects. To identify clusters of genes with similar age-trajectories of expression, we performed a singular value decomposition (SVD) of total genome expression (Alter 2006; Alter *et al.* 2000) on this pooled dataset. The SVD cluster analyses revealed continuous changes difficult to observe with simple pair-wise comparisons between stages and ages. We then considered as correlated gene clusters those sets of genes whose expression closely correlated with the dominant trajectories revealed in the SVD analysis. We also performed pair-wise comparisons, e.g., comparing expression at adjacent stages/ ages, with the primary aim of mapping gene expression levels into functional domains as in previous studies (Kim*et al.* 2005; Koutsos *et al.* 2007; McCarroll *et al.* 2004; Pletcher *et al.* 2002; Remolina *et al.* 2012).

Second, we searched for evidence of divergence in gene expression patterns at each stage and age in our four population X cactus treatment groups. By teasing out expression differences into shifts due to population, host plant, and their interactions, we revealed gene expression/regulatory changes potentially responsible for their recent divergence in life histories.

Materials and Methods

Origin of Stocks

Populations of *D. mojavensis* were collected in nature by baiting over fermented bananas or by collecting adults emerged from cactus rots returned to the lab. A total of 465 adults were baited in Punta Prieta, Baja California in January 2008, and 1264 baited adults plus 9 adults that emerged from sina, *S. alamosensis*, rots were collected from Las Bocas, Sonora in March 2009. All flies were returned to the lab and each population was cultured on banana food (Brazner & Etges 1993) in 8 dr shell vials at room temperature until the experiments began in September 2009.

Preadult stage culture conditions

Thousands of adult flies from each population were introduced into separate population cages (12,720 cm^3) for 7-10 days and allowed to choose mates. Population cages were maintained in an incubator programmed for a 14:10 LD photoperiod and 27:17 ° C. Flies were allowed to oviposit in cups containing fermenting agria or organ pipe cactus (see below). We used both cacti for egg oviposition because we were also interested in the effects of alternate cactus substrates on gene expression at all stages, including fertilized eggs. Thousands of eggs (~200 μg) were collected for six hours and briefly rinsed in deionized water to remove cactus media, snap frozen in liquid nitrogen, and stored at −80° C prior to RNA extraction. For larval and pupal stages, approximately 200 eggs were transferred to cups containing fermenting cactus media (see below) and allowed to develop to the stage of interest. Development times for the pre-adult stages were estimated from analysis of the duration of stage specific differences in larval mouth hook morphology and pupal periods (D. White and W. J. Etges, unpubl. results). A total of six pre-adult stages were used: fertilized embryos (6 hr), first instar larvae (48 hr), second instar larvae (144 hr), third instar larvae (240 hr), early pupae (288 hr), and late pupae (384 hr). Egg hatch is ca

24-25 hr under these conditions. In addition to age in hours, we verified each larval and pupal stage morphologically and discarded individuals that were early or advanced for each developmental stage. Each sample of larvae consisted of thousands of individuals for the first and second instars and hundreds of individuals for the third instar. For early and late pupae, 30 individuals were used in each sample.

Cactus media for rearing pre-adult stages was prepared with 400 g of cactus (either agria or organ pipe), 600 mL of deionized water and 4 g of agar. First, fresh cactus tissue was blended using 2/3 of the water, boiled, and then strained twice to remove large cactus fibers. This media was strained a third time using a fine mesh to remove excess fibers and the resulting liquid paste-like solution was added to the agar dissolved in boiling water. This media was then boiled for 10 min, autoclaved for 8 min and poured into food cups. After the medium cooled, it was inoculated with a pectolytic bacterium, *Erwinia cacticida* (Alcorn *et al.* 1991), and a mixture of seven cactophilic yeasts: Dipodascus starmeri, Candida sonorensis, C. valida, Starmera amethionina, Pichia cactophila, P. mexicana, and *Sporopachydermia cereana*. One mL of yeast and bacterial solution was injected into the cactus media every 48 hr to yield constant fermentation of the cacti. The final media was soft enough to separate the larvae (especially the first and second instars) from the cactus media.

Adult culture conditions

Flies were raised for one generation in population cages (described above), and eggs collected from these cages were reared to eclosion on banana food at moderate larval densities in half-pint bottles. Emerged adults were transferred to 8 dr shell vials in small same sex groups containing banana food until they were sexually mature (8-10 days). Approximately 400 adults (200 females and 200 males) from each population were introduced into separate oviposition chambers and allowed to mate and oviposit for 10 h each day. Eggs were collected from a 5.5 cm diameter petri dish containing agar-cactus media attached to each oviposition chamber, and washed in sterile deionized water, 70% ethanol, and again in deionized water. Eggs were counted into groups of 200, transferred to a 1 cm^2 piece of sterilized filter paper, and placed in bottles containing 75 g of fermenting cactus tissue in the incubator described above. All unhatched eggs were counted to allow calculation of egg to adult viability. Eclosed adults from each replicate culture were counted daily, allowing determination of egg-to adult development time, separated by sex, and immediately transferred to vials containing fermenting cactus (see below) in same sex groups of 30 flies. All cultures were maintained in an incubator (described above).

Cactus media for rearing adults for RNA extraction was prepared by mixing cactus (agria or organ pipe), water, and agar homogenized in a blender in the following proportions: 953 g cactus, 486 ml deionized water, and 5 g agar. This mixture was autoclaved for 15 min, cooled, and inoculated with bacteria and yeasts (see above). This cactus media was prepared one week prior to use and kept in an incubator at 37 °C to maximize microbial fermentation. This media was then loaded into individual cup-like 2.2 cm diameter plastic barrel plugs (Alliance Express, Little Rock, Arkansas USA) that were pressed into one end of autoclaved 25 × 95 mm glass tubes. An additional inoculating loop containing a mixture of bacteria and

seven cactophilic yeasts was added to the fermenting cactus tissue in each food cap to supplement nutrition. After adding 30 adult females or males to each tube, the other end of each tube was closed with a barrel plug that had been drilled with a 1.75 cm hole sealed with fine mesh to allow air circulation. Flies were fed atmospheric ethanol vapor by placing tubes in sealed desiccators containing 1 L of 4% ethanol (Etges 1989; Etges & Klassen 1989) from 8:00 AM to 6:00 PM in the incubator described above. For the remaining 14 hr each day, all tubes were removed from each desiccator and kept in the incubator to minimize condensation inside the tubes. Plugs containing fermenting cactus were replaced every four days.

Adult females for RNA extraction were sampled at 8 time intervals: 0, 3, 6, 10, 14, 18, 24, and 28 days. Each adult sample consisted of 24 virgin females that were snap frozen in liquid nitrogen and stored at −80° C. Additional tubes of females and males sampled at each time interval were frozen at −20° C and used for cuticular hydrocarbon analysis (Etges & de Oliveira 2014). Overall, we planned 24 treatment combinations for pre-adult stages (2 populations × 2 cacti × 6 stages) and 32 combinations (2 populations × 2 cacti × 8 ages) for adult females (Fig. 1). Each combination was replicated four times for RNA extraction and microarray analysis; however, samples of 28 day old females were missing because few flies survived past 28 days in these conditions (Etges & Heed 1992; Jaureguy & Etges 2007), so we pooled them resulting in 7 ages sampled. A number of missing replicates resulted in 86 (pre-adult) and 86 (adult) samples (Suppl. Table 1).

cDNA synthesis, hybridization and visualization

Total RNA was isolated from each sample using RNeasy mini-kits (Qiagen, Valencia, California USA) and stored at −80 °C until cDNA was prepared. Double-stranded cDNA was synthesized using Invitrogen Superscript Double-Stranded cDNA Synthesis kits, and cDNA concentrations were measured using a NanoDrop spectrophotometer (NanoDrop Technologies) to verify that all cDNA samples were   100ng/ul, A260/A280   1.8, and A260/A230   1.8. All cDNA samples were Cy3 labeled using a NimbleGen One Color DNA Labeling kit.

Our Roche NimbleGen microarray design contained a total of 14,528 unique transcripts based on the *D. mojavensis* genome (http://flybase.net/genomes/Drosophila_mojavensis/current/fasta/dmoj-all-transcript-r1.3.fasta.gz ; 4/14/2009) with nine probes per transcript for a total of 130,705 probes (each microarray in the 12-plex design included 135K probes; see Gene Expression Omnibus entry GSE43220 for details). Hybridizations were performed with a NimbleGen Hybridization System (Hybridization System 4, BioMicro Systems, Inc.) and spot intensity scanning was carried out with a GenePix 4000B scanner (Molecular Devices) and GenePix Pro software. All hybridization intensities were normalized using quantiles (Bolstad *et al.* 2003) with NimbleScan v2.5 software. Gene call files were generated using the Robust Multichip Average (RMA) algorithm as described by Irizarry *et al.* (2003).

Data Analysis

Whole-dataset analysis—We assessed time-series gene expression dynamics using Singular Value Decomposition (SVD) analysis (Alter *et al.* 2000; Alter *et al.* 2003). SVD is a linear transformation of expression data from genes × arrays space to a reduced "eigengenes" × "eigenarrays" space. In our case, the SVD took our 14528 gene × 13 stage/age data matrix and returned a 13 × 13 matrix where each row is an eigengene. Each of these eigengenes represents a consensus trajectory of gene expression, similar to a principal component, encompassing a proportion of the overall variation in gene expression over time. This application of SVD is closely analogous to its usual use in signal processing, with each eigengene representing a common trajectory of expression with a strong signal in the data. These eigengene profiles provide a way to cluster genes according to their correlation with these dominant trajectories of gene expression across the life cycle.

SVD analysis was performed on an averaged *D. mojavensis* dataset, consisting of mean within-life-stage gene expression values for all genes at each stage/age to evaluate overall gene expression variation changes. Preliminary analysis revealed a single eigengene representing steady-state expression that accounted for 99.6% of all variation in the data. The entropy of this dataset was also low (d = 0.012 <<< 1) suggesting that stage-specific changes in expression were relatively small deviations from lifetime mean expression (Alter *et al.* 2000). We therefore mean-centered the data by filtering out this eigengene (Alter *et al.* 2000), and all further analyses were undertaken on the resulting normalized dataset. After normalization, the stage-specific expression levels for each gene had values between −1 and 1, with positive relative expression levels indicating overexpression and negative expression indicating under-expression relative to the lifetime mean.

SVD analysis contains an inherent sign ambiguity, thus for each eigengene its complementary (i.e., equal and opposite relative expression level at each stage and age) trajectory is equally significant. While heuristic methods do exist to try to work around this ambiguity, we chose to exploit it by treating significant eigengenes as paired sets of correlated and anticorrelated gene expression trajectories. For each significant eigengene pair, we arbitrarily designated the "positive" eigengene to be the trajectory with positive relative expression in adult stages (Fig 2). The corresponding "negative" eigengene trajectory is a mirror image about zero of its complementary "positive" eigengene. Thus, genes significantly correlated with a "positive" eigengene will be significantly anticorrelated with the corresponding negative eigengene and vice versa.

Serial resampling of the biological replicates was used to assess variation within stage/age samples and its impact on eigengenes revealed by SVD analysis. 10,000 resampled datasets were created by randomly selecting one biological replicate from the available samples at each life stage/age. These resampled datasets were subjected to SVD analysis just as with the averaged dataset, and were used to form 95% confidence bounds around the original eigengenes (Ghosh 2002). To determine which transcripts were contributing most to each eigengene pattern, genes were sorted by their correlation with the eigengene's trajectory over the life history (top 10%), and then these transcripts were sorted again by the magnitude of their projection onto the eigengene (Alter *et al.* 2000) to arrive at 5% or 726 predicted genes.

For simplicity, we included the top 750 genes with the highest +/− rank in this sorting with respect to each eigengene at each stage and age for gene annotation and functional clustering.

Peaks and variance in gene expression

At each life stage and age, we calculated the mean and variance of expression for each transcript across populations and diets. We then determined when each gene was at its highest observed level of expression allowing us to characterize differences in maximum gene expression across the life cycle. We were also interested in the variability of gene expression across our replicate samples to determine whether gene expression may become less tightly controlled with age (cf. Pletcher *et al.* 2002). Thus, we plotted changes in genome-wide variances in gene expression characterized as the stage or age-specific variance of all predicted genes in their expression levels. Different numbers of individuals were sampled at different pre-adult stages that might affect genome-wide variance estimates, but only gene expression variance increases in second instar larvae and late pupae (384 hr) were observed (see Results).

Pair-wise stage and age comparisons

We also assessed a set of specific pair-wise comparisons using datasets pooled in a different way, e.g., comparing expression at two ages, or comparing expression under two environments with the primary aim of mapping gene expression levels into functional domains as in previous studies (Kim *et al.* 2005; Koutsos *et al.* 2007; Pletcher *et al.* 2002) and to search for shared components of gene co-expression underlying development and aging (McCarroll *et al.* 2004). We chose to analyze targeted pairwise interactions rather than use a traditional linear model approach, since a fully parameterized model of our data would involve $2 \times 2 \times 13$ possible comparisons which, in the end, would have needed to be assessed with the same set of pairwise tests. A full linear model for all genes across the life cycle produced stage/age differences in gene expression that were > 99 % similar to our pair-wise comparisons (results not shown). We identified transcripts that significantly increased or decreased in expression between each pair of consecutive life stages using t-tests corrected for false discovery rate (FDR) $P < 0.05$ (Benjamini & Hochberg 1995) and that had absolute fold-changes > 1.5 (the absolute value of the ratio of normalized intensities between two samples). These comparisons helped to tease out individual gene expression changes potentially responsible for, or caused by, important age-stage transitions.

We also identified transcripts that significantly increased or decreased in expression between 3 day old (young adult) and > 18 day old (senescent) adults because many aging studies have focused on such pair-wise comparisons between 'young' and 'old' age classes (e.g. de Magalhães *et al.* 2009; Landis *et al.* 2004; Southworth *et al.* 2009). We pooled samples from ages 18+ days to increase sample sizes, since at older ages only enough flies remained to produce one or two replicate samples per treatment. We also assessed numbers of genes differentially expressed due to host cacti in preadult and adult stages by using a dataset with all preadult stages pooled together and all adult stages pooled together.

Host cactus and population effects—For each preadult stage and adults on day of eclosion, we assessed all gene expression differences due to cactus with t-tests with FDR P < 0.05 and absolute fold-changes > 1.5. The remaining adult data were assessed by ANOVA with cactus, population, and cactus by population interaction included with ages pooled (Etges 2014).

Ortholog search and functional annotation clustering—Submission of the 14,528 *D. mojavensis* transcripts to Flybase (Tweedie *et al.* 2009) produced 9117 *D. melanogaster* orthologs, i.e. only ~ 63 percent of predicted *D. mojavensis* genes could be functionally analyzed. Reciprocal BLAST searches with the other 10 available *Drosophila* genomes did not increase this number (results not shown). These 9117 orthologs were used in gene ontology analyses using DAVID Bioinformatics Resources 6.7 (Huang *et al.* 2009). Thus, for a given list of *D. mojavensis* transcripts of interest, we first determined the subset of those transcripts that had *D. melanogaster* orthologs, and used the corresponding *D. melanogaster* genes in our gene ontology analysis.

Gene annotation clusters were determined by DAVID's clustering algorithm with initial classification stringencies set to 'Moderate'. We also used GO-Module (Yang *et al.* 2011) to reduce redundancy in numbers of annotated clusters when there were several overlapping functional clusters produced by DAVID. Further inspection of annotated gene function was enabled by identifying KEGG pathways (Kanehisa & Goto 2000).

Due to limited annotation of the *D. mojavensis* genome, our gene ontology analysis has two main potential sources of error. First, we could only include genes that have known *D. melanogaster* orthologs. Thus, the gene lists used in our analyses are missing ca 37 percent of the original transcripts of interest. The addition of this missing data could change the significance of the clusters reported here, and could also contain enriched clusters undetectable in our current dataset. Second, our enrichment analyses compared gene lists of interest with the list of 9117 orthologs as background, not with the entire *D. melanogaster* genome. An 'enriched' cluster of GO-terms, then, means that terms within that cluster were proportionately overrepresented in the subset of the original transcript list of interest that had known *D. melanogaster* orthologs, as compared to the total set of orthologs. Given these limitations, we interpret our gene ontology results with caution and focus primarily on broader trends. We performed the same annotation cluster analysis with the top 5% of genes corresponding to each eigengene, genes with maximal expression over the life cycle, and genes differing in expression between consecutive life cycle stages/ages.

Results

Singular Value Decomposition (SVD) analysis

SVD analysis revealed four eigengenes that explained 95% of the variation in the normalized dataset (Fig. 2). All four eigengenes showed life cycle shifts in gene co-expression associated with transitions from egg to larval stages and pupae to day of eclosion, with relatively little change from eclosion to adults of older ages (Fig. 2). We pooled replicates from population and cactus diet treatments because there were no significant differences observed in eigengene structure between these groups, as revealed by overlap in

their 95% bootstrap confidence intervals at all life stages (results not shown). SVD analysis of overall life cycle variation in gene expression was thus insensitive to differences due to rearing substrates or population origin, likely in part because the number of replicates for each age-population-diet combination was limited to four.

The first eigengene accounted for 63.5 % of the overall variation in gene expression and so represented the largest correlated "structure" of life cycle gene expression in the normalized dataset. This trajectory was characterized by a negative relationship between pre-adult and adult gene expression patterns – transcripts that were down-regulated in pre-adult stages were up-regulated in adults and vice versa (Fig. 2). Interestingly, gene expression in 6 h embryos was concordant with expression in adult ages (Suppl. Table 2). This was expected in part because adult females contained developing eggs (cf. Graveley *et al.* 2011). Of the top 5% of all genes with the highest positive correlation with eigengene 1, just 38.7 percent were annotated, and were significantly enriched for general growth and metabolic function including protein synthesis, cell division, and secretory functions (Table 1).

Of the genes with transcription levels negatively correlated with eigengene 1, 86.4 percent were annotated, and were enriched for protease activity, G-protein coupled receptor function, ion transport, sensory perception, and transcriptional regulation (Suppl. Table 2). These functional groups were expressed from first instar larvae to late pupae consistent with protein degradation, larval molting, tissue remodeling in pupation, and increased larval expression of sensory and gustatory genes (Vosshall & Stocker 2007). Many of these genes in this cluster were olfactory (Or) and gustatory receptor (Gr) orthologs that were up-regulated in first instar larvae (Table 2). Thus, the largest sources of life cycle gene co-expression variation for orthologs with inferred functions were due to increased expression of ribosomal-associated translation capacity in embryo and adult stages with correspondingly increased expression of gene clusters with protein degradation and sensory perception function in larval through pupal stages.

The second significant eigengene accounted for 17.7 % of the variation in lifetime gene expression. The 'positive' complement of this trajectory was characterized by down-regulated expression in egg and early pupa stages, with close to mean expression levels during larval stages, strongly up-regulated expression in late pupae and day of eclosion, then a slow monotonic decrease in expression with adult age (Fig. 2). The negative complement showed, conversely, up-regulated expression in egg and early pupal stages, mean expression levels in larval stages, down-regulation at late pupae and eclosion, and monotonic increases in expression with adult age. Thus, increased transcription in 6 h embryos and in aging, post-eclosion adults likely involved common gene clusters.

The largest positive loadings on this eigengene occurred from late pupae to eclosion and in young adults. Of the top 5% positively correlated genes, 574 genes were annotated and enriched for functional clusters involved with plasma membrane structure and ion transport, glycoprotein metabolism, neural development and function, sensory perception and oxidative phosphorylation (Table 1). This enrichment is consistent with expression of developmental genes in late pupae, as well as peak neural and metabolic function in young adults with decreases in neural and metabolic function with increasing adult age. Negative

associations with eigengene 2 included RNA processing and transport, transcriptional regulation, protein folding, chromosomal organization, and epigenetic control of gene regulation (Suppl. Table 3). This enrichment pattern is consistent with protein synthesis in egg and pupal stages, and interestingly, again in late adult life. Thus, eigengene 2 included a significant component of lifetime gene co-expression associated with embryonic gene clusters and the pupa-eclosion transition that then shifted with adult age. This suggested that eigengene 2 structure was driven by post-eclosion shifts in gene cluster transcription associated with slowing of protein metabolism, reduction in neural function, detoxification activity, and chromatin silencing associated with aging, including *Sirt6*, a known determinant of adult lifespan (Kusama *et al.* 2006). Eigengene 2 is therefore an excellent genelet to pursue in order to understand expression of aging genes.

While the third and fourth significant eigengenes accounted for far smaller proportions of the total variation in our data, eigengene 3 was associated with contrasting larval and pupal patterns of gene expression and an overall lack of deviation from mean gene expression levels after eclosion (Fig. 2). The "positive" trajectory of this eigengene had peak expression in larval stages, with strong down-regulation in egg and pupal stages. Transcripts correlated with this trajectory were enriched for peptidase activity and endoplasmic reticulum function. The increased expression of these genes in larvae, with decreasing expression in pupal stages, is consistent with decreases in metabolic rates from early to late pupal stages (Lebo *et al.* 2009; Merkey *et al.* 2011)

The negative trajectory of eigengene 3 was characterized by peak expression in egg and pupal stages, with down-regulated expression in larvae. Transcripts with correlated with this trajectory were enriched for brain and organ development, and metamorphosis consistent with up-regulation of developmental processes in the embryo and pupae (Table 1).

The "positive" trajectory of the fourth eigengene was characterized by down-regulated expression in 6 hr embryos, weaker down-regulation in larval stages, peak expression in pupal stages followed by strong down-regulation of expression at eclosion and slowly increasing expression at adult ages (Fig. 2). Transcripts correlated with this trajectory were enriched for ribosomal function, consistent with the tissue remodeling during pupal stages.

Transcriptional variation correlated with the negative trajectory of this eigengene was associated with pattern formation and larval development, and enriched for *Hox* genes, organ system formation, segmentation and neuron development genes, as well as *wnt* signaling (Table 1). Enrichment for developmental genes is consistent with expression patterns in the embryonic stage, and likely has little to do with the eigengene's expected peak expression in young adults. *wnt* signaling was also associated with embryogenesis, and this enrichment is likely driven by overexpression of *wnt* associated genes in 6 hr embryos. All *wnt* signaling homologs showed peak expression early in embryogenesis, but some, e.g. *boca, WntD, pangolin, and wingless* (Suppl. Table 2), also showed increased expression in adults consistent with the positive expression in young adults for eigengene 4 (Fig. 2) similar to modENCODE expression levels in *D. melanogaster* (Tweedie et al. 2009).

Peak expression and transitions in gene expression levels over the life cycle

Both maximum expression data (percent of all genes at maximum lifetime expression levels, Fig. 3, Suppl. Table 4) and expression change data (percent of all genes with significant (FDR P < 0.05 and >1.5 X fold changes, Suppl. Table 5) between successive life stages/ages showed the same three distinct peaks over the life cycle of *D. mojavensis* (Fig. 4). There was a clear burst of genome-wide levels of expression in 6 h embryos that declined throughout larval stages, an increase in pupae to day of eclosion, and an almost monotonic decline until adults were 14 days old (Table 3; Fig. 4). A slight late-life peak in gene expression levels was apparent from 14 to 18 and 24 days, a peak also seen for genes at their maximum lifetime expression levels (Fig. 4).

Genome-wide variance in expression levels peaked at second larval instar and late pupal stages and remained relatively unchanged over adulthood (Fig. 4). Since sexes were pooled until day of eclosion, and only female adults were analyzed here, we could not separate variation due to sex-specific expression in preadult stages from other causes as a contributing factor to these variance increases. However, sex-specific differences in expression, particularly in pupae, were likely greater in germline than somatic tissues (Lebo *et al.* 2009).

Almost 21% of all predicted genes were at their maximum transcription levels in 6 h embryos (n = 2999, Fig. 3), and were significantly enriched for 26 different functional gene clusters (Table 3). The second transcriptional peak in late pupae to day of emergence involved 1886 and 1947 genes, respectively, and a third peak in 18 day old females revealed 1224 genes at maximum lifetime expression (Fig. 3). This lifetime pattern was quite similar to that of eigengene 1 (Fig. 2). Numbers of functionally annotated clusters identified in DAVID (Huang *et al.* 2009) in each life stage were strongly correlated with numbers of *D. mojavensis* genes with *D. melanogaster* orthologs (Pearson r = 0.95, t = 10.09, P < 0.0001). Here, the average proportion ± 1 SD of annotated genes was 0.64 ± 0.18, with a range from 0.37 in early pupae to 0.80 on the day of eclosion (Table 3).

Annotation clustering of genes with maximal lifetime expression levels uncovered the largest number of functional terms in the 6 h embryo stage (Table 3), in part because 79% of these early developmental genes were annotated. A diverse set of gene clusters involved with development, segmentation, nucleic acid metabolism, oogenesis, cellular metabolism, negative and positive regulation of biosynthesis and transcription, mitosis, morphogenesis, and imaginal disc development were significantly enriched. That meiotic gene expression was enriched in embryos has been previously observed (Mukai *et al.* 2006), and was due to genes associated with meiotic chromosome segregation, microtubule binding, and cell cycle dynamics (Table 3).

The transition from 6 h embryo to first instar revealed a precipitous decline in the numbers of genes with maximal expression, the proportion of genes with significant changes in expression from the embryo stage (Fig. 3, 4), and numbers of enriched gene clusters of diverse function (Table 3). The most enriched gene cluster in the first instar stage was associated with formation of the peritrophic membrane, a lining of a specialized extracellular matrix in the gut, indicating significantly increased expression of genes

associated with feeding and digestion. Other enriched clusters included those annotated for ribosome assembly, increased metabolism, and development (Table 3). Genes that increased in expression from embryo to first instar stages were significantly enriched for functional clusters with membrane, chitin, cuticle and a number of other metabolic pathways and sensory systems associated with larval development (Suppl. Table 6A). This transition was also characterized by significant decreases in expression of many of the embryonic gene clusters with maximal gene expression (Table 3). Thus, the embryo to larval transition involved the largest down-regulation of genome-wide expression across the life cycle in *D. mojavensis*.

Maximum expression of second and third instar larval genes was enriched for similar functional clusters associated with growth and development (Table 3). Membrane receptor function, HOX gene regulation, and cuticle formation gene clusters were at maximum expression levels in second instar larvae accompanied by significantly increased transcription of cellular respiration, energy production, and fatty acid metabolism genes as in first instar larvae (Suppl. Table 6A), while expression of cell division and DNA repair genes significantly declined. Third instar larvae showed enrichment for genes at maximum expression for sensory perception of chemical stimuli and increased membrane receptor activity (Table 3) with decreased expression of metabolic pathways including energy, sugar, amino acid, lipid, and P450 metabolism (Suppl. Table 6A). Decreased transcription of endoplasmic reticulum genes and increased expression of cuticle structure, fat body associated ADH, and odorant binding genes was consistent with the continued trajectory of increasing larval growth and size prior to pupation.

From first instar to late pupae, the numbers of genes at maximum expression increased (Fig. 3) while the fractions of annotated genes at maximum expression levels decreased from 0.66 (432/658) in first instars to as low as 0.37 (602/1641) in early pupae (Table 3) suggesting increases in expression of lineage–specific *D. mojavensis* genes, i.e. those with no *D. melanogaster* orthologues, during late preadult development. The third instar to early pupa transition revealed a drastic reduction in metabolic rates where mitochondrial, oxidative phosphorylation, citric acid cycle, and sugar, lipid and amino acid metabolism genes showed significant decreases in expression (Suppl. Table 6A). DAVID also identified a gene cluster with 54 annotated *D. melanogaster* orthologs enriched for spermatogenesis that was significantly up-regulated from L3 to EP (Suppl. Tables 6A, 7) consistent with the known timing of testis development in *D. melanogaster* (Cooper 1950).

Significantly increased transcription of gene clusters enriched for mitochondrial and aerobic respiration function, the TCA cycle, and glycolysis (Suppl. Table 6A) was consistent with increases in metabolic rates in late pupae (Merkey *et al.* 2011). Increased expression of flight muscle genes (DeSimone *et al.* 1995; Fernandes *et al.* 1991) and associated mitochondrial genes, as well as gene clusters enriched for glycolysis, were accompanied by significant decreases in transcripts associated with DNA replication and RNA processing, DNA repair, sensory perception and steroid synthesis.

Almost 2000 genes were at maximum expression levels on the day of eclosion that accounted for 15 significantly enriched gene clusters, second only to the diversity of genes

expressed in 6 h embryos across the entire life cycle (Fig. 3, Table 3). Highly significant GOterms included cellular respiration, mitochondrial and TCA cycle function, vision, adult movement, and other metabolic functions (Table 3). Eighty percent of genes showing increased expression from late pupa to day of emergence were annotated, and were functionally enriched for a number of metabolic functions including membrane transport, ATP binding, protein transport and catabolism, mitochondrial function and biogenesis, growth, and others (Suppl. Table 6A). Up-regulation of fatty acid metabolism was also apparent in KEGG pathway analysis (Kanehisa & Goto 2000). The transition from late pupae to emergence was characterized by decreases in cytoskeleton formation, ion transport, peptidase activity, and KEGG pathways involving carbohydrate and glutathione metabolism, and oxidative phosphorylation. An annotated cluster of 21 taste and olfactory receptor genes showed significant decreases in expression from late pupa to eclosion (Suppl. Table 6A). This was a subset of the 94 sensory, taste, and olfactory orthologs that were significantly up-regulated from egg to first instar (Table 2). Thus, the pupa-adult transition involved a large decrease in expression of sensory genes that were up-regulated in early larval stages.

From eclosion into adulthood, far fewer genes were expressed at maximum lifetime levels, except in day 18 adults, and there was a corresponding decrease in the numbers of genes showing significant decreases/increase in expression between sampling points (Fig. 3, Suppl. Table 6). In three day old adults, genes at maximum lifetime expression were functionally enriched for diverse metabolic functions including fatty acid metabolism, iron ion binding, sugar metabolism, carboxylic acid catabolism, and P450 activity (Table 3), and there were significant increases in gene expression for DNA replication, cell division, ribosome manufacture, egg production, and DNA repair (Suppl. Table 6A). In six day-old adults, fewer genes were at maximal expression (Fig. 3) and these were enriched for vitamin and cofactor binding, steroid hormone manufacture, and oogenesis. Three to six days is approximately the age at first reproduction for *D. mojavensis* females depending on temperature and nutrition (Etges & Klassen 1989; Markow 1982). Also seen in the transition from three to six day old adults were 621 genes associated with oogenesis, meiosis, cell division, DNA repair, and down-regulation of metabolism, as well as decreased expression of cuticle formation, immune response, melanin metabolism, sugar transport, and muscle development genes (Suppl. Table 6A) suggesting decreasing gene expression associated with somatic maintenance with the onset of female reproduction.

From the 6 to 10 day and 10 to 14 day intervals, there were continuing decreases in expression for cuticle gene expression, immune response, melanin metabolism, and muscle formation, and few significant increases in gene expression. Sixty-seven genes were at maximal expression levels at day 14 (Fig. 3) that were enriched for translation and oogenesis (Table 3). A larger number of genes, 1224, were at maximal lifetime expression at day 18 that were enriched for genes associated with aging including DNA repair, protein chaperones, DNAase activity, and apoptosis regulation, as well as control of gene regulation and oocyte development. Significant decreases in expression from 14 to 18 days involved gene clusters enriched for signal peptides, lipid synthesis, microsome-associated iron binding, immune response, and a number of other cellular catalytic functions (Suppl. Table 6A). Many of these same gene clusters were then up-regulated from 18 to 24 days (Fig. 2),

including antimicrobial peptides, immune response, lipid metabolism, and microsome-associated iron binding, as well as amino acid metabolism, glutathione metabolism (KEGG), and P450 activity suggesting further regulatory changes associated with aging, increased oxidative stress, and immune response to microbes (Table 3, Suppl. Table 6A). This "late life" transition in gene expression from 14 to 18 to 24 days was also observed in both eigengenes 2 and 3 (Fig. 2).

Assessing differential expression between 'young' and 'old' adults (3-6 day vs. 18+ day) revealed changes consistent with other studies of aging (see de Magalhães *et al.* 2009 for a review). In 'old' samples, there was increased gene expression in DNA repair, DNA replication, stress response, mitosis and meiosis, and decreased expression of electron transport chain, muscle development, signaling and transport, hormone binding and locomotor genes (Suppl. Table 6B). However, as seen above, this simple young-old comparison missed the non-monotonic trajectories of expression through adulthood, particularly the 'late life' transitions observed between ages 14 and 18, and 18 and 24 (Fig. 2).

Host cactus effects on gene expression across the life cycle—Both host cactus and population effects influenced preadult stage-specific patterns of gene expression (fold change > 1.5 X and FDR P < 0.05). From the embryo stage to eclosion, there were significant differences between these two populations in the timing of differentially expressed genes due to host cactus (Table 4). Variation in egg to adult development time and viability in this experiment (Suppl. Tables 8, 9; Suppl. Fig. 1) was consistent with previous studies, so transcriptional variation here should help to identify causes of cactus-influenced shorter development times and higher viabilities of Baja populations vs. those on the mainland (Etges 1990; Etges *et al.* 2010). Cactus rearing substrates caused expression levels to differ in first and second instar stages in the mainland population, but in the Baja California population most transcriptional differences due to cactus occurred in early and late pupal stages (Table 5; Suppl. Table 10). There were no differences in the numbers of genes showing significant up/down transcription differences due to agria or organ pipe substrates (paired t = 0.66, P > 0.05), and the average proportion of predicted *D. mojavensis* genes with *D. melanogaster* orthologs that were influenced by rearing substrates for these two populations ranged from 55 to 64 percent.

While just eight genes in 6 h embryos showed significant expression differences due to cactus (Table 5), just GI22080 was annotated, a Cep78 homolog, a centrosomal protein (The-UniProt-Consortium 2011) that showed increased transcription due to agria cactus (Table 5). A larger number of orthologs in first (n = 1201) and second (n = 76) instar larvae were differentially expressed in this mainland population than in the Baja population due to cactus substrates. Organ pipe cactus caused increased transcription of cuticle and odorant binding-related orthologs in first instar larvae, and moderate increases in expression in genes associated with cytoskeleton and ribosome function in second star larvae compared to agria-reared larvae. Agria cactus caused greater expression of a broad range of significantly enriched genes in first instar larvae associated with growth and development including protein transport, cell division, and ion transport than organ pipe cactus (Table 5). Just four genes of diverse function in Baja California first instar larvae were significantly over-

expressed due to agria cactus, and few third instar genes showed any effect of cactus on expression levels. Of the 381 early and late pupal genes showing expression differences due to cactus, significant enrichment for ubiquitin conjugation function (proteolysis) genes was observed, as well as genes responsible for cuticle structure and mitochondrial membrane function. Thus, agria cactus caused increased expression of a broader spectrum of genes in different parts of the preadult life cycle than organ pipe cactus, particularly those associated with early larval development and metabolism, but the overall number of genes with significantly different levels of expression influenced by cactus was small.

For adults, samples were pooled across ages and variation in expression levels was assessed with a mixed model ANOVA with population, cactus, and population × cactus effects where cactus was a fixed effect. Organ pipe cactus caused increased expression of genes enriched for neurotransmitter binding, circadian rhythm, and courtship and mating behavioral functions. Mainland females showed significantly increased expression of genes enriched for fatty acid metabolism genes and iron binding functions, such as P450 genes associated with xenobiotic detoxification. Baja females had higher overall expression of genes associated with transcription than mainland females (Etges 2014). Overall, these patterns of differential gene expression in populations of *D. mojavensis* were influenced by cactus rearing substrates in both preadult and adult stages, where different cactus substrates influenced expression of a greater number of orthologs in preadult stages than in adults.

Discussion

The holometabolous life cycle of *D. mojavensis* is marked by major transitions in the expression of functional clusters of genes similar to those in *D. melanogaster* (Arbeitman *et al.* 2002; Graveley *et al.* 2011; Tennessen & Thummel 2011). SVD captured a portrait of gene expression throughout preadult development and adult aging in *D. mojavensis* expressed in environments designed to simulate natural conditions, yet these eigengenes were not overly sensitive to population or host cactus differences. This suggests that population origin and cactus substrates influenced expression for relatively small numbers of genes in relation to the major developmental transitions in gene expression, i.e. the life cycle transcriptome of *D. mojavensis* is relatively well buffered from differences in its host plants and has yet to become strongly geographically differentiated.

Population and cactus differences have previously been shown to influence both egg to adult development time and average longevity in adults in *D. mojavensis* (Etges 1990; Jaureguy & Etges 2007). Our sampling from egg to eclosion was stage-based, not age-based, and so would not reveal expression differences related to rate of development. Our sampling of adults was necessarily destructive, so it was not possible to infer whether gene expression levels of adults at different ages were related to their ultimate lifespans.

However, our experimental design included just four replicate samples at each age/stage with often surprisingly high within-age variance, so perhaps some influences of these environmental factors may be distinguishable with increased replication. The top four eigengenes revealed three major transitions, i.e., from embryos to larvae, larvae to pupae, and pupae to adults (Fig. 2). Thus, the latent patterns of biological organization and function

revealed in eigengene analysis (Alter 2006; Ponnapalli *et al.* 2011) of the variation in lifetime gene expression occurred between, rather than within life stage types, and were uncovered through functional gene ontology clustering.

All four eigengenes were significantly influenced by variation in ribosome function and protein production (Table 1) suggesting that life cycle SVD analyses, when used to compare patterns of life gene expression in this and other organisms, may reveal fundamental insights into the general processes of development and senescence. Eigengene 1 encompassed most of overall transcriptional variation (63.5 %) due to increased expression of larval and pupal endopeptidases, embryonic and adult protein production and transport, as well as sensory perception (Fig. 2, Tables 1, 3, Suppl. Table 6). While biased due to the lack of annotation for ca. a third of predicted genes, life cycle shifts in protein metabolism and tissue remodeling were not surprisingly major sources of variation in lifetime gene expression.

In addition, a large number of gustatory, odorant binding, olfactory receptor, ion transport, and photoreceptor gene orthologs that increased in expression from embryo to first instar larvae, and then were down-regulated in adults were also highly correlated with eigengene 1 and revealed in comparisons of consecutive life cycle stages (Table 2). While adult sensory perception has been intensively studied because of its roles in chemical, host plant attraction/repulsion, and adult mating behavior (Amrein 2004; Carlson 1996; Olsson *et al.* 2006; Stokl *et al.* 2010; Thistle *et al.* 2012), sensory perception in preadult stages has been less well studied, but is known to be a determinant of successful larval feeding behavior, growth, and attainment of body critical mass prior to pupation (Beadle *et al.* 1938; Tennessen & Thummel 2011). The functional consequences of sensory genes across the life cycle in *Drosophila* species have been documented (Cobb 1999; c.f. de Belle *et al.* 1989; Gerber & Stocker 2007; Kent *et al.* 2009; Matsuo *et al.* 2007), but rarely in flies reared under natural conditions. In larvae, foraging behaviors are facilitated by chemical perception (Fishilevich *et al.* 2005) and thus resource acquisition during larval development. While *D. mojavensis* larval behavior in the wild has not been well studied (but see Fogleman *et al.* 1981), our results suggest that further study of the expression and evolution of these sensory gene families may help to unravel sensory behavior variation in nature and how it is related to resource exploitation, i.e. the cactus-influenced preadult life history differences between Baja California and mainland populations (Etges 1990, 1993; Etges *et al.* 2010). These patterns were far more subtle in the stage-specific GO clustering analyses (Table 1, 2) exemplifying the utility of SVD, and also emerged in analyses of host cactus effects (Table 4, 5).

Most emphasis on understanding host cactus preferences and subsequent larval and adult performance in desert *Drosophila* has been on production of and attraction to cactus fermentation by-products (Etges & Klassen 1989; Fanara *et al.* 1999; Newby & Etges 1998; Starmer 1982; Starmer *et al.* 1977) xenobiotic metabolism of cactus secondary compounds (Fogleman *et al.* 1998; Fogleman & Heed 1989; Matzkin 2008), and host cactus resource availability (Etges 1990; Etges *et al.* 2010; Heed & Mangan 1986). For *D. mojavensis*, use of agria and organ pipe cacti in the Sonoran Desert is due largely to its tolerance of medium sized fatty acids (C_6 - C_{18}, but most are C_{10} - C_{12} (Fogleman & Kircher 1986)), sterol diols, and high levels of triterpene glycosides. It can also tolerate the isoquinoline alkaloids

present in the rarely used alternate hosts saguaro, *Carnegiea gigantea*, and cardón, *Pachycereus pringlei*, cacti (Fogleman & Danielson 2001), but secondary compounds of other alternate hosts, e.g. California barrel cactus, *Ferocactus cylindraceus*, sina cactus, *S. alamosensis*, cochal cactus, *Myrtillocactus cochal* in Baja California, and *Opuntia* species on Santa Catalina Island have not been as intensively studied. While differences between agria and organ pipe cacti on overall gene expression were sometimes small, there were significant preadult stage-specific differences in gene expression between populations (Table 5) and population and cactus effects on adult gene expression (Etges 2014). There was little evidence of cactus-induced differences in expression of detoxification genes in preadult stages (Table 5), but there was significantly greater enrichment of P-450 genes in adult mainland females reared on organ pipe cactus. Thus, larvae were less sensitive to differences in cactus secondary compounds than adults, perhaps helping to explain genetic evidence for host plant generalism in larval performance in *D. mojavensis* (Etges 1993).

Other ecological aspects of cactus rots influencing larval growth and development involving sensory perception include selective foraging and predator/parasite avoidance. Larval *D. mojavensis* prefer particular yeast species over others in naturally occurring rots, so larval olfactory and gustatory receptors are likely to be directly involved with foraging preferences (Fogleman *et al.* 1981). In addition to bacteria and yeasts, cactus rots comprise a complex fermenting environment of degraded cactus tissues, secondary compounds, volatiles, and other invertebrates as rots progress from early bacterial fermentation, but interactions between these organisms and drosophilids have only been partially assessed (Escalante & Benado 1990; Kiontke*et al.* 2011; Mangan 1979; Polak 1998). Thus, understanding patterns of gustatory, odorant binding, olfactory receptor, and photoreceptor gene expression throughout the life cycle in *D. mojavensis* may contribute to our general understanding of patterns of resource use, life history variation, and host plant adaptation in natural populations of *Drosophila*.

Expression of life histories in contrasting environments

Central to a general understanding of life history evolution are the consequences of lifetime differences in environmental variability on survivorship and reproduction, and uncovering the environment-dependent expression of genetic variation underlying these components of fitness. Genetic differences in life histories between Baja California and mainland populations of *D. mojavensis* are host plant dependent, and thought to be influenced by differences in resource predictability at different stages of the life cycle (Etges 1990, 1993; Etges *et al.* 1999; Heed 1978, 1981). Cactus substrate-influenced development time differences between populations (Suppl. Fig. 1, Suppl. Table 8) were accompanied by larval, stage-specific differences in gene expression (Table 4, 5). Consistent with the increased development times of organ pipe-reared Las Bocas (mainland) flies and a Population × Cactus interaction, organ pipe-reared first instar larvae were enriched for 14 down-regulated gene clusters associated with larval development and metabolism. A handful of annotated orthologs were also down-regulated in the Punta Prieta, Baja California population due to organ pipe cactus, including GI17029, a *D. melanogaster* ortholog of *split ends*, involved in nucleic acid binding and postembryonic development (Table 5). Thus, decreased expression of developmental genes due to organ pipe cactus and increased expression of larval cuticle

and olfactory reception genes in first instar larvae (Table 4, 5) suggests longer mainland development times result in part from transcriptional events early in larval development. Just a few gene clusters were functionally enriched for proteolysis associated with metamorphosis including the ubl conjugation pathway, and cuticle structure, where organ pipe cactus again caused reduced transcription levels in Baja flies. Several of these functional clusters including genes responsible for nucleic acid binding, cuticle proteins, and larval growth and metabolism were correlated with a tradeoff between larval mass and survival in *D. melanogaster* (Bochdanovits & de Jong 2004) suggesting there may be a shared genetic basis for preadult growth rates in these species.

In adults, co-expression of genes associated with aging and age-specific reproduction was revealed by different eigengenes, patterns of maximum lifetime gene expression (Table 3) and in pairwise comparisons between adjacent ages (Suppl. Table 6). From a positive eigengene 2 peak at eclosion through 18 to 24 days (Fig. 2), there was a monotonic shift from eclosion onwards reflecting shifts in neural functioning, cellular maintenance, metabolic rates, and P450 activity through adulthood (Suppl. Table 2). Also at day 18, there were 14 significantly enriched gene clusters based on genes at maximum lifetime expression levels (Table 3), most that were associated with aging related traits, patterns strikingly similar to those in *D. melanogaster* and *Caenorhabditis elegans* (McCarroll *et al.* 2004). After 18 days, there was significantly increased expression of five gene clusters enriched for antimicrobial peptides, immune response, lipid metabolism, membrane function, and further P450 activity (Suppl. Table 6) similar to replicate lines of *D. melanogaster* selected for late life reproduction (Remolina *et al.* 2012). The noticeable late life shift in eigengene 2 and 3 expression at 18 days was due in part to increased expression of genes responsible for DNA repair, protein chaperones, signal transduction, ATP production, apoptosis, and others. Thus, *D. mojavensis* females at ca three weeks of age reared on fermenting cactus exhibited transcriptional shifts associated with physiological signs of increased cellular maintenance and protection from microbes and harmful chemical compounds.

A classic life history tradeoff between somatic maintenance and reproduction was evident in decreases in gene expression associated with somatic maintenance with the onset of female reproduction. At the onset of sexual maturity at three to six days (Supple. Table 6A), increased expression of 621 genes were functionally enriched for reproduction and DNA repair, and showed decreased expression of cuticle formation, immune response, melanin metabolism, sugar transport, and muscle development genes (Supple. Table 6A). However, there was little evidence for down-regulation of genes associated with egg production as in *D. melanogaster* where decreases in transcript abundance of chorion formation genes with increasing age has been observed (Pletcher *et al.* 2002). Likely explanations for this are; 1) *D. mojavensis* females cultured on fermenting cactus and ethanol vapor rarely live more than 30 days and so may not reach reproductive senescence vs. the 60 + day survivorship of *D. melanogaster* cultured on artificial media, 2) our adult female *D. mojavensis* were unmated, so it is unlikely that we would expect to observe realistic lifetime shifts in expression of gene clusters associated with mating and egg production because female longevity, fecundity, and metabolism are significantly influenced by mating and remating (Etges & Heed 1992; Markow *et al.* 1990).

Conclusions

Comparative life cycle studies of genomic expression in different organisms are imperative for characterizing the genetic architecture and ontogeny of gene expression responsible for the life history variation we seek to understand. Only then can we evaluate the expression of genomic elements responsible for fitness tradeoffs and senescence in relation to phenotypic variation in life histories. Despite the limitations of genome annotation for most non-model species, SVD analysis successfully resolved many of the major developmental and adult shifts in the expression of correlated groups of genes from embryogenesis through senescence in this model insect. Ideally, future whole genome expression SVD studies should involve direct comparisons of the same life stages and ages under controlled environmental conditions. Although few whole genome studies assessing such life cycle variation have been performed under natural conditions for comparison, the transcriptome of *D. mojavensis* reared on two of its major host cacti throughout its life cycle has revealed similar core developmental transitions to those in *D. melanogaster*. However, there remains a significant fraction of the genome that is still unknown due to limited gene annotation, much that is necessary for understanding subtle expression differences due to population or host plants (Table 5). This will limit future comparative studies whether microarrays or other transcriptome methods are used.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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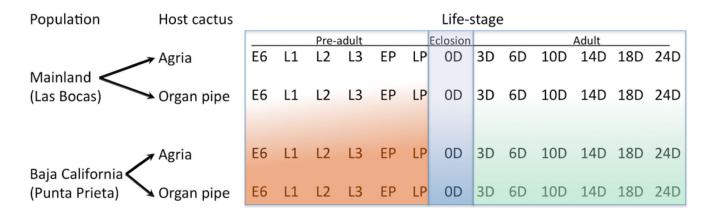
Figure 1.
Experimental design for RNA sampling of the two populations of *D. mojavensis* reared on two host plants throughout the life cycle where L1 = first instar, L2 = second instar, L3 = third instar, EP = early pupae, LP = late pupae, 0 D = adult day of emergence, 3 D = 3 day old adults, etc. Day 24 adults were pooled with Day 28 adults because so few Day 28 adults were available due to mortality.
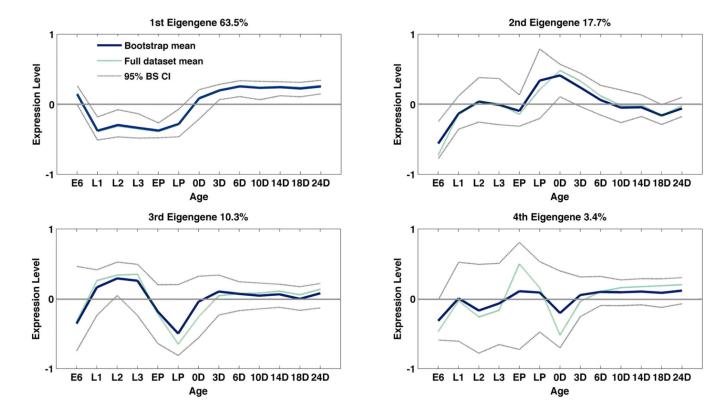
Figure 2.
The first four eigengenes plotted across the life cycle in *D. mojavensis.* The proportion of the total variation explained by each eigengene is listed. Plus/minus correlations with each eigengene are plotted for the overall data set means, bootstrap means, and 95 % bootstrap mean confidence intervals. The X axis represents the six preadult stages: embryo, larval, and pupal plus adults defined in Figure 1 and the text.
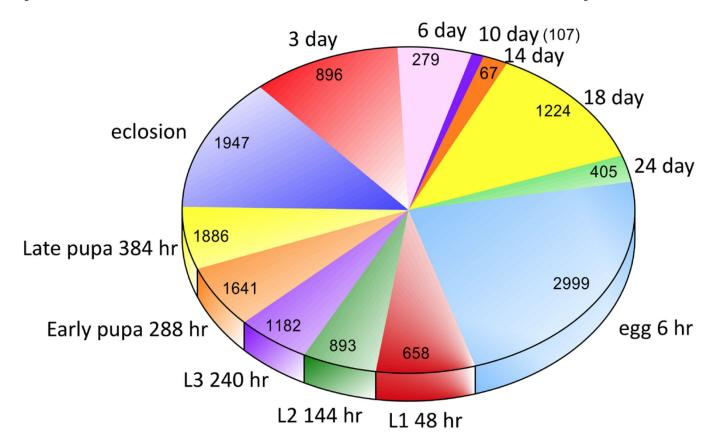
Figure 3.
Pie chart showing the numbers of genes at their maximum lifetime expression levels at each stage and age in this study. Stages and ages are defined in Figure 1 and in the text.
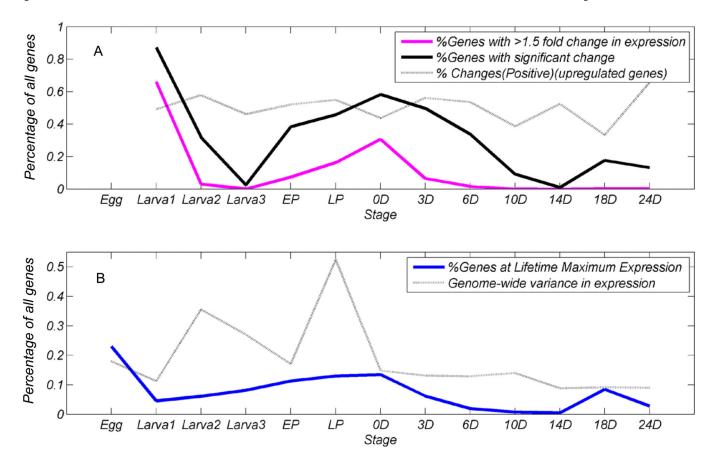
Figure 4.
A. Plots of the changes in gene expression compared with previous stages/ages for the proportion of all 14,528 genes with 1.5 × fold changes, significant changes (P < 0.05), percent of all genes that were upregulated from the previous stage/age. B. Plot showing the percentage of all genes at maximum lifetime expression levels and changes in the variance in gene expression for all genes at each stage/age.

Table 1

Gene ontology terms identified with DAVID using the top 5% (750 genes) of all predicted *D. mojavensis* genes. Results for the top 4 significant eigengene clusters based on all life stages and ages are shown. Numbers in parentheses are the percentages of the top 750 genes with *D. melanogaster* orthologs.

Eigengene 1 (63.5%)

Positive correlation 290 (38.7%)+		Negative correlation 648 (86.4%)	
GOTerm	enrich	GOTerm	enrich
protein transport, localization	3.78 ****	G-protein coupled receptor protein sensory perception, taste, chemical stimulus	5.82 ****
Vesicle transport, endocytosis, secretion	2.88 ***	Cell morphogenesis	3.35 ***
		Nucleotide binding	2.74 *
		Transcriptional regulation	2.06 *
		Translation in mitochondrion	1.99 **

Eigengene 2 (17.7%)

Positive correlation 574 (76.5)		Negative correlation 531 (70.8)		
GOTerm	enrich	GOTerm	enrich	enrich
Membrane structure	4.62 ****	RNA, mRNA, tRNA processing, splicing, transport	8.9 ****	10.38 ****
Glycoproteins	2.1 **	Zinc finger, metal ion binding	4.79 ****	6.05 ****
ATP transmembrane metabolism, ion transport	1.96 *	Chromosomal structure	4.32 ***	4.24 ****
Plasma membrane	1.64 *	Transcription, protein synthesis	3.2 *	4.1 ****
Larval behavior	1.25 *	Chromosome organization, modification	2.35 *	3.86 ***
		Protein folding	2.33 **	3.21 **
		Ribonuclease activity	2.1 ***	2.92 *
		Epigenetic regulation of gene expression	1.54 *	1.36 *
		Detection of external stimulus	1.38 **	
		Cell-cell signaling and neurological function		
		Oxidative phosphorylation		
		g-protein coupled receptors		

Eigengene 3 (10.3%)

Positive correlation 457 (60.9%)		Negative correlation 422 (56.3%)	
GOTerm	enrich	GOTerm	enrich
Peptidase activity	3.78 ****	Neuron differentiation & development, axonogenesis	5.4 ****
Endoplasmic reticulum	2.88 ***	Cell migration	5.32 ****

Eigengene 4 (3.4%)

Positive correlation 406 (54.1%)		Negative correlation 545 (72.7%)	
GOTerm	enrich	GOTerm	enrich
Ribosome	6.27 ****	HOX/homeobox, development protein, DNA binding, transcriptional regulation	7.98 ****
Structural constituent of ribosome	2.81 ****	Mitochondrion, mitochondrial ribosome	5.13 ****

Eigengene 3 (10.3%)

Positive correlation		Negative correlation	
457 (60.9%)		422 (56.3%)	
GOTerm	**enrich**	**GOTerm**	**enrich**
		Organ formation	4.58 *
		Eye development	4.22 *
		Instar larva or pupal morphogenesis, metamorphosis	3.23 *
		Salivary gland development	2.22 ***

Eigengene 4 (3.4%)

Positive correlation		Negative correlation	
406 (54.1%)		545 (72.7%)	
GOTerm	**enrich**	**GOTerm**	**enrich**
		Organ formation	4.52 ****
		Regionalization, pattern specification	4.24 ****
		Neuron development	3.8 ***
		Transcription repressor activity, negative regulation of transcription	3.67 ***
		Head segmentation	3.57 **
		Oxidative phosphorylation	3.49 **
		Gut development	3.14 **
		Respiratory system development	2.31 *
		Wnt signaling	2.16 **

FDR

+ Number (percentage of top 750) of genes annotated

* $P < 0.05$

** $P < 0.01$

*** $P < 0.005$

**** $P < 0.0001$

Table 2

Changes in expression (all 1.5 X fold change, FDR $P < 0.05$) of sensory genes from 6 h embryos to first instar larvae (Egg-L1), second to third instar (L2-L3), and late pupae to day of eclosion (LP-0D) in two populations of *D. mojavensis*. All significant changes were identified in enriched functional clusters of genes differing in expression between consecutive life stages identified by DAVID (Huang et al., 2009). Direction of arrows indicates increased or decreased transcript abundance.

D. melanogaster ID	gene	Egg-L1	L2-L3	LP-0D	GO annotation
FBgn0000120	*Arr1*	↑↑			deactivation of rhodopsin mediated signaling
FBgn0000206	*boss*	↑↑			R7 cell fate commitment; GO:0007465 compound eye development; GO:0048749
FBgn0000313	*chp*	↑↑			homophilic cell adhesion; GO:0007156 rhabdomere development; GO:0042052
FBgn0066293	*CheB42b*	↑↑			detection of pheromone; GO:0043695
FBgn0040726	*dpr*	↑↑			salt aversion; GO:0035199, sensory perception of salty taste
FBgn0004623	*Gbeta76C*	↑↑			deactivation of rhodopsin mediated signaling; GO:0016059, G-protein coupled receptor protein signaling pathway; GO:0007186
FBgn0028433	*Ggamma30A*	↑↑			G-protein coupled receptor protein signaling pathway; GO:0007186, phototransduction; GO:0007602
FBgn0004618	*gl*	↑↑			response to red light; GO:0010114, entrainment of circadian clock by photoperiod
FBgn0045502	*Gr10a*	↑↑			sensory perception of taste; GO:0050909
FBgn0041250	*Gr21a*	↑↑			sensory perception of taste; GO:0050909
FBgn0041248	*Gr23a*	↑↑			sensory perception of taste; GO:0050909
FBgn0041247	*Gr28a*	↑↑		↓↓	sensory perception of taste; GO:0050909
FBgn0045495	*Gr28b*	↑↑			sensory perception of taste; GO:0050909
FBgn0032416	*Gr33a*	↑↑		↓↓	sensory perception of taste; GO:0050909
FBgn0041236	*Gr59d*	↑↑		↓↓	sensory perception of taste; GO:0050909
FBgn0035468	*Gr63a*	↑↑		↓↓	sensory perception of taste; GO:0050909, response to carbon dioxide; GO:0010037
FBgn0045479	*Gr64a*	↑↑		↓↓	sensory perception of taste; GO:0050909, detection of glucose; GO:0051594
FBgn0045478	*Gr64b*	↑↑		↓↓	sensory perception of taste; GO:0050909
FBgn0045477	*Gr64c*	↑↑		↓↓	sensory perception of taste; GO:0050909
FBgn0045476	*Gr64e*	↑↑		↓↓	sensory perception of taste; GO:0050909
FBgn0052255	*Gr64f*	↑↑		↓↓	sensory perception of taste; GO:0050909
FBgn0035870	*Gr66a*			↓↓	sensory perception of taste; GO:0050909
FBgn0046885	*Gr98d*	↑↑		↓↓	sensory perception of taste; GO:0050909, sensory perception of taste; GO:0050909
FBgn0011672	*Mvl*			↓↓	sensory perception of taste; GO:0050909, taste receptor activity; GO:0008527

D. melanogaster ID	gene	Egg-L1	L2-L3	LP-0D	GO annotation
FBgn0013972	Gycalpha99B	↑↑			positive phototaxis; GO:0046956 rhodopsin mediated phototransduction; GO:0009586, guanylate cyclase complex, soluble; GO:0008074
FBgn0004784	inaC	↑↑			adaptation of rhodopsin mediated signaling; GO:0016062 phototransduction; GO:0007602
FBgn0001263	inaD	↑↑			deactivation of rhodopsin mediated signaling; GO:0016059, phototransduction; GO:0007602
FBgn0053197	mbl	↑↑			embryonic development; GO:0009790, muscle organ development; GO:0007517, compound eye photoreceptor cell differentiation; GO:0001751
FBgn0036414	nan	↑↑			calcium ion transport; GO:0006816, sensory perception of sound; GO:0007605
FBgn0002936	ninaA	↑↑			rhodopsin biosynthetic process; GO:0016063
FBgn0002938	ninaC	↑↑			cytoskeleton organization; GO:0007010, phototransduction, visible light; GO: adaptation of rhodopsin mediated signaling; GO:0016062
FBgn0002940	ninaE	↑↑			phototransduction; GO:0007602, photoreceptor cell morphogenesis; GO:0008594
FBgn0037896	ninaG	↑↑			retinoid metabolic process; GO:0001523
FBgn0016047	nompA	↑↑			dendrite morphogenesis; GO:0048813, detection of mechanical stimulus involved in sensory perception of sound; GO:0050910
FBgn0016919	nompB	↑↑			flagellum assembly; GO:0009296, sensory cilium assembly; GO:0035058
FBgn0016920	nompC	↑↑			calcium ion transport; GO:0006816, mechanosensory behavior; GO:0007638, sensory perception of sound; GO:0007605
FBgn0031110	Obp19b	↑↑			sensory perception of chemical stimulus; GO:0007606
FBgn0033268	Obp44a	↑↑			sensory perception of chemical stimulus; GO:0007606
FBgn0033508	Obp46a	↑↑		→	sensory perception of chemical stimulus; GO:0007606
FBgn0033573	Obp47a	↑↑			sensory perception of chemical stimulus; GO:0007606, odorant binding GO:0005549
FBgn0050067	Obp50a	↑↑			sensory perception of chemical stimulus; GO:0007606
FBgn0033931	Obp50e	↑↑			sensory perception of chemical stimulus; GO:0007606
FBgn0034468	Obp56a	↑↑			sensory perception of smell; GO:0007608, response to pheromone; GO:0019236
FBgn0046879	Obp56c	↑↑		→	sensory perception of chemical stimulus; GO:0007606
FBgn0034471	Obp56e	↑↑			sensory perception of chemical stimulus; GO:0007606
FBgn0034474	Obp56g	↑↑			sensory perception of chemical stimulus; GO:0007606
FBgn0034475	Obp56h	↑↑			sensory perception of smell; GO:0007608 response to pheromone; GO:0019236
FBgn0034768	Obp58b	↑↑		→	sensory perception of chemical stimulus; GO:0007606
FBgn0034769	Obp58c	↑↑		→	sensory perception of chemical stimulus; GO:0007606
FBgn0034770	Obp58d	↑↑		→	sensory perception of chemical stimulus; GO:0007606
FBgn0046876	Obp83ef			→	sensory perception of chemical stimulus; GO:0007606
FBgn0046875	Obp83g	↑↑		→	olfactory behavior; GO:0042048, response to pheromone; GO:0019236
FBgn0038859	Obp93a	↑↑		→	sensory perception of chemical stimulus; GO:0007606

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D. melanogaster ID	gene	Egg-L1	L2-L3	LP-0D	GO annotation
FBgnt039685	Obp99b	↑↑	↑↑	→→	autophagic cell death; GO:0048102, salivary gland cell autophagic cell death; GO:0035071, olfactory behavior; GO:0042048, olfactory behavior; GO:0042048, response to pheromone; GO:0019236
FBgnt039682	Obp99c	↑↑			sensory perception of chemical stimulus; GO:0007606
FBgnt026396	Or22c			→→	sensory perception of smell; GO:0007608
FBgnt026394	Or24a	↑↑			sensory perception of smell; GO:0007608
FBgnt032096	Or30a	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt026390	Or33c	↑↑		→→	olfactory behavior; GO:0042048
FBgnt033041	Or42a	↑↑			sensory perception of smell; GO:0007608
FBgnt026389	Or43a	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt033422	Or45b	↑↑			sensory perception of smell; GO:0007608
FBgnt026388	Or46a	↑↑			olfactory behavior; GO:0042048, sensory perception of smell; GO:0007608
FBgnt033727	Or49a	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt028963	Or49b	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt034473	Or56a	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt026384	Or59a	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt035382	Or63a	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt036078	Or67c	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt036709	Or74a	↑↑		→→	integral to membrane; GO:0016021, olfactory receptor activity; GO:0004984, odorant binding; GO:0005549
FBgnt037322	Or83a	↑↑		→→	sensory perception of smell; GO:0007606
FBgnt037324	Or83b	↑↑		→→	olfactory behavior; GO:0042048 sensory perception of smell; GO:0007608 response to pheromone; GO:0019236
FBgnt037576	Or85a	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt037594	Or85d	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt038798	Or92a	↑↑		→→	sensory perception of smell; GO:0007608
FBgnt030204	Or9a	↑↑			sensory perception of smell; GO:0007608
FBgnt060296	pain	↑↑			calcium ion transport; GO:0006816, sensory perception of pain; GO:0019233, response to mechanical stimulus; GO:0009612, feeding behavior
FBgnt011283	Pbprp5	↑↑			sensory perception of chemical stimulus; GO:0007606
FBgnt065109	ppk11	↑↑			sodium ion transport; GO:0006814, liquid clearance, open tracheal system; GO:0035002, sensory perception of salty taste; GO:0050914
FBgnt085373	rdgA	↑↑			diacylglycerol kinase activity
FBgnt004366	rdgC	↑↑			phototransduction; GO:0007602, photoreceptor cell maintenance; GO:0045494, visual perception; GO:0007601
FBgnt003248	Rh2	↑↑			phototransduction; GO:0007602
FBgnt003249	Rh3	↑↑			phototransduction, UV; GO:0007604

D. melanogaster ID	gene	Egg-L1	L2-L3	LP-0D	GO annotation
FBgn0014019	Rh5	↑↑			phototransduction; GO:0007602
FBgn0019940	Rh6	↑↑			phototransduction; GO:0007602
FBgn0036260	Rh7	↑↑			G-protein coupled receptor protein signaling pathway; GO:0007186, phototransduction; GO:0007602
FBgn0003380	Sh	↑↑			flight behavior; GO:0007629, potassium ion transport; GO:0006813, courtship behavior; GO:0007619
FBgn0086367	t	↑↑			flight behavior; GO:0007629, cuticle pigmentation; GO:0048067, visual perception; GO:0007601, dopamine biosynthetic process; GO:0042416
FBgn0014395	tilB	↑↑			sensory perception of sound; GO:0007605, male courtship behavior, veined wing generated song production; GO:0045433
FBgn0005614	trpl	↑↑			calcium ion transport; GO:0006816, response to abiotic stimulus; GO:0009628, response to light stimulus; GO:0009416
FBgn0004514	TyrR	↑↑			sensory perception of smell; GO:0007608, octopamine/tyramine signaling pathway; GO:0007211
FBgn0039482	CG14258		↑↑		pheromone/odorant binding GO:0005549
FBgn0051345	CG31345	↑↑			detection of calcium ion; GO:0005513, phagocytosis, engulfment; GO:0006911
FBgn0147028 (Dmoj)	Dmoj_GI24305		↑↑		IPR004272: Odorant binding protein, IPR013053: Hormone binding, (no D. melanogaster ortholog)

Table 3

Functional annotation clustering of genes with maximum lifetime expression levels in two populations of *D. mojavensis* using DAVID. Numbers of transcripts having their maximum expression at each life stage are indicated with the number of *D. melanogaster* orthologs (in parentheses) used for GO clustering. FDR P values associated with top GOterms are indicated. L1 = first instar, L2 = second instar, L3 = third instar, EP = early pupae, LP = late pupae, 0 D = adult day of emergence, 3 D = 3 day old adults, etc. See text for details.

Life stage							
Embryo 6 h n = 2999 (2363)		**L1 n = 658 (432)**		**L2 n = 893 (345)**		**L3 n = 1182 (451)**		**EP n = 1641 (602)**	
GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score
1. RNA splicing, processing	22.6 ****	1. peritrophic membrane structure, chitin binding	4.4 ****	1. G-protein coupled peptide receptor activity	4.2 ****	1. G-protein coupled receptor, sensory perception	12.3 ****	1. cuticle structure	4.2 ****
2. nuclear lumen, nucleoplasm	19.6 ****	2. signal peptide	3.1 ****	2. HOX , DNA binding	3.0 ****	2. detection of chemical stimulus	6.5 ****	2. salivary gland histolysis	3.6 ***
3. DNA binding, transcription regulation	19.3 ****	3. cell adhesion	2.6 *	3. membrane receptor	2.7 ****	3. sodium channel activity	5.6 ****	3. chitin binding	2.7 **
4. Chromosome	16.1 ****	4. proton transport ATPase	2.6 ****	4. carbohydrate binding	2.1 **	4. peptide receptor activity	2.5 *	4. neuron development	2.6 *
5. Down regulation of gene expression	13.5 ****	5. transmembrane function	2.6 **	5. cuticle structure	1.7 *	5. ligand-gated channel activity	2.5 ****	5. Serine protease	1.8 **
6. Mitosis, cell cycle	12.5 ****	6. ribosome structure	2.5 ****	6. serine-type peptidase activity	1.7 *	6. chitin binding	2.3 **	6. dynein complex	1.7
7. Embryonic morphogenesis	10.5 ****	7. procollagen-proline dioxygenase activity	2.0 **					7. cell adhesion	1.7 *
8. Chromatin organization/ modification	8.8 ****	8. neurotransmitter, gated channel activity	1.9 **						
9. Up regulation of gene expression	8.1 ****								
10. Oogenesis	7.9 ****								
11. Transcription factor binding	7.9 ****								
12. DNA replication	6.9 ****								
13. DNA repair	6.2 ****								
14. Neuron differentiation, morphogensis	5.8 ****								

Life stage

Embryo 6 h n = 2999 (2363)		L1 n = 658 (432)		L2 n = 893 (345)		L3 n = 1182 (451)		EP n = 1641 (602)	
GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score
15. Ribosome biogenesis	5.5 ****								
16. Nucleocytoplasmic transport	5.3 ****								
17. RNA helicase activity	4.9 ****								
18. DNA packaging, nucleosome assembly	4.8 ****								
19. Meiosis	4.8								
20. cell migration, motion	4.7 ****								
21. compound eye development	4.7 ****								
22. Nucleotidyl-transferase activity	4.5 ****								
23. Stem cell maintenance	4.4 ****								
24. Imaginal disc development	4.1 ****								
25. Gene silencing	3.5 ****								
26. Embryonic segmentation	3.4 ****								

Life stage

LP n = 1886 (818)		0 day n = 1947 (1562)		3 day n = 896 (613)		6 day n = 279 (213)		10 day n = 107 (79)	
GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score
1. cuticle structure	3.1 ***	1. cellular respiration	20.6 ****	1. fatty acid biosynthesis	5.8 ****	1. cofactor binding	2.5 ****	1. ribosome, translation	2.8 ****
2. microtubule cytoskeleton	2.6 ****	2. transit peptide: mitochondrion	6.6 ****	2. iron ion binding	5.0 ****	2. vitamin binding	2.3 ****		
3. ion channel activity	2.2 ***	3. TCA cycle	6.2 ****	3. cofactor binding, acyl-CoA DH activity	4.3 ****	3. steroid metabolism	2.3 ****		
4. muscle development	1.8 ****	4. photo-transduction, light detection	5.5 ****	4. hexose metabolism	4.0 ****	4. Golgi vesicle membrane	1.9 ***		

Life stage

LP n = 1886 (818)		0 day n = 1947 (1562)		3 day n = 896 (613)		6 day n = 279 (213)		10 day n = 107 (79)	
GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score
5. membrane transport	1.8 **	5. ion transport	4.9 **	5. transaminase activity	3.8 ***	5. membrane, endocytosis	1.8 *		
6. mitochondrial membrane	1.5	6. ATP metabolic process	4.6 ****	6. glycosidase, lysosome	3.8 ****	6. oogenesis	1.8		
7. neuropeptide binding	1.5 *	7. mitochondrial electron transport	4.4 ****	7. signal peptide secretion	3.1 ***				
		8. adult locomotory behavior	4.3 ****	8. carboxylic acid catabolism	3.0 ***				
		9. membrane transport	4.2 ****	9. glutathione transferase activity, P450	2.9 ***				
		10. proteasome accessory complex	3.7 *****	10. galactose metabolism	2.9 **				
		11. glycolysis, alcohol metabolism	3.7 ****	11. unsaturated FA synthesis	2.9 ***				
		12. synaptic transmission	3.3 ****	12. oxidoreductase activity, AA metabolism	2.3 ***				
		13. glycogen biosynthetic process	2.6 ***						
		14. ion transport	2.5 ****						
		15. axon projection	2.2 **						

14 day n = 67 (50)		18 day n = 1224 (997)		24 day n = 405 (316)	
GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score
1. ribosome, translation	1.2	1. DNA repair	7.8 ****	1. peroxisomal membrane	3.0 ****
2. oogenesis	1.1	2. zinc finger, ion binding	7.1 ****	2. translation, ribosome	3.0 ****
		3. ATP binding	4.3 ****	3. protein transport	2.1 *
		4. chaperonin, protein folding	3.6 ***		
		5. mitosis	2.5 **		
		6. Golgi apparatus	2.2 ***		

14 day n = 67 (50)		18 day n = 1224 (997)		24 day n = 405 (316)	
GOTerm	enrich score	GOTerm	enrich score	GOTerm	enrich score
		7. phosphoinositide binding	2.1 ***		
		8. apoptosis regulation	2.1 *		
		9. protein transport	2.1 **		
		10. DNA helicase activity	2.0 **		
		11. meiosis	2.0 *		
		12. recombination	1.8		
		13. deoxyribonuclease activity	1.7 *		
		14. oocyte development	1.7 **		

FDR

*
$P < 0.05$

**
$P < 0.01$

***
$P < 0.005$

****
$P < 0.0001$

Table 4

Total numbers of genes differentially expressed (fold change > 1.5 X and FDR < 0.05) due to cactus rearing substrates in preadult stages, from egg to eclosion, in two populations of *D. mojavensis* reared on two cactus hosts. Contrasts indicate numbers of genes overexpressed due to organ pipe (OP) vs. agria (AG) cactus, OP>AG, and vice versa. Life stages are eggs (6 hr), L1 - first instar larvae (48 hr), L2 - second instar larvae (144 hr), L3 - third instar larvae (240 hr), EP - early pupae (288 hr), LP - late pupae (384 hr), and 0D – adults at day of eclosion.

Population							Life stage							
	Egg		L1		L2		L3		EP		LP		0D	
Las Bocas [*]	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP
D. mojavensis genes	-	-	570	631	61	15	-	3	3	2	-	-	1	-
D. melanogaster orthos	-	-	222	529	53	9	-	3	1	2	-	-	0	-
Proportion annotated	-	-	0.389	0.838	0.869	0.6	-	1	0.33	1	-	-	0	-
Average % annotated	0.637													

Population														
	Egg		L1		L2		L3		EP		LP		0D	
Punta Prieta [#]	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP	OP>AG	AG>OP
D. mojavensis genes	4	4	1	4	-	-	-	-	15	21	213	132	-	4
D. melanogaster orthos	0	1	0	4	-	-	-	-	2	14	137	59	-	3
Proportion annotated	0	0.25	0	1	-	-	-	-	0.13	0.67	0.643	0.447	-	0.75
Average % annotated	0.553													

[*] mainland Sonora

[#] Baja California

Table 5

Gene ontology results for predicted genes that were differentially expressed (>1.5 fold difference, FDR $P < 0.05$) from the egg stage to day of eclosion in two populations of *D. mojavensis* reared on agria and organ pipe cacti. Life stages are defined in Table 3 and individual genes and their *D. melanogaster* ortholog names are listed when only several genes differed in expression and no functional clustering was observed in DAVID (Huang et al., 2009).

	Population							
	Las Bocas (mainland)				**Punta Prieta (Baja California)**			
	OP>AG		**AG>OP**		**OP>AG**		**AG>OP**	
	GOTerm (gene)	enrich score	GOTerm (gene)	enrich score	GOTerm (gene)	enrich score	GOTerm (gene)	enrich score
Egg	-	-	-	-	-	-	GI22080 - Cep78 protein	-
L1	1. cuticle, chitin structure 2. Odorant binding, olfactory reception	5.9**** 2.1***	1. transport 2. cell metabolism 3. protein transport 4. Oxphos process/ ion transport 5. mitotic spindle/ribosome protein 6. cell morphogenesis 7. RNA binding 8. larval development 9. actin filament processes 10. protein synthesis 11. RNA splicing 12. axon development 13. mitochondria transport peptide 14. germ cell develop.	5.9**** 4.3**** 3.5**** 3.4**** 3.3**** 3.0*** 3.0** 2.9** 2.9** 2.5** 2.5* 2.2** 2.1** 1.8**	-	-	GI24491 – Rheb GI24142 - Rab-protein 7 – GTPase mediated signal transduction GI10537 - alternative testis transcripts open reading frame A GI17029 - split ends - post-embryonic morphogenesis	-
L2	1. cytoskeleton, ribosomal protein	1.1	1. metal ion binding	0.5	-	-	-	-
L3	-	-	GI16482 - ribosomal protein L40 GI12755 – Rh50 (membrane transport) GI11050 – yu (prn and RNA binding)		-	-	-	-

	Population							
	Las Bocas (mainland)				Punta Prieta (Baja California)			
	OP>AG		AG>OP		OP>AG		AG>OP	
	GOTerm (gene)	enrich score	GOTerm (gene)	enrich score	GOTerm (gene)	enrich score	GOTerm (gene)	enrich score
EP	GI11311 - GNBP2 (Gram-negative bacteria binding protein 2) GI24782^{+} GI12221^{+}	-	GI18191 – fbp (fructose-1,6-bisphosphatase) GI15376 - mesoderm development	-	GI24313 - Cpr30F (pupal chitin structure) GI19286	-	1. ubl conjugation pathway, proteolysis	2.0 *
LP	-	-	-	-	1. mitochondrial membrane	1.6	1. cuticle structure	1.2
0D	GI23541[+]	-	-	-	-	-	GI16633 – heat shock ptn/ metal ion binding GI24251 – nucleotide binding GI16531 - Dpy-30 (adenylate kinase) GI19042	-

False Discovery rate P values

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

**** $P < 0.0001$

[+] gene of unknown function