Ecological Genomics of Host Shifts in *Drosophila mojavensis*

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Abstract

Advances in next-generation sequencing technologies have liberated our dependency on model laboratory species for answering genomic and transcriptomic level questions. These new techniques have dramatically expanded our breadth of study organisms and have allowed the analysis of species from diverse ecological environments. One such species is the cactophilic *Drosophila mojavensis* that inhabits the deserts of western North America. These insects feed and develop in the necrotic cacti, feeding largely on the microflora of the necrotic plant tissues. *Drosophila mojavensis* is composed of four geographically and ecologically separated populations. Each population (Baja California peninsula, mainland Sonoran Desert, Mojave Desert and Santa Catalina Island) utilizes the necrotic tissues of distinct cactus species. The differences in the nutritional and chemical composition of the necroses include a set of toxic compounds to which resident population must adapt. These ecological differences have facilitated many of the life history, behavior, physiological and genetic differences between the cactus host populations. Genomic resources have allowed investigators to examine the genomic and transcriptional level changes associated with the local adaptation of the four *D. mojavensis* populations, thereby providing further understanding of the genetic mechanism of adaptation and its role in the divergence of ecologically distinct populations.

Keywords

Ecological genomics • Cactophilic drosophila • Adaptation • *Drosophila mojavensis* • Transcriptomics

12.1 Introduction

One of the great advantages in studying model laboratory species (e.g. *Drosophila melanogaster*, *Caenorhabditis elegans*,

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Saccharomyces cerevisiae, *Arabidopsis thaliana* and *Escherichia coli*) is the presence of a completely sequenced and annotated genome (Adams et al. 2000; *C. elegans* Sequencing Consortium 1998; Blattner et al. 1997; Goffeau et al. 1996; Arabidopsis Genome Initiative 2000). These model systems offer the availability of many mutant and transgenic stocks, which allows for the manipulation and genetic dissection of the traits of interest. The lack of much ecological information about these species prevents in many instances the correlation of patterns of genomic variation to the ecological factors influencing its variance. The recent feasibility of large-scale sequencing, due to the development of next-generation platforms, has allowed the genomic and transcriptomic investigation of a greater number of ecologically diverse species. The placing of genomic data in an ecological context has been an instrumental approach in our understanding of the adaptation and evolution of species (Feder and Mitchell-Olds 2003). This ecological genomic approach has been embraced in many fascinating and ecologically well-studied systems such as the water flea (*Daphnia pulex*) and stickleback fish (*Gasterosteus aculeatus*) (Colbourne et al. 2011; Jones et al. 2012).

The ecological characteristic of certain species facilitates the change or shift in resource use. This is no more evident that in the many species of phytophagous insects (Agrawal et al. 2009; Janz 2011; Berenbaum 2002). Genetic and genomic analysis of host shifts in phytophagous insects has allowed investigators to peer into the mechanism of the adaptation process. In general, such as observed in the apple maggot fly (*Rhagoletis pomonella*) and the pea aphid (*Acyrthosiphon pisum*), host shifts involve not only changes associated with the use of an alternative host, but as well in several correlated life history, physiological and behavioral traits (Caillaud and Via 2012; Dambroski and Feder 2007; Linn et al. 2003; Via 1999). Genome level studies have begun to identify the loci and genomic regions associated many of these host shift related adaptations (Michel et al. 2010; Richards et al. 2010; Schwarz et al. 2009; Smadja et al. 2012) and in the case of *Drosophila pachea* investigators have been able to identify the genetic loci responsible for obligate host use (Lang et al. 2012). Furthermore, when correlated with reproductive isolation between host populations, the changes associated with host shifts could eventually lead to ecological speciation (reviewed in Nosil 2012). Among the many insect groups, the ecologically diverse and specious genus *Drosophila* offers a vast number of study systems to assist in the understanding of the genetic basis of host adaptation and its relationship to speciation.

The genus *Drosophila* is comprised of over 2,000 described species inhabiting a wide variety of ecological habitats from tropical rainforests to deserts (Markow and O'Grady 2006). The vast majority of these species are saprophytic, mainly feeding as larvae and adults on yeasts and bacteria growing in a variety of tissues, such as fruits, tree sap fluxes, leaves, cactus and mushrooms (Throckmorton 1975; Sturtevant 1921; Jaenike 1978; Kaneshiro et al. 1973; Heed 1978). A few species are known to utilize live flowers, nutrient-soaked soils and even land crabs for feeding (Brncic 1983; Carson 1974; Kaneshiro et al. 1973). Although yeasts are a major source of the *Drosophila's* nutrition, they are also exposed to chemical compounds found in the host. In certain systems these compounds are toxic and resident *Drosophila* species must adapt to their presence, such as in the case of α-amanitin tolerance in the mycophagous species *D. putrida*, *D. recens* and *D. tripunctata* (Jaenike et al. 1983) or octanoic acid in the *Morinda citrifolia* fruit, the host of *D. sechellia* (Legal et al. 1994).

Another example of *Drosophila* exposed to the toxic chemical profile of its host, are those species inhabiting cactus necroses. Cactophilic *Drosophila* species feed as adults, oviposit and develop in necrotic stems and/or fruits of cacti, with some fascinating exceptions such as *D. mettleri* which feeds as an adult in the necrotic tissues of cardón (*Pachycereus pringlei*), saguaro (*Carnegiea gigantean*) and occasionally senita

(*Lophocereus schottii*) cactus, but oviposits, develops and pupates in the necrotic exudate soaked soil (Heed 1977). These Drosophilids, including specialists and generalists, are found throughout the Americas utilizing a wide variety of plants within the family Cactaceae (Hasson et al. 1992; Heed 1978; Oliveira et al. 2012; Ruiz et al. 1990; Carson and Wasserman 1965; Heed and Kircher 1965; Markow and O'Grady 2008; Fontdevila et al. 1988). One exception to the New World distribution of cactophilic *Drosophila* is *D. buzzatti*, which has been introduced to the Mediterranean region and Australia (Carson and Wasserman 1965).

With the exception of the *D. nannoptera* species group, cactophilic *Drosophila* are members of the *D. repleta* species group, which is comprised of approximately 100 described species (Throckmorton 1975; Oliveira et al. 2012). Both of these groups are part of a larger species radiation (virilis-repleta) that occurred within the genus approximately 36 MYA (Throckmorton 1975). Among the species within the *D. repleta* species group is *D. mojavensis,* which has proven to be a powerful system for understanding the ecological genomics of adaptation.

12.2 The *Drosophila mojavensis* Study System

12.2.1 Evolutionary History

Drosophila mojavensis is one of four cactophilic species endemic to the Sonoran Desert of western North America (Heed 1978). Its distribution includes four geographically separated populations, or host races, each utilizing a distinct necrotic cactus host for both oviposition and adult feeding. *Drosophila mojavensis* utilizes the agria cactus (*Stenocereus gummosus*) in the Baja California peninsula, organ pipe cactus (*S. thurberi*) in the mainland Sonoran Desert, Red Barrel cactus (*Ferocactus cylindraceus*) in the Mojave Desert and Coastal Prickly Pear (*Opuntia littoralis*) in Santa Catalina Island(hereafter Catalina Island) (Fig. 12.1) (Heed 1978; Ruiz et al. 1990). In the mainland Sonoran Desert and in Baja California *D. mojavensis* is sympatric with its sister species, *D. arizonae*, a generalist cactophile known to utilize the same hosts as *D. mojavensis* in addition to the cina cactus (*S. alamosensis*) (Fellows and Heed 1972). In fact, the presence of *D. arizonae* across Baja California appears to be a relative recent observation. Field collections in the

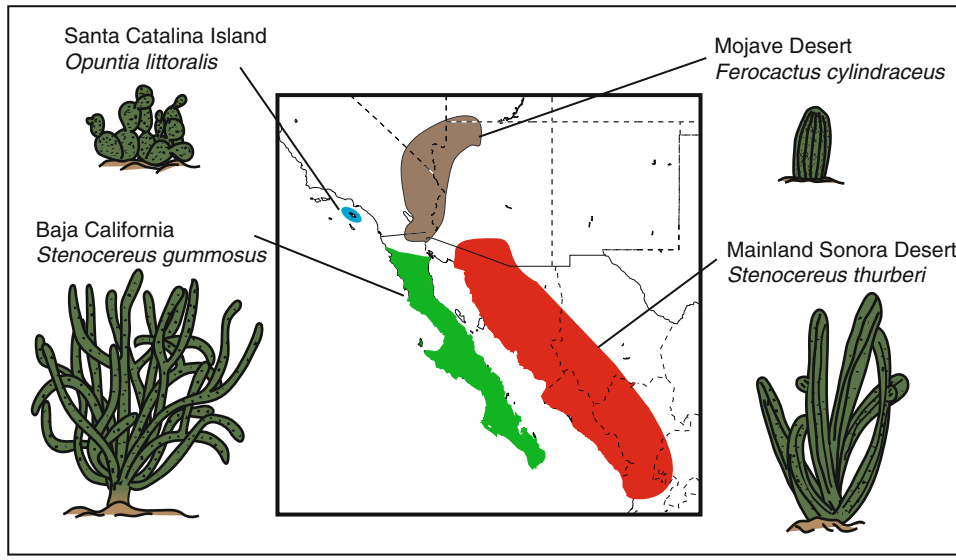

Fig. 12.1 Distribution and cactus host use of the four *D. mojavensis* host races

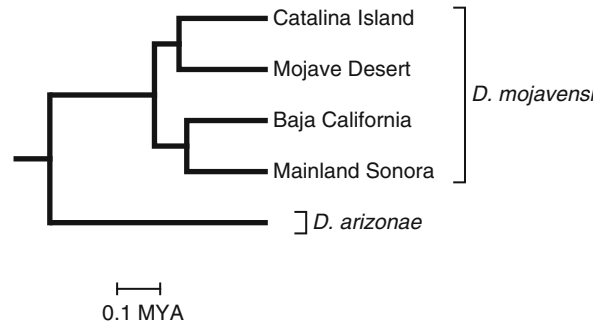

Fig. 12.2 Phylogenetic relationship of the four *D. mojavensis* host races. Population and species relationship and divergence times estimates based on previous molecular studies (Smith et al. 2012; Matzkin 2008; Machado et al. 2007; Reed et al. 2007)

1970s and 1980s rarely observed *D. arizonae* in Baja California, and when collected, all localities where in the Cape region of the peninsula (Heed 1982). Currently, *D. arizonae* can be collected across the peninsula and into Southern California, USA (Reed et al. 2007; Matzkin pers. obs.).

Drosophila mojavensis was originally collected by Warren Spencer from necrotic barrel cactus in the Mojave Desert of California (Patterson and Crow 1940; Spencer 1941). *Drosophila mojavensis*, *D. arizonae* and their sibling species *D. navojoa*, also a cactophile, are all members of the mulleri complex, which spans South and North America (Wasserman 1962, 1982). Cytologically, *D. mojavensis* and *D. arizonae* differ by inversions in the X, second and third chromosomes (Muller elements A, E and B, respectively) (Wasserman 1962). Polymorphic inversions in the second and third chromosome exist both within and between populations of *D. mojavensis*, with the greatest karyotypic diversity found within the Baja California population (Johnson 1980; Mettler 1963). On the basis of the cytological data it was originally proposed that the Baja California peninsula was likely the location of origin of *D. mojavensis* (Ruiz et al. 1990; Johnson 1980). Following its divergence from *D. arizonae*, *D. mojavensis* colonized and subsequently shifted cactus hosts in mainland Sonora, Mojave and Catalina Island. The levels and pattern of sequence variation in *D. mojavensis* largely support this model of evolution (Machado et al. 2007; Matzkin 2008; Reed et al. 2007).

The first molecular phylogenetic analysis was based on the alcohol dehydrogenase (*Adh*) paralogs and estimated the divergence time of *D. mojavensis* and *D. arizonae* to be approximately 4 MYA (Russo et al. 1995), subsequent population genetics analysis of the same loci demonstrated a more recent divergence time of approximately 1 MYA (Matzkin 2004; Matzkin and Eanes 2003). Further population genetic analysis of nuclear and mitochondrial genes suggest an even more recent divergence time of less than 0.5 MYA (Matzkin 2008; Reed et al. 2007). The relationship of the four host populations has been examined using both cytological and molecular data. Recently, given the level of morphological and molecular differences, the host races of *D. mojavensis* have been described as subspecies (Pfeiler et al. 2009). Evidence from multiple nuclear markers spanning all chromosomes suggests that soon after the establishment of *D. mojavensis*, the species diverged into two clades, the southern populations (Baja California and Sonora) and the northern populations (Catalina Island and Mojave Desert) (see Fig. 12.2) (Machado et al. 2007; Matzkin 2008). The relationship between the host populations is slightly altered when utilizing either only X-linked (Smith et al. 2012) or mitochondrial genes (Reed et al. 2007), although all analyses of genetic diversity support the placement of the Baja California population as the center of diversity of the species. One possibility for the slight discrepancy is the fact that the different modes of inheritance (e.g. uniparental vs. diparental) between genes can strongly influence their effective population size and therefore the pattern of evolution (Chesser and Baker 1996). Sex-biased dispersal would also affect levels of variation between uniparental and diparental inherited

loci (Chesser and Baker 1996), although, in the population studied (Sonora) no difference in dispersal was observed to occur between the sexes (Markow and Castrezana 2000). The ongoing sequencing of the genomes from all *D. mojavensis* host populations will provide a better understanding of the history and relationship among them (Matzkin unpub.)

12.2.2 Ecology

The different cacti utilized by each of the host races offers distinct biotic and abiotic environments to the resident *D. mojavensis*. Much of the chemical composition of the necrotic cactus is a function of both the host plant as well as the resident microflora (Fogleman and Starmer 1985; Starmer 1982a, b; Starmer et al. 1990). It is the bacterial and yeast communities found in the cactus necrosis that are instrumental in setting up the chemical environment for the flies (Starmer et al. 1986). The greatest microflora and chemical similarities are between organ pipe and agria, both columnar cactus species (Kircher 1982; Starmer and Phaff 1983). Cactus hosts can differ in a variety of compounds such as triterpene glycosides, unhydrolyzed glycosides, sterol diols, free fatty acids, sugars, many volatiles and in the case of *Opuntia* sp., alkaloids (Fogleman and Abril 1990; Kircher 1982; Starmer and Phaff 1983; Meyer et al. 1980). The compounds associated with cactus necroses have been shown to be detrimental, even lethal to other non-cactophilic species and in certain cases deleterious to non-native cactophilic *Drosophila* (Fellows and Heed 1972; Kircher et al. 1967).

In addition to the chemical composition, there are distinct differences in the physical properties of the cacti and their necroses (Etges 1989; Mangan 1982). The total size of the plant is positively correlated with the persistence and biomass of the necrosis, but negatively correlated with the density of the necroses in the desert landscape (Breitmeyer and Markow 1998). This would suggest that individuals that utilize necroses from small plants would have an easier task in discovering oviposition sites, but such sites would be available to adults and developing larvae for a shorter period of time. In contrast, individuals utilizing larger host would need to travel longer distances to locate a potential oviposition site. With respect to *D. mojavensis*, *Opuntia* cladodes (cactus pads) are significantly smaller than the arms of an organ pipe or agria cactus, which would influence the evolution of life history characters such as developmental time, dispersal rate, starvation and desiccation resistance and chemosensory behavior. Furthermore, abiotic factors such as the thermal environment differ across the populations, and data suggests that the genetic mechanisms underlying resistance to thermal stress might be distinct in each of the populations (Krebs and Thompson 2005). The many biotic and abiotic differences in the ecology of the four *D. mojavensis* host populations have influenced their evolutionary trajectory.

12.3 Genetic Variation and Population Genetics

The environmental conditions experienced by the *D. mojavensis* host races have shaped many aspects of their biology. Genetic variation in life history, morphological, physiological and behavioral characteristics exist across the populations. Furthermore, for several of these characters, there exists a significant interaction with environmental variables.

Many life history comparisons have involved the Baja California and Sonora populations, showing differences in developmental time and adult size (thorax length) between the two populations (Etges 1990, 1998; Etges et al. 2010). Thorax length is a significant life history trait, given its correlation with other characteristics such as flight performance, stress resistance, ovariole number (which is correlated with lifetime fecundity) and mating success (Markow and Ricker 1992; Azevedo et al. 1998; Hoffmann et al. 2001; Mangan 1978). Several morphological and pigmentation differences have been identified across all four host races, most notably divergence in features of the male genitalia such as the shape of the aedeagus

(Richmond et al. 2012; Pfeiler et al. 2009). Overall, *D. mojavensis* is highly resistant to water stress relative to other Drosophila (Gibbs et al. 2003; Gibbs and Matzkin 2001; Matzkin et al. 2009), but yet significant differences in desiccation resistance exist between Catalina, Baja California and Sonora populations (Rajpurohit et al. 2013; Matzkin et al. 2007). Furthermore, interpopulation differences extend to the composition of the hydrocarbons in the fly cuticle. The composition of these hydrocarbons is distinct between Baja California and Sonora populations and, although composition could be influenced by host utilization and temperature, it also affects courtship behavior both within and between populations (Havens and Etges 2013; Markow and Toolson 1990; Etges and Jackson 2001). Courtship behavior differences exist between several of the host races, which contributes to a reduction in gene flow (Markow et al. 1983; Etges et al. 2006).

Utilization of alternative cactus hosts can elicit negative fitness effects. Development of flies from Baja California and Sonora populations on non-native hosts (necrotic agria or organ pipe, respectively) results in significant life history consequences, affecting such characters as thorax length and developmental time (Etges 1990, 1993, 1998). Larval viability can be drastically reduced when Sonora flies develop in necrotic agria or cina (Matzkin and Markow 2013; Bono and Markow 2009). These viability differences are amplified when *D. mojavensis* utilize more chemically distinct cactus hosts (Fellows and Heed 1972). For example, relative to organ pipe or agria, the larval viability of *D. mojavensis* is 2 % in the senita cactus (*L. schottii*), a Sonoran and Baja California plant with high levels of alkaloids (Fellows and Heed 1972).

12.3.1 Candidate Gene Studies

Fermentation by the resident yeast communities largely contributes to the volatile concentration variation across cactus hosts (Fogleman 1982; Heed 1982; Kircher 1982; Vacek 1979). The cactus-specific substrates used in the fermentation process affect the concentration and composition of many of the volatiles such as alcohols. Relative to the organ pipe cactus (Sonora), necroses of agria (Baja California) contain relatively greater levels of 2-propanol than 1-propanol (Heed 1978; Kircher 1982; Starmer et al. 1986; Vacek 1979). In *Drosophila*, Alcohol Dehydrogenase (ADH) is a major pathway for the metabolism of small alcohol molecules (e.g. ethanol) (Chambers 1988). In *D. mojavensis* the *Adh* locus is duplicated, having a larval and adult ovarian tissue expressed locus (*Adh-1*) and a late larval stage and adult (non-ovarian tissue) expressed locus (*Adh-2*) (Batterham et al. 1983; Atkinson et al. 1988). Population genetic analyses date the duplication event to approximately 4 MYA (Matzkin 2004). Earlier studies have shown the presence of two major allozyme alleles (Fast and Slow) at the *Adh-2* locus. While in the Sonora population the Slow allele is at high frequency (>90 %), in Baja California the Fast allele is most frequent (>90 %) (Heed 1978). Resistance to specific alcohols is associated with *Adh-2* genotype, with *Adh-2* Fast homozygotes having increased resistance to 2-propanol relative to *Adh-2* Slow flies (Heed 1978; Starmer et al. 1977). Subsequent studies have shown that the mutation responsible for the Fast/Slow allozyme class (serine to arginine change at residue 28) is associated with as many as four other amino acid substitutions (Matzkin 2004; Matzkin and Eanes 2003). These amino acid differences between allozyme class alleles confer significant substrate specificity differences, with the ADH-2 Fast allele having greater activity on 2-propanol relative to 1-propanol, matching the alcohol concentration of the cactus necrosis in which the *Adh-2* Fast allele is commonly found (Matzkin 2005).

The different metabolic environment experienced by a larval and adult expressed ADH paralogs has distinctly shaped their evolution. Studies using *D. melanogaster* have shown that the control of metabolic flux of ADH in larvae is significantly greater relative to when expressed in adult tissues (Freriksen et al. 1991, 1994; Middleton and Kacser 1983). In any given pathway, the effect of activity changes of a single

enzymatic step on the overall metabolic rate or flux through that pathway is known as the flux control coefficient (Kacser and Burns 1973). Activity perturbations of an enzyme with a high flux control coefficient will produce greater changes on the overall metabolic flux of that pathway relative to an enzyme with little control.

Interestingly, *D. melanogaster* lacks a similar *Adh* duplication, although it produces two distinct transcripts (larval and adult), which resemble the expression pattern of *D. mojavensis* paralogs (*Adh-1* and *Adh-2*, respectively) (Benyajati et al. 1983; Savakis et al. 1986). In the lineage leading to *D. mojavensis*, the larval/ovarian expressed paralog (*Adh-1*) is under positive selection, unlike what is observed for *Adh-2* (Matzkin 2004; Matzkin and Eanes 2003). Furthermore, several amino acid substitutions have occurred between the *Adh* paralogs and are responsible for the observed substrate specificity and kinetic differences between the genes (Matzkin 2005). The non-overlapping expression pattern and functional differences of the *Adh* paralogs in *D. mojavensis*, coupled with the expression pattern of *Adh* in species with a single copy, strongly supports a subfunctionalization model of evolution for the *D. mojavensis* paralogs (Force et al. 1999; Hughes 1994; Lynch and Force 2000).

12.4 *Drosophila mojavensis* in the Genomic Era

The sequencing, assembly and annotation of the *D. melanogaster* genome was a major leap in the understanding of the genetics and evolution of a species in which a tremendous amount of information was already known (Adams et al. 2000). This was later followed by the genome sequencing of a relatively distant species, *D. pseudoobscura* (Richards et al. 2005). These accomplishments lead to the subsequent genome sequencing and comparative analysis of ten additional *Drosophila* species (Drosophila 12 Genomes Consortium 2007). Together these 12 species encompassed a wide breadth of the genus, with nine members of the Sophophora subgenus, and three from the Drosophila subgenus, including *D. mojavensis*.

Although a cDNA microarray was developed for *D. mojavensis* prior to the genome sequencing (Matzkin et al. 2006), knowledge of the genome allowed the construction of complete transcriptome oligonucleotide microarrays (Bono et al. 2011; Matzkin 2012; Matzkin and Markow 2009, 2013; Rajpurohit et al. 2013; Smith et al. 2013). Additional tools for *D. mojavensis* include a BAC library and the availability of transgenic stocks (Song et al. 2011; Holtzman et al. 2010). Although not as extensive as those available for *D. melanogaster*, the *D. mojavensis* transgenic stocks could be used to create null alleles via RNA interference to begin to identify the role of candidate loci in host adaptation. The expanse of ecological information of *D. mojavensis* and the addition of genomic and transcriptomics tools vastly increased the power of this system to help answer many fundamental questions in biology such as the genomic basis of adaptation, the role of local ecological adaptation in speciation and the genomic basis of the evolution of reproductive incompatibilities.

12.4.1 Host Adaptation

The four chemically distinct hosts of *D. mojavensis* have influenced the evolution of the populations at many levels. The genome level changes include structural (e.g. chromosomal inversions), coding sequence, and transcriptional changes. One clear advantage of the *D. mojavensis* system is the vast ecological information that has been previously gathered, which allows for genomic analyses in the ecological context of the species. In the case of *D. mojavensis* the necrotic cactus is a major component of its ecology. The creation of ecologically realistic breeding substrates using lab-generated cactus necroses incorporating the natural microflora has been extensively used in many prior life history studies (Etges 1989, 1990, 1993; Etges and Heed 1987). Similarly, recent microarray studies in *D. mojavensis* allow flies to develop in lab-generated necrotic cactus also including the natural yeast and bacteria

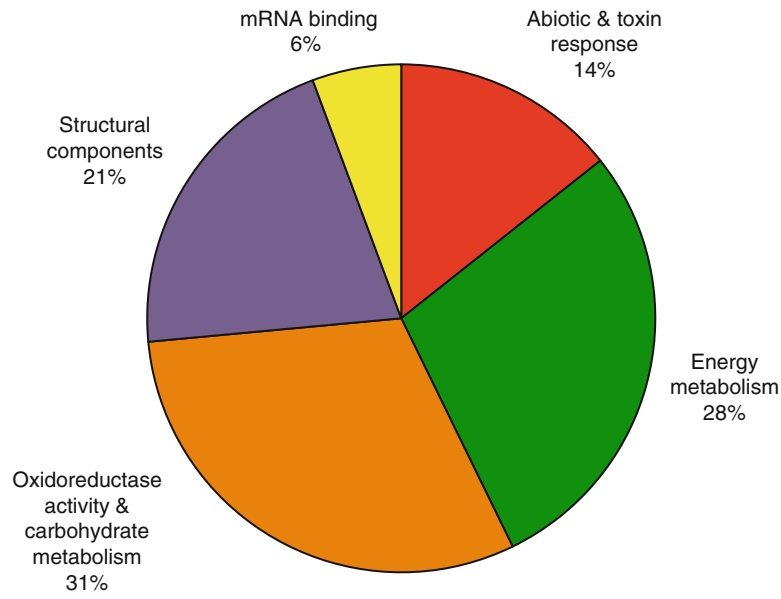

Fig. 12.3 Overrepresented (FDR < 0.05) molecular function and biological process gene ontology categories of differentially expressed genes (Data from Matzkin (2012) examining gene expression of third instar larva from nine isofemale lines from Sonora when reared in either necrotic organ pipe (native host) or agria)

microflora (Matzkin 2012; Matzkin et al. 2006; Rajpurohit et al. 2013; Smith et al. 2013).

In Matzkin et al. (2006) and Matzkin (2012) *D. mojavensis* larvae from either Baja California or Sonora were exposed to necrotic agria or organ pipe cactus. Development and exposure to necrotic cactus elicited a complex transcriptional response, with a variety of loci being differentially expressed (Matzkin 2012; Matzkin et al. 2006). The differentially expressed genes correspond to several gene ontology groups, including xenobiotic metabolism and detoxification (Fig. 12.3). Among the xenobiotic metabolism genes, Glutathione *S*-transferases, Cytochrome P450, and UDP-glycosyltransferase were modulated in response to cactus use (Table 12.1). In other insect systems, members of these three gene families have been known to play a central role in detoxification (Luque and O'Reilly 2002; Ranson and Hemingway 2005; Ranson et al. 2001; Feyereisen 2005; Li et al. 2007).

Although the ability to modulate gene expression in response to environmental change would be advantageous, over evolutionary time constant exposure to an environment, such as a host shift, may produce fixed expression differences (Waddington 1953; West-Eberhard 2003). There are a number of genes whose expression difference appear to be fixed when comparing across the *D. mojavensis* host races (Matzkin and Markow 2013). Recently, Matzkin and Markow (2013) examined the expression profile of third instar larvae reared in media lacking cactus compounds (i.e. standard banana media). In addition to detoxification genes, genes associated with metabolism were differentially expressed across the cactus host races. In fact, these metabolic genes included a large proportion of central metabolism enzymes located both at and outside branch points (Fig. 12.4). Branch enzymes are important control points of flux through pathways (LaPorte et al. 1984). For example, the activity of the enzyme Glucose-6-dehydrogenase (G6PD, see Fig. 12.4) not only influences flux through the pentose shunt, but given that its substrate (glucose-6-phosphate) is used by other enzymes (PGM, HEX and PGI), it also could modulate flux through those other pathways. Given the greater control of flux of branch point enzymes, it would be expected that these enzymes be involved in adaptation (Eanes 1999; Flowers et al. 2007; Rausher 2013). This suggests that the nutritional differences between hosts could have influenced flux through this pathway. Further analysis is needed to examine the functional consequence of these gene expression differences.

Table 12.1 Summary of known detoxification genes that are differentially expressed in response to cactus utilization

D. mojavensis annotation	*D. melanogaster* ortholog	Gene name	Gene family
GI16623	CG17523	GstE2	GST
GI16624	–	GstE2b	GST
GI19388	CG17522	GstE10	GST
GI20124	CG17534	GstE9	GST
GI24379	CG10045	GstD1	GST
GI23193	–	GstD1b	GST
GI23196	CG17639	CG17639	GST
GI10234	CG9716	Cyp313b1	P450
GI13002	CG33503	Cyp12d1-d	P450
GI16117	CG3656	Cyp4d1	P450
GI16990	CG9964	Cyp309a1	P450
GI18674	CG3540	Cyp4d14	P450
GI18951	CG8859	Cyp6g2	P450
GI20221	–	Cyp9h1b	P450
GI20230	CG13977	Cyp6a18	P450
GI20590	CG8453	Cyp6g1	P450
GI24047	CG14680	Cyp12e1	P450
GI10119	CG4739	Ugt86Dc	UGT
GI10120	CG18578	Ugt86Da	UGT
GI10122	CG4772	Ugt86Dh	UGT
GI14390	CG11289	CG11289	UGT
GI17058	CG13271	Ugt36Bb	UGT
GI17522	CG11012	Ugt37a1	UGT
GI22627	CG6644	Ugt35a	UGT
GI22628	CG6649	Ugt35b	UGT
GI22630	CG6633	Ugt86Dd	UGT

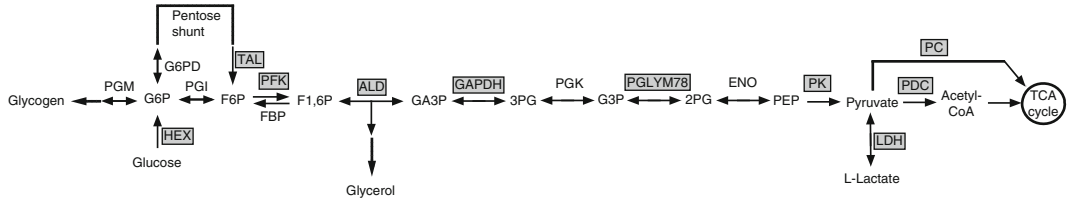

Fig. 12.4 Fixed expression differences in central metabolism genes across the four host races. The enzymes with fixed expression differences: Hexokinase (*HEX*), Transaldolase (*TAL*), Phosphofructose Kinase (*PFK*), Aldolase (*ALD*), Glyceraldehyde 3-phosphate Dehydrogenase (*GAPDH*), Phosphoglyceromutase (*PGLYM78*), Pyruvate Kinase (*PK*), Lactate Dehydrogenase (*LDH*) and Pyruvate Carboxylase (*PC*) are highlighted with a shaded box. The enzyme without fixed expression differences are: Phophoglucose Mutase (*PGM*), Glucose-6-phosphate Dehydrogenase (*G6PD*), Phosphoglucose Isomerase (*PGI*), Phophoglycerate Kinase (*PGK*) and Enolase (*ENO*) (Data from Matzkin and Markow (2013))

In conjunction with transcriptional differences, host adaptation has also been facilitated by changes in the coding region of detoxification genes. Interestingly, there appears to be an association between transcriptional modulations and coding sequence evolution. *Drosophila mojavensis* genes that lack an orthologous call to *D. melanogaster* were found disproportionally among the genes whose expression was significantly affected by cactus host use (Matzkin 2012). A specific example of a gene that fits this pattern is *Glutathione S-transferase D1*

(*GstD1*), a gene whose expression was observed to be affected by alternative host use (organ pipe cactus) in a population from Baja California (Matzkin et al. 2006). Population genetic analysis of *GstD1* suggests that this gene has been under positive selection in the lineage leading to the Baja California and Sonora populations (Matzkin 2008). Among the eight amino acid substitutions occurring in this lineage, two (Leu-7-Gln and His-39-Gln) occur in the active site pocket of the enzyme. Biochemical analysis suggests that these amino acid substitutions result in functional differences between the GSTD1 isoforms from Baja California/Sonora and Catalina Island/Mojave (Matzkin unpub.)

12.4.2 Genomics of Desiccation Resistance

Across the four *D. mojavensis* host races, the chemical composition of the host (including nutritional and toxic compounds) has shaped the pattern of variation at the genomic, transcriptional and functional levels. A major abiotic stress for *D. mojavensis* and other desert endemics is the adaptation to a desiccating environment. Desiccation resistance is significantly greater in *D. mojavensis* compared to other mesic adapted Drosophilids (Matzkin et al. 2009; Gibbs and Matzkin 2001). The increase in resistance is largely due to an overall decrease in the rate of water loss (Gibbs and Matzkin 2001), which is achieved via a decrease in respiratory rate (i.e. metabolic rate) (Gibbs et al. 2003). Analysis of gene expression differences during the desiccating process suggests that key points in central metabolism are modulated in a manner that would suggest a decrease in metabolic flux (Matzkin and Markow 2009). In a recent study, Rajpurohit et al. (2013) observed that in both Baja California and Sonora populations desiccating conditions were associated with the up-regulation of genes associated with the structure of the cuticle and sensory pathways.

12.4.3 Chemosensory Adaptation

Given the deleterious fitness consequences associated with developing in a non-native host, it is predicted that there would be strong selective pressure in *D. mojavensis* to correctly identify a cactus before oviposition. The chemosensory system in *Drosophila* is composed of a number of sensory neurons with transmembrane receptors distributed across the insect's body (Vosshall and Stocker 2007). In adults, odorant receptors are expressed in sensory neurons (ORN) housed in sensilla in the antenna and maxillary palps, while gustatory receptors are expressed in neurons (GRN) not only in the proboscis, but also the legs, wings and ovipositor (Stocker 1994; Vosshall and Stocker 2007). These transmembrane proteins initiate a signal cascade that leads to the perception of taste and smell. Unlike GRN, in ORN, a universal co-receptor (*Or83b*) is expressed which interacts with the neuron-specific odorant receptor (Benton et al. 2006). Overall, both odorant and gustatory receptors can specialize to interact with only a subset of ligands (Laissue and Vosshall 2008; Hallem and Carlson 2006).

Drosophila mojavensis females have the ability to assess host type prior to oviposition. Although there is some variation across studies, overall there is a large amount of genetic variation for oviposition preference. In several populations there still appears to be a preference for the ancestral agria cactus, even though agria is not present in all locations (Lofdahl 1985, 1986; Newby and Etges 1998). In contrast, there are also examples of adult preference to its native host, such as in the Mojave population (Newby and Etges 1998). Prior expression studies examining interpopulation and host-induced changes in *D. mojavensis* focused on the larval stage, and, interestingly, both odorant and gustatory receptors were significantly differentially expressed (Matzkin 2012; Matzkin and Markow 2013). *Drosophila mojavensis* larvae have been shown to selectively feed on certain cactophilic yeast species while ignoring others (Fogleman et al. 1981). Therefore,

it is quite possible that larvae, using their chemosensory system (including odorant and gustatory receptors), are selecting microhabitats within individual cactus necroses. Preliminary evidence shows that some *D. mojavensis* odorant receptors have been under positive selection (Matzkin unpub.), and thus might be candidates for involvement in the location of host-specific microhabitats. Further functional and behavioral studies are needed to fully understand the consequence and role of these receptors in cactus host adaptation.

12.5 Conclusion

The technological and computational advances of recent years have not only revolutionized the study of model laboratory organisms but have dramatically expanded our choice of organisms. These new methods have allowed for the investigation of ecologically defined species, those species in which ecological information is known and genomic information could be analyzed in an explicit ecological context.

In *D. mojavensis* genomic and transcriptomic tools have allowed us to peer into the genomic mechanisms of the adaptive process. We have seen how the transcriptome has been shaped by the various host shifts that have occurred in the history of *D. mojavensis*. These changes include the modulation and fixed expression pattern of a wide variety of genes, some of which we would have expected to be involved in the host adaptation process, such as detoxification, chemosensory and metabolic genes. The few analyses of candidate genes have shown how selection has shaped the pattern of genetic and functional variation, and its possible link to performance in the field. More studies are necessary to make the connection between the genetic, functional and life history variation in the ecological context of this fly. Furthermore, genome-wide surveys of sequence and structural variation will help us elucidate large-scale changes in the *D. mojavensis* populations and determine for example the presence of genomic islands of divergence between them (Turner et al. 2005, 2008). Several of these questions will begin to be answered using the previously sequenced Catalina Island genome (Drosophila 12 Genomes Consortium 2007) and the recently sequenced Baja California, Mojave and Sonora *D. mojavensis* genomes (Matzkin unpub.). Furthermore, meta-genomic analysis of the microflora of both the cactus necroses and the *D. mojavensis* gut, could shed light into the role of these interspecific interactions and host adaptation. Finally, a future aim is to examine links between local adaptation occurring in the four cactus host races, with their behavioral and genetic divergence and the ongoing pattern of incipient speciation (Nosil 2012).

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