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Evolutionary genomics of host plant adaptation: insights from *Drosophila*

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Variation in gene expression in response to the use of alternate host plants can reveal genetic and physiological mechanisms explaining why insect-host relationships vary from host specialism to generalism. Interpreting transcriptome variation relies on well-annotated genomes, making drosophilids valuable model systems, particularly those species with tractable ecological associations. Patterns of whole genome expression and alternate gene splicing in response to growth on different hosts have revealed expression of gene networks of known detoxification genes as well as novel functionally enriched genes of diverse metabolic and structural functions. Integrating trancriptomic responses with fitness differences and levels of phenotypic plasticity in response to alternate hosts will help to reveal the general nature of genotype-phenotype relationships.

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Introduction

Comparisons within and among plant feeding insect species that vary in the range of host plants that they feed and lay eggs on have been central to addressing behavioral and physiological mechanisms underlying host plant-insect specificity [1–4]. In this review, I focus on how insect-host plant relationships have been shaped at the genomic level, centering on transcriptome-wide responses to current and alternate hosts that 1) help explain how gene expression differences facilitate the use of different plant hosts, 2) elucidate the role of host plant specialization on insect divergence and speciation, 3) describe how carefully designed and replicated laboratory experiments have helped uncover host-related transcriptional differences, and 4) reveal the degree of transcriptional plasticity as host diversification evolves [5]. An ultimate goal is to dissect the role of gene network variation and gene expression influencing fitness differences that determine the use of different plant hosts.

Better understanding of genotype-phenotype relationships requires more than studies of transcriptome expression in different environments, yet patterns of differential gene expression are excellent proxies of how population level trancriptomic responses to different host plants can be tied to differences in fitness. Downstream studies of tissue-specific proteomes, metabolomes, and so on, will facilitate connections to phenotypic expression [6] of host plant-related fitness differences, but transcriptome studies remain widely used [7]. Further, anonymous interrogation of transcriptomes without regard to particular candidate genes should reveal a wider spectrum of gene families and networks that would have otherwise been ignored. While numerous studies of xenobiotic detoxifying cytochrome P450 gene families [8••,9,10] and other detoxification enzymes including hydroxylases, transferases, and so on [11,12] have shown how regulation of detoxification gene expression responds to particular plant compounds, more recent transcriptome studies have revealed multigene transcriptional responses including previously unknown genomic responses. An obvious limitation is the incomplete annotation of most genomes making functional and ecological interpretation much more difficult.

A step-wise approach to understanding transcriptomic responses to different host plants includes host finding behaviors, neurological/sensory responses to plant attractants, that is, chemical, morphological, color, and so on [13–15], physiological responses to host plant feeding and oviposition, responses to host plant tissue quality and secondary compounds, and connecting offspring fitness with differences in gene expression. Unfortunately, few studies have provided integrative understanding at all levels, with emphasis on transcriptomic variation on offspring exposed to different hosts. An immediate implication is the degree to which host use patterns drive population divergence leading towards adaptation, reproductive isolation and ecological speciation [16–23]. Further, transcriptome responses to alternate host plants are essential organismal aspects of phenotypic plasticity [24–28], although inferring the adaptive value of plastic responses to new environments remains difficult [29,30]. Nevertheless, a detailed understanding of

transcriptome responses to novel/alternate host plants will lead to a better understanding of how potential plastic responses play a role in host plant use, the evolution of host plant specialization or generalism, and host plant-mediated divergence. Here, I will review studies of insect transcriptome responses to alternate host plants, exploring how they reveal the roles of known detoxification genes and identify new candidates through the analysis of functional groups of annotated genes identified by gene ontology (GO) analyses [31].

Genomics of host plant use

Chemically related host plants may be easier to add to the menu of oligophagous and polyphagous insects given their evolutionary history of host use [32,33]. Several model systems, including species of *Drosophila*, have provided insights into the roles of gene networks allowing detoxification or sequestering of host allelochemicals and toxins. Sequenced genomes of *Drosophila* species depend on the *D. melanogaster* reference genome for homology and gene annotation, and the associated wealth of comparative and functional genetic resources. These resources should facilitate more comprehensive transcriptome analyses of host plant use. However, *Drosophila* are typically saprophytic, feeding and breeding on decomposing, fermenting plant, fruit, and fungus tissues that have shaped toxin gene evolution [34•], so these cases may not closely align with herbivorous species. Here, I review genomic and transcriptomic analyses of mycophagous *Drosophila*, cactophilic *Drosophila*, the *D. sechellia-Morinda* fruit association, and Scaptomyzid leaf-miners using species of Brassicaceae, the best drosophilid examples of host plant mediated transcriptome variation.

Mycophagous *Drosophila*

Mushrooms contain a large number of toxin classes, including α-amanitin in toxic *Amanita* species [35]. There are 17 known species in the *D. immigrans-tripunctata* radiation that are tolerant of α-amanitin [36], a potent inhibitor of eukaryotic RNA polymerase II. Mushroom specialization and α-amanitin tolerance are ancestral in this group [37,38] and tolerance has been lost in one species, *D. quinaria*. Mushroom and non-mushroom breeding species showed no differences in α-amanitin-RNA pol II binding [37,39], implying other mechanisms of detoxification and/or sequestration must be responsible. Jaenike [35] proposed that α-amanitin tolerant fly species had evolved to escape nematode parasitism: these nematodes are not α-amanitin tolerant [40].

Absent genetic analyses of α-amanitin tolerance in mycophagous *Drosophila* species, contrasting patterns of genotype-phenotype association and gene expression have been shown in different strains of *D. melanogaster* [reviewed in Ref. 41••]. After the discovery of the α-amanitin tolerant mutant of *D. melanogaster*, a lab-induced RNA pol II mutant [42], screening for naturally occurring α-amanitin resistant populations revealed three Asian *D. melanogaster* lines that showed at least 2 dominant third chromosome loci were involved [43]. Study of another population revealed two loci mapped to the same regions as in [43] involved with α-amanitin tolerance, Multidrug resistance 65 (*Mdr65*) and Protein kinase C98E (*Pkc98E*) [44]. Microarray analysis of one of the original Asian lines revealed no upregulation of *Mdr65* or *Pkc98E*, but suggested involvement of several different blockage, detoxification, and peptidase cleaving gene groups [45]. In particular, 3 cytochrome P450 genes, *Cyp6a2*, *Cyp12d1-d*, and *Cyp12d1-p* were upregulated over 200 X in larvae fed α-amanitin. Mitchell *et al.* [46] then carried out a genome wide association study (GWAS) with ∼180 of the Drosophila Genetic Reference Panel (DGRP) lines in order to fine map α-amanitin resistance. Their results showed no overlap with their microarray study of the Asian population, but instead identified associated variants in the genes *tequila* (*teq*), *megalin* (*mgl*), and *widerborst* (*wdb*). These genes are thought to be associated with the TOR pathway, a repressor of autophagy and endocytosis, suggesting lysosome/cytoplasmic elimination of α-amanitin. While these results suggest convergent evolution of α-amanitin detoxification mechanisms in *D. melanogaster* and reinforce the absence of modified RNA pol II as a factor, future comparative genomic and transcriptomic analyses with mycophagous *Drosophila* species are necessary to understand α-amanitin metabolism.

Cactophilic *Drosophila*

Including about one-half of the >100 species in the new world *D. repleta* group that are cactophilic, species of *Drosophila* invaded the cactus niche of fermenting tissues (rots) and fruits of flat leaf *Opuntia* and columnar cacti ca 17 mya [47–50]. While most flat leaf *Opuntia* species contain fewer toxic secondary compounds, columnar cacti have been widely studied because of their repertoire of species-specific secondary compounds including alkaloids, sterol diols, triterpene glycosides, medium chain fatty acids (C_8–C_{18}), and sterols [8••,51–54]. One extraordinary case involves Sonoran Desert populations of *D. pachea*, a species restricted to a single host, senita cactus, *Lophocereus schotti*, because of an altered ecdysone biosynthetic pathway and tolerance of senita alkaloids [55,56]. *D. pachea* cannot convert cholesterol to 7-dehydrocholesterol; instead it uses lathosterol, a Δ^7 sterol produced by senita, due to several amino acid changes in the neverland oxygenase gene (*nvd*). Thus, *D. pachea* is a single host cactus specialist due to ca 2–4 *nvd* gene mutations [57••]. Another Sonoran Desert endemic species, *D. mettleri*, is tolerant of allelochemicals in several cacti, but oviposit only in soil soaked with cactus rot exudates [58] that can contain 25 X the amounts of alkaloids as fresh tissues. Larval *D. mettleri* metabolize the tetrahydroisoquinoline alkaloid carnegine from saguaro cacti, *Carnegiea gigantea*, and lophocerine in senita by upregulation of P450 genes [59]. These include *Cyp28A1* induced by senita cactus

[60], and *Cyp4D10* induced by saguaro cactus [61]. RNA-seq studies of *Opuntia* and saguaro using populations of *D. mettleri* revealed differential expression of a host of P450 genes, carboxylesterases, one GST and six UGT-glycosyltransferases [62].

Species of cactophilic desert *Drosophila* using more than one host have also revealed population, species, and host specific differences in transcriptome responses. Sonoran Desert populations of *Drosophila mojavensis* use different host cacti in different parts of their range, originating in Baja California where they use agria cactus, *Stenocereus gummosus*, and several other secondary hosts. After colonizing northwest Mexico ca 250 kya by shifting to organ pipe cactus, *Stenocereus thurberi*, they later invaded what is now the Mojave Desert by shifting to barrel cactus, *Ferocactus cylindraceus* [63,64]. Populations of *D. mojavensis* inhabit Santa Catalina Island, California using *Opuntia* cactus with likely Baja California origins [65]. Most interest has centered on the Baja California-Sonora, Mexico host plant shift as this has been accompanied by adaptation to these hosts and evolution of cactus-influenced premating reproductive isolation [66–68].

Study of P450 gene family and glutathione transferase gene evolution first suggested potential mechanisms of the agria to organ pipe cactus host switch, as detoxification of cactus secondary compounds is thought to play a major role in host plant use by cactophilic *Drosophila*. Induction of P450 monooxygenases has been observed in larvae and adults that both feed on fermenting cactus tissues, but was far larger in adults [69]. Seven adaptive amino acid substitutions in Baja California/mainland populations in *D. mojavensis* glutathione *S*-transferase D1 (*GstD1*) suggested adaptive protein evolution in response to agria and organ pipe cactus [11]. Third instar larvae from mainland inbred lines derived from a population using organ pipe cactus exhibited transcriptome variation when reared on fermenting agria versus organ pipe cactus in the lab [70]. A total of 2066 genes were differentially expressed in response to feeding on these cactus hosts [FDR $P < 0.01$; 71], involving ca >13% of coding genes in this population of *D. mojavensis* [70,72]. Annotated genes fell into 16 biological processes categories and 5 different gene ontology (GO) groups (clusters of overexpressed genes with inferred biological functions) including oxidoreductase/carbohydrate metabolism, energy metabolism, abiotic/toxin response, structural (chitin, cuticle) and mRNA binding. The detoxification group included 25 homologs in the *Gst*, P450 and *UGT* (UDP-glucuronosyltransferases that catalyze the glucuronidation of toxins) gene families.

Thus, transcriptome responses of *D. mojavensis* to fermenting agria and organ pipe tissues have revealed a comprehensive portrait of gene expression pointing to a much larger repertoire of gene function than detoxification gene families alone. A series of microarray experiments with *D. mojavensis* revealed adult transcriptome variation in response to desiccation and temperature variation [73,74], effects of egg-to-adult development time on adult gene expression [75], and transcriptome variation over the life cycle [76•]. All experiments used replicate outbred populations reared on fermenting agria and organ pipe cactus tissues, and in all but the latter study, adults were reared to sexual maturity on artificial media because previous studies revealed significant carryover effects of cactus rearing on adults [66,77]. In adults from two Baja California and two mainland populations exposed to 0, 9, or 18 hours of low humidity, 18 genes were differentially expressed due to cactus rearing. Of these, 16 genes were overexpressed in organ pipe reared flies and were enriched for cation function and anion transport activity [73]. In addition to humidity effects, ANOVA revealed hundreds of genes that were also differentially expressed due to interaction effects with cactus, that is, population X cactus, desiccation X cactus interactions, and so on, emphasizing how fully replicated experimental designs are needed to uncover more subtle interaction effects with host plants affecting transcriptome variation.

In adults exposed to 15, 25, and 35°C for 12 hours, 2457 genes were differentially expressed when pre-adult stages were reared either on replicate cultures of agria and organ pipe cacti (FDR $P < 0.01$), with 2094 transcripts overexpressed in agria-reared flies. These transcripts were enriched for 18 clusters of GO terms, including peptidases, secondary metabolism, and six mitochondrial function clusters. GO analysis of 363 genes overexpressed in organ pipe-reared flies included five clusters enriched for DNA repair, DNA replication, chromatin assembly, and ATP binding. Again, there were many differentially expressed genes due to interaction effects such as population X cactus, temperature X cactus, and so on, revealing the manifold effects of host cactus differences on gene expression [74].

Cactus substrates also had significant stage/age and population-specific influences over the life cycle on gene expression in *D. mojavensis* [76•]. Mainland, first instar larvae showed significant differential expression of hundreds of genes where organ pipe reared flies showed upregulation of cuticle/chitin and olfactory reception enriched gene clusters compared to agria-reared flies. Agria cactus caused upregulation of 631 genes significantly enriched for 14 different GO categories annotated for transport, cell metabolism, protein synthesis and transport, OXPHOS processes, and so on. Compared to first instar larvae, far fewer genes differed in expression due to cactus in second instars, third instars, and early pupae. In contrast, most differentially expressed genes were observed in eggs and pupal stages due to cactus rearing in a Baja California population, where agria cactus caused upregulation of ubiquitin conjugation pathway

genes involved with proteolysis and genes enriched for cuticle structure. Thus, transcriptome responses to alternate host cacti were stage-specific and population-specific from egg to pupal stages.

Organ pipe and agria cactus had contrasting influences on transcriptome expression and patterns of alternate splicing in adult female *D. mojavensis* [5,78]. With ages pooled (3–24 days old), organ pipe cactus caused increased expression of 66 genes enriched for two GO clusters including a small group of gated ion channel genes associated with neurotransmission, circadian rhythm, and courtship behavior. As organ pipe cactus causes increased male mating success in mainland *D. mojavensis* and sexual isolation between mainland and Baja California populations, these differences in gene expression suggest candidate gene clusters responsible for host cactus influences on premating isolation [5]. There were significant cactus X population interactions for 302/514 annotated orthologs enriched for iron binding/P450 function and fatty acid synthesis, some of which showed differences in alternate splicing [78]. Thus, cactus substrates caused significant differences in gene expression for fatty acid synthesis, xenobiotic metabolism, and courtship behavior revealing a link between cactus-induced gene expression and reproductive isolation.

In South American cactophilic *Drosophila buzzatii* and *Drosophila koepferae*, evolution of host cactus use has focused on a shift from necrotic *Opuntia* tissues to the columnar cactus *Echinopsis* (*Trichocereus) terscheckii* containing allelochemicals including mescaline, trichocereine, and related phenylethylamine alkaloids [54,79]. As *D. buzzatii* uses both *Opuntia* and *E. terscheckii*, but *D. koepferae* is restricted to the latter host, RNAseq studies were designed to study transcriptome responses to *E. terscheckii* alkaloids by rearing *D. buzzatii* on tissues of both cacti with or without added alkaloids [80•]. In third instar larvae, 3556 genes were upregulated in *E. terscheckii* and 61 were downregulated in *O. sulphurea*, accounting for 28% of all protein encoding genes. Across all treatments, 62 homologs were differentially expressed including cuticular proteins, detoxification (two ADHs, three GSTs and four P450s), oxidation–reduction, development and neurobiological processes, and other genes [80•]. Thus, alternate host cactus use by *D. buzzatii* involved differential expression of nearly a third of all predicted genes, including known candidate detoxification genes.

The *Drosophila sechellia-Morinda citrifolia* association

Endemic to the Seychelles islands, *D. sechellia* is restricted to the fruits of *Morinda citrifolia* that are toxic to other drosophilids [81]. The fruits contain high concentrations of octanoic acid (OA) that *D. sechellia* prefers and is resistant to [82,83]. RNAseq analyses of adults exposed to 0.7% OA revealed 132 differentially expressed genes, including upregulation of transcripts enriched for body morphogenesis and chitin-based cuticle development containing six *Osiris* family genes, and five *Tweedle* genes [84,85••]. One *Osiris* gene, *Osiris 6* (*Osi6*), lies in a previously described QTL for OA resistance [86,87]. Decreased expression by RNA interference of *Osi6*, *Osi7* and *Osi8* in adults and *Osi6* in larvae (all are located in the OA resistance QTL) resulted in altered resistance to OA in *D. melanogaster* [85••,88]. Differential *TwdlY* expression, associated with cuticle development, suggested another mechanism of OA resistance. Downregulated genes were enriched for response to bacteria and antibacterial humoral response including chorion proteins. A recently discovered population of the mainland Africa generalist *Drosophila yakuba* has been found using *M. citrifolia* on the island of Mayotte off the coast of Madagascar. Genome scans comparing this island and mainland population suggested positive selection on *Osiris* and *Tweedle* genes, as well as several serine proteases consistent with convergent evolution of OA resistance by *D. yakuba* [89].

The *Scaptomyza flava-Arabidopsis* system

The genus *Scaptomyza* contains 272 described species [90], including herbivorous leaf mining species that use host plants in the order Brassicales containing inducible defenses [91] such as glucosinolates. These amino acid-derived thioglucosides can increase in concentration in leaves up to 40 X due to insect damage. In *Scaptomyza flava* populations, *Arabidopsis thaliana* is used as a host, in which the glucosinolate biosynthetic pathway has been characterized [92]. RNAseq studies with *A. thaliana* with and without glucosinolates revealed 341 DE *S. flava* transcripts, of which 278 were upregulated and 63 were significantly downregulated in larvae reared on normal versus glucosinolate knockout plant tissues [93]. Of 121 transcripts with homologs in *D. melanogaster*, functional enrichment yielded four significant GO categories including hemolymph coagulation, body morphogenesis (including 4 *Tweedle* homologs), plasma membrane (including 8 *Osiris* genes), and cuticle/chitin structure (including several cuticle protein genes and 3 *Tweedle* homologs). Thus, larval transcriptional responses induced or repressed by dietary glucosinolates share similarities with the OA response in *D. sechellia*. However, this is likely an incomplete picture of the transcriptome because 36% (5,967/16,476) of all *S. flava* transcripts had no homologs in other species [93]. Interestingly, *Osiris* genes were among differentially expressed genes in Lepidopteran larvae feeding on plants versus artificial diets [94]. One paralogous copy of *Osi9* was upregulated in all four Lepidopteran species studied, specifically in the larval gut. This suggests that *Osiris* genes may have ancient, conserved roles in detoxification or resistance, and it emphasizes how the same genetic mechanisms can underlie host use in *Drosophila*.

Conclusions

Causal genetic mechanisms underlying host plant shifts and evolution of host plant specialists/generalists require interrogation of fully annotated transcriptomes expressed in contrasting environments. *Drosophila* species are excellent model systems because of their phylogenetic affinity with *D. melanogaster* and the hope that homology with this completely annotated genome [95] will help to identify gene clusters and networks involved with host plant use. While many candidate detoxification genes show significant patterns of differential expression, *Drosophila* host shifts have revealed manifold transcriptomic responses in other gene families and networks that have provided insights into the connections between host plant use, diversification, and reproductive isolation. Future gene knockdown experiments and improved genome annotation based on these transcriptome analyses will help to resolve the precise genetic mechanisms underlying patterns of host plant use in nature in these and other insects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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