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Transcriptional variation associated with cactus host plant adaptation in *Drosophila mettleri* populations

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Abstract

Although the importance of host plant chemistry in plant–insect interactions is widely accepted, the genetic basis of adaptation to host plants is not well understood. Here, we investigate transcriptional changes associated with a host plant shift in *Drosophila mettleri*. While *D. mettleri* is distributed mainly throughout the Sonoran Desert where it specializes on columnar cacti (*Carnegiea gigantea* and *Pachycereus pringleii*), a population on Santa Catalina Island has shifted to chemically divergent coastal prickly pear cactus (*Opuntia littoralis*). We compared gene expression of larvae from the Sonoran Desert and Santa Catalina Island when reared on saguaro (*C. gigantea*), coastal prickly pear and laboratory food. Consistent with expectations based on the complexity and toxicity of cactus relative to laboratory food, within-population comparisons between larvae reared on these food sources revealed transcriptional differences in detoxification and other metabolic pathways. The majority of transcriptional differences between populations on the cactus hosts were independent of the rearing environment and included a disproportionate number of genes involved in processes relevant to host plant adaptation (e.g. detoxification, central metabolism and chemosensory pathways). Comparisons of transcriptional reaction norms between the two populations revealed extensive shared plasticity that likely allowed colonization of coastal prickly pear on Santa Catalina Island. We also found that while plasticity may have facilitated subsequent adaptive divergence in gene expression between populations, the majority of genes that differed in expression on the novel host were not transcriptionally plastic in the presumed ancestral state.

Keywords: Baldwin effect and genetic assimilation, detoxification, gene expression, genetic accommodation, metabolism, plasticity

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Introduction

Ecological specialization is a fundamental process involved in generating biological diversity (Forister *et al.* 2012). This is exemplified by the remarkable diversity of plant-feeding insects, the majority of which are relatively specialized on a subset of available plant species (Jaenike 1990; Janz *et al.* 2006; Forister *et al.* 2012). The colonization of a novel host exposes insect populations to a variety of selection pressures, with adaptation to the new environment often promoting population divergence (Nosil 2012). Of primary importance is adaptation to host plant chemistry, which involves both behavioural and physiological mechanisms associated with performance on the new host (Jaenike 1990). This is particularly relevant for larvae, as they are relatively immobile and must acquire nutritional resources from plants while also circumventing plant defences.

While the importance of insect adaptation to host plant chemistry has long been recognized, the genetic basis of adaptation to new hosts is not well understood

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(Vogel *et al.* 2014). Previous studies have demonstrated transcriptional plasticity associated with insects feeding on different diets, with Celorio-Mancera *et al.* (2013) suggesting that plasticity in the expression of digestive proteases, ribosomal proteins and detoxification enzymes is particularly common. It is unclear, however, to what degree this transcriptional plasticity may facilitate or constrain adaptive differentiation following a host plant switch. In fact, the role of plasticity in adaptive evolution has been the subject of ongoing debate. On one hand, when organisms are faced with novel environmental challenges (e.g. a new host plant), plasticity could produce phenotypes that are optimal in the new environment (Ghalambor *et al.* 2007). For example, in the context of a host plant switch, transcriptional plasticity in metabolic pathways involved in processing and detoxifying food resources may produce a phenotype that is well matched to the new environment. In such cases, plasticity not only allows the population to persist in the novel environment, but may also constrain adaptive genetic differentiation between populations from ancestral and novel environments (Ghalambor *et al.* 2007). However, plasticity may also facilitate adaptive evolution if a phenotype is produced that is in the same direction as the phenotype favoured by natural selection, but not yet at the optimum (Crispo 2007; Ghalambor *et al.* 2007). In this case, directional selection is expected to further alter the plastic response in the direction of the optimum, resulting in adaptive differentiation (often referred to as the Baldwin effect; Crispo 2007). For example, if upregulation of a gene was initially beneficial in a novel environment but transcription was not at the optimal level, further upregulation of this gene may subsequently evolve. Another possibility is that the elevated transcriptional response becomes fixed regardless of the environment through the process of genetic assimilation (Waddington 1959; Crispo 2007). In this case, the level of transcription in the novel environment does not change, but plasticity is lost. Alternatively, adaptation may involve altered regulation of genes that were not transcriptionally responsive to the new host initially or that were initially regulated maladaptively. Identifying transcriptional changes associated with the early stages of host plant shifts is clearly an important step in understanding plant–insect interactions and the role of transcriptional plasticity in facilitating adaptation. Nevertheless, relatively few studies have examined genomewide transcriptional changes associated with adaptation to novel host plants (Vogel *et al.* 2014).

Drosophila mettleri is a cactophilic fruit fly that uses soil soaked in rotting cactus exudates as its breeding substrate (Meyer & Fogleman 1987). In the Sonoran Desert, *D. mettleri* specializes mainly on saguaro cactus (*C. gigantea*; northern Mexico and southwestern United States) and cardón cactus (*Pachycereus pringleii*; Baja peninsula and isolated areas in northern mainland Mexico) (Fogleman & Danielson 2001), which are columnar cacti from the tribe Pachycereeae. *Drosophila mettleri* is also found on Santa Catalina Island off the coast of southern California, where it mainly utilizes chemically divergent coastal prickly pear cactus (*Opuntia littoralis*) as saguaro and cardón are unavailable (Markow *et al.* 2002; Hurtado *et al.* 2004; Bono *et al.* 2008). Genetic evidence indicates that the *D. mettleri* population on Santa Catalina Island is derived and highly genetically differentiated from populations in the Sonoran Desert (Hurtado *et al.* 2004; Richmond *et al.* 2013). This, coupled with the fact that several species of prickly pear cacti are abundant but not utilized as hosts in the Sonoran Desert, suggests that the ancestors of the Santa Catalina Island population likely utilized columnar cacti. Further evidence suggests that the Santa Catalina Island population has experienced a 'host plant shift', whereby adaptation to the new host is associated with fitness trade-offs on the ancestral host, as opposed to a 'host range expansion', where there are no associated trade-offs (Diegisser *et al.* 2009). Specifically, a previous study demonstrated that Santa Catalina Island larvae had higher survivorship on some prickly pear species relative to Sonoran Desert larvae (although coastal prickly pear was not used in the study), while also exhibiting lower survival on saguaro (Castrezana & Bono 2012).

Survival differences likely at least in part reflect divergent host plant chemistry of columnar and prickly pear cacti. This includes major differences in lipid content, and prickly pear species have much higher amounts of free sugars and water available compared to columnar cacti (Kircher 1982; Fogleman & Abril 1990). In addition, toxicity of columnar cacti and prickly pear is likely to vary qualitatively and/or quantitatively. The main toxins in cardón and saguaro are isoquinoline alkaloids (Kircher 1982), which can be nearly 30 times more concentrated in soaked soil used by *D. mettleri* larvae relative to cactus tissue. Although only volatile compounds (e.g. terpenoids), from *O. littoralis*, have been studied (Wright & Setzer 2014), other species of prickly pear are known to contain novel alkaloids not found in other cacti (Meyer *et al.* 1980; Stintzing & Carle 2005).

Previous research has demonstrated the likely role of detoxification and other metabolic genes in adaptation of *D. mettleri* and related cactophilic *Drosophila* to their hosts. For example, studies in *D. mettleri* have shown reduced survivorship in the presence of the cytochrome P450 inhibitor, piperonyl butoxide, when larvae were reared on food containing cactus compounds (Frank & Fogleman 1992; Danielson *et al.* 1998). Moreover,

Danielson *et al.* (1997, 1998) found induction of specific *D. mettleri* cytochrome P450 genes in response to cactus, including some that were induced by compounds from a variety of cacti and others that responded only to compounds from a specific cactus. Research on host shifts in another cactophilic fly, *Drosophila mojavensis*, has demonstrated functionally significant allelic variation in *alcohol dehydrogenase 2* in populations using different hosts (Heed 1978; Matzkin 2005) and has identified cytochrome P450s, glutathione *S*-transferases (GSTs) and UDP-glycosyltransferases as important gene classes likely involved in cactus utilization (Matzkin *et al.* 2006; Matzkin 2012). Specific genes induced by exposure to host plant compounds in both *D. mettleri* and *D. mojavensis* also show evidence of positive selection, lending more support to their significance in these flies (Bono *et al.* 2008; Matzkin 2008). While most of the focus has been on genes involved in detoxification, some evidence also suggests a role for other metabolic genes in adapting to the nutritional content of host plants. For example, a previous study identified fixed transcriptional differences in several genes involved in central metabolism between *D. mojavensis* populations that specialize on different host plants (Matzkin & Markow 2013).

Differences in plant chemistry and geographic variation in host plant specialization make the *D. mettleri* system especially amenable for studies on the genetic basis of host plant adaptation. We expect that adaptation to chemically divergent hosts in *D. mettleri* has, in part, involved regulatory evolution, particularly in metabolic and detoxification pathways. To examine this possibility, we compare genomewide transcriptional profiles of larvae from Santa Catalina Island and from the Sonoran Desert when reared on saguaro, coastal prickly pear and laboratory fly food. First, we compare gene expression of larvae from each population when reared on their native host vs. laboratory food. This comparison allows us to identify general plastic transcriptional responses when larvae feed on host plants relative to a benign feeding substrate. To investigate how transcriptional variation may contribute to local adaptation, we also compare gene expression between populations to find genes that differ in expression regardless of food type (environment-independent genes) and those that are differentially regulated on saguaro and coastal prickly pear specifically (environment-dependent genes). Given that the Santa Catalina Island population is derived from ancestors that were likely associated with columnar cacti, we also compare transcriptional reaction norms (Aubin-Horth & Renn 2009) of plastic genes in both populations to gain insight into the importance of plasticity during the early stages of a host plant shift.

Methods

Rearing experiment

Flies used in experiments were collected from Santa Catalina Island, CA, and Tucson, AZ (heretofore 'Sonoran'), in April of 2009 (3 isofemale lines from each population). Flies were maintained in the laboratory on rotting cactus of the native host mixed with Formula 4–24, Instant *Drosophila* Medium (Carolina Biological Supply Company). Our experimental design involved rearing larvae from each population on the following: (i) native host, (ii) alternative host and (iii) laboratory fly food (Formula 4–24, Instant *Drosophila* Medium), for a total of 18 samples (2 populations/3 lines per population/3 food types; Fig. 1). Rotting cactus media was prepared for oviposition and larval rearing by inoculating fresh tissue with rotting cactus collected from the field. Tissue was incubated until enough exudates were produced to adequately soak sterilized soil, creating a rearing substrate that mimics the natural environment experienced by *D. mettleri* (Fogleman & Williams 1987). Adult flies from each line were placed in embryo collecting cages and allowed to oviposit on either standard fly food, soil soaked with rotting coastal prickly pear, or soil soaked with rotting saguaro. Newly hatched larvae were transferred to a 60-mm tissue culture dish containing either standard fly food or sterilized soil soaked with cactus exudates. Individual larvae were thus exposed to only one food type during development. For each sample, 10 third-instar larvae were transferred to TRI Reagent on the eighth day after hatching, and RNA was extracted following standard TRI Reagent protocol followed by treatment with Turbo DNA-free (Applied Biosystems).

RNA sequencing and transcriptome assembly

RNA-seq libraries were constructed by BGI Americas and sequenced on two lanes of an Illumina HiSeq 2000 following a paired-end 100-bp sequencing protocol. The 18 bar-coded libraries were pooled prior to sequencing and spread evenly across lanes to eliminate any lane effects. Low-quality sequences were filtered out by removing any reads with unknown nucleotides >5%, or reads with more than 20% of base quality scores <10. We assembled the transcriptome *de novo* from the 18 libraries with Trinity using default parameters (Haas *et al.* 2013). As the initial assembly was expected to contain many contaminant sequences (e.g. microorganisms or cactus), we then filtered the assembled Trinity components using Geneious's (v.5.5) BLAST and binning option to retain only those with top hits to *D. mojavensis*, *Drosophila grimshawi*, *Drosophila virilis* or *Drosophila*

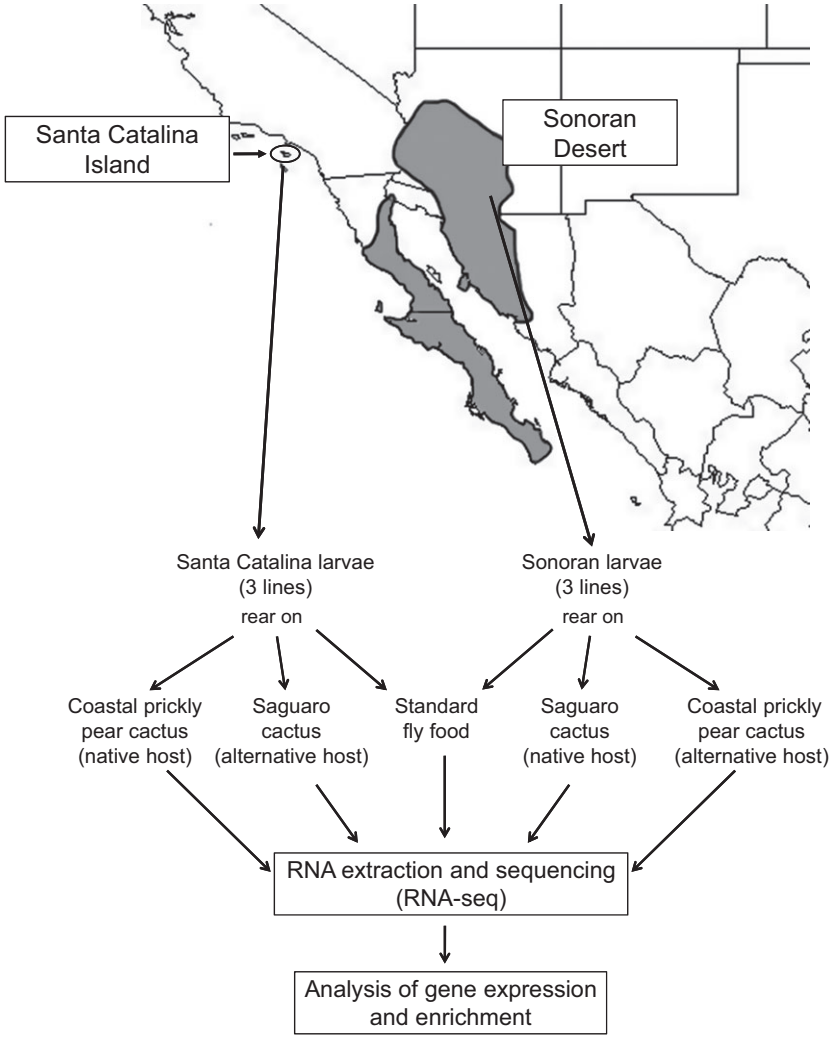

Fig. 1 Overview of experimental set-up. The geographic range of *Drosophila mettleri* is shaded in grey.

melanogaster with e-value cut-off at 1E-10. These components were used in subsequent differential expression (DE) analysis.

Differential expression analyses

We estimated the abundance of transcripts by mapping reads back to the assembly using the RNA-Seq by Expectation Maximization (RSEM) software package bundled with Trinity (Li & Dewey 2011). We used the Bioconductor package edgeR to perform DE analyses (Robinson *et al.* 2010). To exclude genes with low counts in the analysis, we kept only those that were expressed at least once per million in at least three libraries. DE analyses were conducted using generalized linear models (glm) that included food type and population as experimental factors (the dispersion value was 0.1434). We addressed specific questions of interest by constructing and analysing contrasts between different conditions. First, we compared gene expression for larvae within each population when reared on standard laboratory food vs. the native host plant for that population. We next compared gene expression between populations to identify evolved regulatory differences, including those that were fixed regardless of the rearing environment (environmentally independent) and any additional genes that showed host plant-specific differences in expression (environment dependent). We identified environment-independent genes by finding those with a significant population effect (averaging over the three food types), but no significant population × food interaction. To find environment-dependent differences, we analysed genes with a significant population × food interaction to identify those that differed in expression specifically on coastal prickly pear or saguaro.

As the Santa Catalina Island population was derived from ancestors that likely utilized columnar cacti (e.g. saguaro), we also compared transcriptional reaction norms between the two populations to assess the degree to which transcriptional plasticity may have

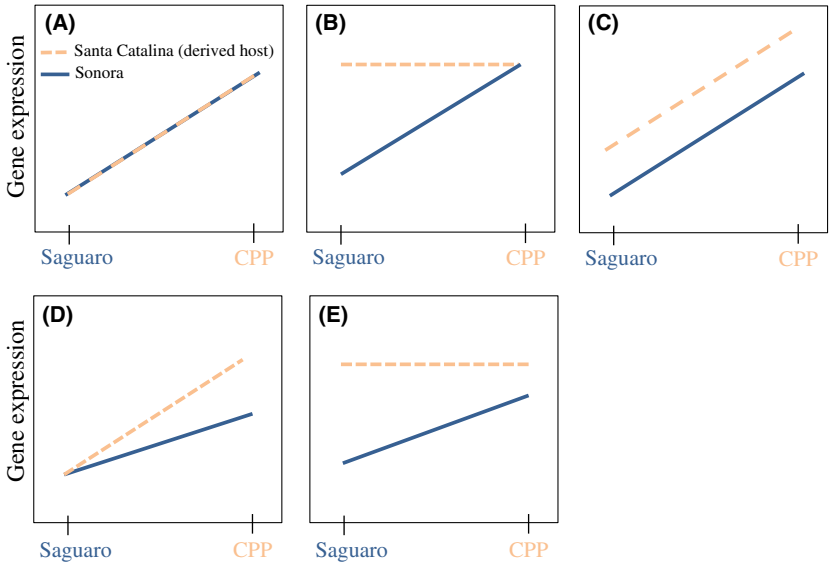

Fig. 2 Transcriptional reactions norms for Sonoran and Santa Catalina Island populations consistent with (A) plasticity, no divergence, (B) genetic assimilation, (C) and (D) Baldwin effect and (E) Baldwin effect with genetic assimilation. Note that only patterns involving upregulation in the Santa Catalina Island population are shown, although patterns involving downregulation were also analysed.

facilitated the host shift to coastal prickly pear (this analysis only included cactus food sources). First, to find plastic genes that did not diverge in expression between populations (i.e. genes with overlapping reaction norms), we identified genes with a significant cactus effect, but no population effect or population × cactus interaction effect (Fig. 2A). We next conducted a series of tests to evaluate the relationship between transcriptional plasticity and divergence. We first used a hypergeometric test to examine whether genes with plastic expression in the Sonoran population were overrepresented in the complete set of genes that differed in expression between the populations. Genes that were plastic in the Sonoran population included the following: plastic genes with overlapping reaction norms as described above, and any additional genes with a significant population × cactus interaction that were plastic between the cacti in the Sonoran population. We next considered cases where transcriptional plasticity may have facilitated subsequent adaptive evolutionary changes in the Santa Catalina Island population, following descriptions of genetic assimilation and the Baldwin effect discussed by Crispo (2007) (Fig. 2B–E). For genetic assimilation, we first identified genes with a significant population × cactus interaction, as this indicated nonequivalent reaction norms in the two populations, which would be necessary to infer genetic assimilation. We then used a series of contrasts to identify the subset of these genes following the specific pattern consistent with genetic assimilation depicted in Fig. 2B. As an example, a gene showing a pattern consistent with genetic assimilation would have a significant population × cactus interaction and would differ between populations on saguaro but not coastal prickly pear. Furthermore, the gene would show plasticity across hosts in the Sonoran population, but not in the Santa Catalina Island population. For the Baldwin effect, we focused on genes that differed in expression on coastal prickly pear in the two populations, as these represent cases where plasticity could have promoted subsequent evolutionary change on the derived host. Figure 2C–E illustrates patterns consistent with different forms of the Baldwin effect (Fig. 2C,D) or the Baldwin effect with genetic assimilation (Fig. 2E). To find genes fitting the pattern in Fig. 2C, we first identified those with a significant population effect (this included all of the environment-independent genes in addition to any additional genes with a significant population effect when averaged over cacti), a significant cactus effect, but no significant population × cactus interaction. We then found the subset of these in which plasticity in the Sonoran population was in the direction of the change observed in Santa Catalina Island population. For patterns depicted in Fig. 2D,E, we first found genes with a significant population × cactus interaction and then further dissected expression differences using a series of contrasts to find the subset of these genes following the illustrated patterns.

Enrichment analyses

To identify specific processes that were overrepresented in DE gene sets, we performed enrichment analyses using CLUEGO (v.2.1.0) (Bindea *et al.* 2009). We first identified genes in our comparisons with suspected biological roles in *D. mettleri* by finding top hit genes in *D. mojavensis* using BLAST+ (v.2.2.28) with e-value cut-off of 1E-10. As we are mainly interested in the general

functional classes that these genes belong to, which we assume to be conserved across *Drosophila*, *D. melanogaster* orthologs of the *D. mojavensis* hits were used in our analyses as the *D. melanogaster* genome is annotated more extensively. We recognize that these *D. mettleri* genes are not necessarily orthologous to top hits in *D. mojavensis*/*D. melanogaster*, but this should nevertheless give us relevant functional information. In CLUEGO, the *D. melanogaster* genes were clustered using GO terms (biological process, cellular component, immune system process, and molecular function, and KEGG pathways) at GO Tree Levels 3–8, with 2 as the minimum number of identifiers found to be associated with a term, and 3 as the minimum percentage that the mapped identifiers are representing from the total associated genes with the term. We used the right-sided hypergeometric test, corrected with the Benjamini–Hochberg procedure (significance cut-off = 0.1).

Results

RNA sequencing and transcriptome assembly

Sequencing resulted in 333 290 115 clean reads after quality filtering, with each library producing an average of approximately 18.5 million reads (range: approximately 16.5–20.5 million reads). Trinity assembles read into transcripts, and clusters transcript together to form components. Assembled components are approximately equivalent to genes (which may have multiple transcripts), so for simplicity, we refer to them as such throughout the remainder of the paper. The *de novo* assembly yielded 66 582 genes and 92 312 Trinity transcripts. Filtering by Blast to other *Drosophila* resulted in 15 947 genes (the total number of protein-coding genes in *D. mojavensis*, the most closely related *Drosophila* with a sequenced genome, is 14 849) (Clark *et al*. 2007). After filtering out genes with low counts, 12 003 genes were used in the DE analyses. The average assembled length of transcripts associated with these genes was 2250 bp (Table S1, Supporting information), and the majority of transcripts were nearly complete, with 72% exhibiting at least 70% alignment coverage with *Drosophila* homologs. Altogether, these statistics suggest a high-quality assembly sufficient for examining differential expression.

Within-population transcriptional differences between larvae reared on cactus vs. laboratory food

Differential expression data for all contrasts conducted in this study are available in Supporting information. In the comparison between Santa Catalina Island larvae reared on coastal prickly pear and laboratory food, 1095 genes differed in relative expression (Table 1, comparison 1). A number of GO terms were overrepresented in the set of DE genes, including several associated with endopeptidase and proteasome activity, which were upregulated on prickly pear, and downregulated terms linked to sugar transport and various processes connected to chitin (Fig. S1, Supporting information). When Sonoran larvae were reared on saguaro vs. laboratory food, 1541 genes differed in expression (Table 2, comparison 2). Overrepresented terms among the DE genes included a large network associated with central meta-

Table 1 Number of genes upregulated or downregulated relative to the first treatment in the comparison

Comparison	Genes upregulated	Genes downregulated
Within populations		
1. Santa Catalina: coastal prickly pear vs. laboratory food	794	301
2. Sonora: saguaro vs. laboratory food	1361	180
Between populations		
3. Environment independent: Santa Catalina vs. Sonora	109	102
4. Environment dependent: Santa Catalina vs. Sonora on coastal prickly pear	18	14
5. Environment dependent: Sonora vs. Santa Catalina on saguaro	70	16

Table 2 Number of genes fitting the patterns depicted in Fig. 1 and a summary of enriched GO terms. The enriched terms for 'plasticity, no divergence' include a subset of all overrepresented terms. Networks including all terms are available as supplemental figures

Pattern	Figure 1 panel	No. genes	Enriched terms
Plasticity, no divergence	A	5803	Proteasome TCA cycle
Genetic assimilation	B	4	Amino acid catabolism
Baldwin effect	C	64	Glycan degradation Galactose metabolism
	D	1	Pentose and glucuronate interconversions
Baldwin effect w/genetic assimilation	E	0	NA

bolism (Fig. S2, Supporting information). The majority of these terms were upregulated on saguaro, although some clusters included genes that were either mostly downregulated or included a mixture of genes that were up and downregulated. In addition, a network of genes associated with processes involving chitin was also downregulated on saguaro (Fig. S2, Supporting information).

Although GO terms associated with detoxification were not overrepresented in either cactus vs. laboratory food comparison, a number of genes belonging to detoxification gene families were differentially regulated between these food types. We were most interested in the subset of these that were induced in each population on its native host (e.g. induced in Santa Catalina Island larvae when reared on coastal prickly pear), as these are strong candidates for involvement in host plant utilization. We recognize that these gene families have diverse functions, so not all identified genes are likely to be involved in detoxification. Nevertheless, 27 different genes belonging to gene families involved in detoxification met this criterion, with only six showing overlapping induction on both host plants (three carboxylesterases and three cytochrome P450s; Table S2, Supporting information). More specifically, eight carboxylesterases and three cytochrome P450s were induced by prickly pear in the Santa Catalina Island population, while nine carboxylesterases, nine cytochrome P450s and two UDP-glycosyltransferases were induced when Sonoran larvae were reared on saguaro (Table S2, Supporting information).

Evolutionary divergence in gene expression between populations

A total of 319 genes differed in expression between the Sonoran and Santa Catalina Island populations when considering both environment-independent and environment-dependent responses. Overrepresented terms included networks associated with sensory perception, endopeptidase activity, chitin binding and multiple networks associated with sugar metabolism and drug metabolism (Fig. 3). Of the 319 genes, 211 exhibited expression differences that were independent of the larval rearing environment (Table 1, comparison 3). Overrepresented GO terms in this subset included a large network of clusters associated with drug metabolism and starch/sugar metabolism, and a small network involved in chemosensory perception (Fig. S3, Supporting information). The remaining environment-dependent genes included 32 that differed in expression on coastal prickly pear (Table 1, comparison 4) and 86 that differed on saguaro (Table 1, comparison 5; 10 genes were included in both sets). For the 32 that differed in expression on coastal prickly pear, the only overrepresented terms were associated with drug metabolism (Fig. S4, Supporting information), while the 86 that differed in expression on saguaro included multiple networks associated with amino acid metabolism, and additional networks linked to the proteasome complex and to chitin-related processes (Fig. S5, Supporting information).

Detoxification genes

All of the population comparisons included overrepresented GO terms associated with detoxification. In total, 19 genes belonging to detoxification gene families were differentially expressed between the populations including six carboxylesterases, six cytochrome P450s, one GST and six UGT-glycosyltransferases (Table S2, Supporting information). Sixteen of the 19 genes differed in expression between the populations regardless of food type, with only one carboxylesterase and one cytochrome P450, showing higher induction in the Sonoran population on saguaro, and one UDP-glycosyltransferase showing a stronger induction pattern in the Santa Catalina Island population on coastal prickly pear (Table S2, Supporting information).

Role of transcriptional plasticity in facilitating host shifts

To assess the potential involvement of transcriptional plasticity in the host shift to coastal prickly pear, we first identified plastic genes with overlapping reaction norms in the two populations (i.e. a cactus effect, but no population effect, and no population × cactus interaction; Fig. 2A). This included a total of 5803 genes with overrepresented clusters associated with central metabolism (e.g. TCA cycle) and the proteasome complex (Fig. S6, Supporting information). Although most transcriptionally plastic genes thus had overlapping reaction norms in the two populations, plastic genes in the Sonoran population were also overrepresented among the 319 genes that differed in expression between the populations. Specifically, while 5909 of 12 003 (49%) of all genes were plastic in the Sonoran population, 180 of 319 genes (56%) that differed between populations fell into this category (hypergeometric test, $P = 0.005$). The remaining 139 (44%) genes that differed in expression between the populations were not transcriptionally plastic in the Sonoran population. We also ran the same test considering only genes with divergent expression on the derived host (coastal prickly pear), which indicated no significant overrepresentation of plastic genes in this set (121/243; hypergeometric test, $P = 0.449$). The remaining 122 genes that

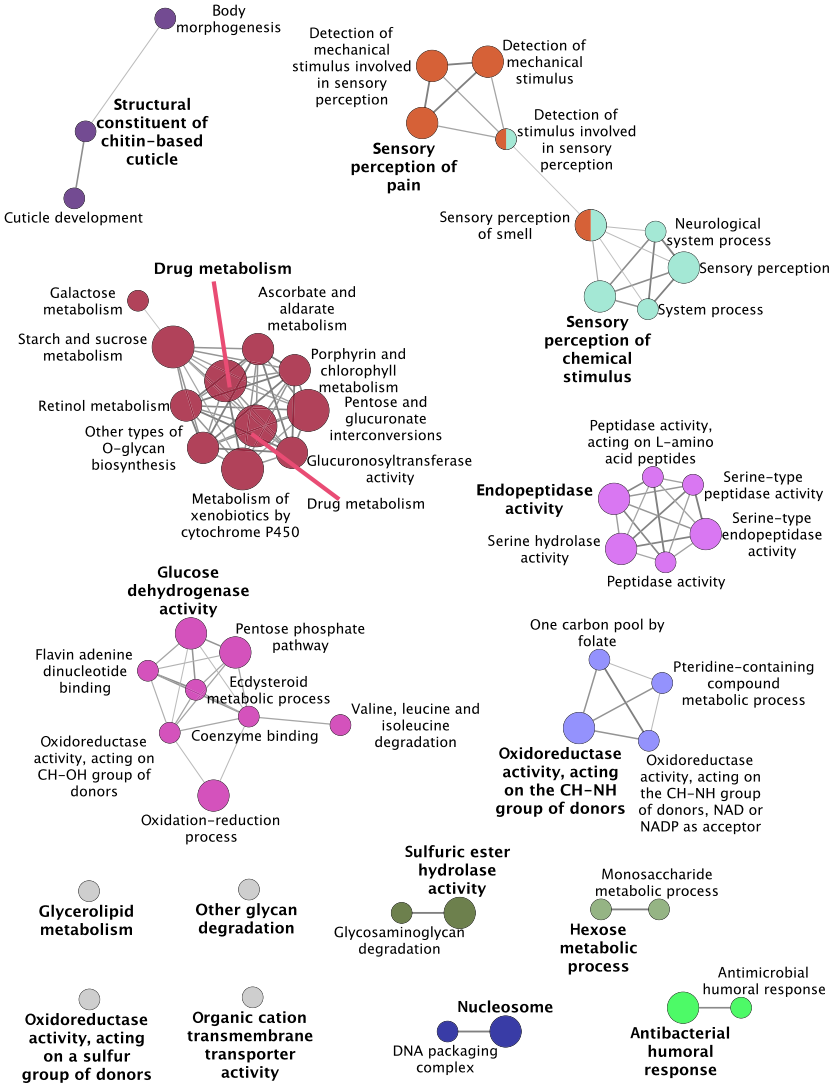

Fig. 3 Functional enrichment analysis for the 319 genes that were differentially expressed between the two populations. A false discovery rate cut-off of 0.1 was used to identify enriched terms. Node size indicates degree of significance, and larger font indicates the most significant term in cluster. Edges indicate degree of connectivity between biological terms.

were differentially regulated between the populations on coastal prickly pear were enriched for terms associated with drug metabolism, starch/sugar metabolism, sensory perception and hormone metabolism (Fig. S7, Supporting information).

To further characterize the relationship between transcriptional plasticity and divergence, we categorized plastic genes into patterns consistent with genetic assimilation (Fig. 2B) and the Baldwin effect (Fig. 2C–E). Only four genes had reaction norms consistent with genetic assimilation (Table 2). Despite the small size, terms associated with amino acid catabolism were overrepresented in this set (Fig. S8, Supporting information). In total, 65 of 121 plastic genes that differed in expression on coastal prickly pear exhibited reaction norms consistent with the Baldwin effect (Table 2), with overrepresentation of several terms associated with sugar metabolism (Fig. S9, Supporting information). Most of the remaining 56 plastic genes had reaction norms that looked like those in Fig. 2C except that the vertical position of the lines on the *y*-axis was switched. For example, a gene might be upregulated in both populations on coastal prickly pear relative to saguaro, but downregulated in the Santa Catalina Island population relative to the Sonoran population. In other words, plasticity produces a level of expression that 'overshoots' the optimal level on coastal prickly pear. In such cases, plasticity could be considered adaptive or maladaptive depending on whether the expression on coastal prickly pear was closer to or further from the optimum than it would be if there was no plasticity. Interpretation of this pattern is further complicated by the fact that it is not clear whether the consequences of expression below or above the optimum would be the same, even if the magnitude of the differences was equal. Given these complications in interpreting this

pattern, we do not attempt to classify genes in this set as being adaptively or maladaptively regulated.

Discussion

The extraordinary diversity of plant-feeding insects is driven, in part, by host plant switching and subsequent adaptation to the new host (Janz *et al.* 2006). Although insects may adapt to host plant chemistry through changes in gene expression, few studies have examined genomewide transcriptional responses of insects to different host plants (Vogel *et al.* 2014). The results of our study thus provide interesting insights that help shed light on the importance of regulatory evolution and transcriptional plasticity in host plant shifts.

Transcriptional responses to host plant feeding

We would expect usage of cactus hosts to be more stressful than laboratory fly food, particularly due to the presence of secondary plant metabolites and other compounds involved in plant defence (e.g. protease inhibitors). In addition, host plants are also assumed to include more complex nutrient mixtures than laboratory food. These differences are reflected in transcriptional changes we observed in comparisons between larvae reared on host plants and laboratory food. In the comparison between coastal prickly pear and fly food in the Santa Catalina Island population, terms involved in proteasome activities were overrepresented among DE genes, with most being upregulated on cactus. The proteasome system is involved in many processes in insects including, programmed cell death, stress response and immune response (Mykles 1999). Previous studies have also found differential regulation of genes coding for proteasome components when insect larvae are reared in different host plant environments (Ragland *et al.* 2015), and some studies have specifically demonstrated involvement of the proteasome complex in detoxification. For example, a subunit of the 26S proteasome complex was found to confer resistance to the insecticide Deltamethrin in mosquitoes (Sun *et al.* 2013), while the same complex was also thought to have been involved in detoxification of a secondary cotton metabolite in *Helicoverpa armigera* (Celorio-Mancera *et al.* 2011). Additionally, a large network of genes associated with serine-type endopeptidase activity was upregulated on coastal prickly pear. Upregulation of serine proteases in response to plant feeding has also been observed in a number of other insects, with Celorio-Mancera *et al.* (2013) highlighting this as an emerging trend in gene expression studies of insect host plant feeding. We also observed transcriptional plasticity in predicted pathways when Sonoran larvae were reared on saguaro or laboratory food. Most notably, these comparisons revealed major differences in the regulation of genes involved in central metabolism, which underscores the divergent nutritional composition of saguaro relatively to laboratory food.

An additional interesting result from the cactus vs. laboratory food comparisons is that both populations showed downregulation of genes associated with chitin when reared on cactus. Chitin is an important component in insect cuticles and peritrophic matrices (a barrier that aids in digestion and provides protection in the insect gut) (Tellam *et al.* 2000; Gagou *et al.* 2002; Merzendorfer & Zimoch 2003). Certain insecticides are known to inhibit chitin synthesis (Leighton *et al.* 1981), and a previous study in aphids demonstrated differential expression of chitin-related genes in insecticide-susceptible and insecticide-resistant strains (Puinean *et al.* 2010). Some plants also contain chitinases, presumably as a mode of defence against herbivorous insects (Vogel *et al.* 2014), and altered regulation of chitin genes on different insect hosts has now been observed in several transcriptional studies of plant-feeding insects (Matzkin 2012; Celorio-Mancera *et al.* 2013; Vogel *et al.* 2014). Taken together, these results highlight a potentially important mode of interaction between insects and their hosts that warrants further study.

Several genes belonging to families associated with detoxification were upregulated on cactus relative to laboratory food. Previous research examining the induction of *D. mettleri* cytochrome P450s in response to alkaloids of different hosts led Danielson *et al.* (1998) to suggest that detoxification of plant allelochemicals likely involved the cooperative action of 'specialist' enzymes that respond selectively to specific substrates and 'generalist' enzymes that respond a variety of chemically similar substrates. Considering induction patterns of all the multigene families associated with detoxification, our results also support this hypothesis, although we found many more 'specialist' genes that were induced on only one of the hosts (20/27) relative to genes induced by both hosts. While we recognize that all of these genes may not function in xenobiotic metabolism, at least one has been linked to detoxification in *D. melanogaster*. Specifically, *cyp304a1* (upregulated on saguaro) was regulated in response to methanol (Wang *et al.* 2013). Moreover, three of the induced genes (*cyp28a5*, *cyp6d5* and *cyp4e3*) were expressed in key metabolic tissues (midgut, fat body or malpighian tubules) in *D. melanogaster*, suggesting that they could also play a role in detoxification (Chung *et al.* 2009). Additionally, homologs of six of the induced cytochrome P450s (*cypt1*, *cyp4e1*/*cyp4e3*, *cyp6d5*, *cyp313a4*, *cyp304a1 and cyp4p1*/*cyp4p3*) were also differentially regulated in comparisons between *Rhagoletis*

larvae reared on different host fruits (Ragland *et al.* 2015). Interestingly, we did not observe upregulation of *cyp4D10* in Sonoran larvae reared on saguaro, which was predicted given earlier studies demonstrating induction of this gene specifically in response to saguaro alkaloids. This discrepancy may be explained by differences in rearing conditions, as the previous study exposed larvae to rehydrated dried saguaro tissue supplemented with additional alkaloids (Danielson *et al.* 1998), while our study involved rearing larvae in soil soaked with exudates generated from actively decomposing tissue. Microorganisms associated with the rotting process have been shown to alter both nutritional and toxic properties of decaying cactus tissues and thus could play a role in the differences we observed (Fogleman & Abril 1990; Starmer & Aberdeen 1990).

Evolutionary divergence in gene expression between populations

An earlier study demonstrated local adaptation to saguaro and various species of prickly pear in Sonoran and Santa Catalina Island populations, respectively (Castrezana & Bono 2012). This pattern could be explained, in part, by transcriptional evolution, with genes differentially regulated in the two populations on each host being the strongest candidates for involvement in local adaptation. Overall, only 2.7% of the 12 003 genes we analysed showed divergence in expression between the populations. Nevertheless, information on their predicted functions suggests that many are associated with processes directly relevant to host plant adaptation. For example, consistent with expectations given the considerable differences in plant chemistry of saguaro and coastal prickly pear, networks of gene clusters associated with sugar metabolism and sensory perception were overrepresented among differentially regulated genes. In addition, a large network of clusters included homologs of several genes with known associations to drug metabolism (*G17323, GstE6, Ugt35b, Ugt36Bb, Ugt86Dd, sepia and esterase-P*), and another 12 genes belonging to gene families involved in detoxification were also differentially regulated between the populations (Table S2, Supporting information). Notably, this set included homologs of several UDP-glycosyltransferases, two of which were also differentially regulated on different cactus hosts of *D. mojavensis* in a previous experiment (*ugt86Dd* and *ugt36Bb*; Matzkin 2012). Interestingly, three *D. mettleri* genes had top Blast hits to the *D. mojavensis* ortholog of *ugt86Dd*. One of the *D. mettleri* genes was upregulated in Santa Catalina Island larvae on coastal prickly pear, while the other two were upregulated in the Sonoran population on saguaro (Table S2, Supporting information). This suggests that duplication events because the split between *D. mettleri* and *D. mojavensis* has resulted in multiple paralogs in *D. mettleri* that have subsequently diverged in expression between populations, although more targeted sequencing is necessary to rule out possible transcriptome assembly errors. A similar scenario was also observed for three *D. mettleri* genes that had top Blast hits to the *D. mojavensis* ortholog of *Cyp4d5*. All of these genes were highly upregulated in the Sonoran population and are thus candidates for involvement in adaptation to saguaro.

The majority of expression differences between populations were independent of the larval rearing environment. While some of these differences could be unrelated to larval–cactus interactions, enrichment analyses indicate that predicted functions of a significant portion of this set of differentially regulated genes are relevant to host plant utilization. For example, many of the genes linked to detoxification and starch and sugar metabolism were differentially expressed in the two populations across all food types. While somewhat surprising, these results may reflect the predictability of the larval environment in populations that specialize on a specific host plant, as constitutive expression of key metabolic/detoxification genes could be advantageous in such cases. This may, however, lead to trade-offs in performance in the ancestral environment, where gene expression would no longer be optimal. Castrezana & Bono (2012) did, in fact, report a reduction in performance on saguaro for Santa Catalina Island larvae reared on this host. Such trade-offs in performance may have important evolutionary consequences, as this could further promote population divergence following colonization of new hosts.

Role of transcriptional plasticity in facilitating host shifts

Comparisons of reaction norms between the Sonoran population and the Santa Catalina Island population, which uses the derived host (coastal prickly pear), provide insight into whether transcriptional plasticity may have facilitated adaptation to the novel host plant. Strikingly, nearly half of all genes analysed (5803/12 003) were differentially regulated between the two hosts and had completely overlapping reaction norms in the two populations. Furthermore, many of these genes are linked to processes directly relevant to host plant utilization (e.g. central metabolism and the proteasome complex). This suggests that ancestral transcriptional plasticity likely resulted in expression levels for many genes that were already well matched to the new environment. Such plasticity may be crucial during the initial stages of colonization of a new host, as it could

allow the population to persist long enough for directional selection to further fine-tune the response to the new environment. We note that while we did not quantitatively analyse larval survivorship in this study, we did not notice dramatic differences between the populations as observed previously on one prickly pear species (e.g. Sonoran larvae had almost no survivorship on *Opuntia phaeacantha*) (Castrezana & Bono 2012), which would be consistent with the extensive shared plasticity between populations we observed. Smaller, but significant, reductions in survival were also observed when Sonoran larvae were reared on other prickly pear species in that study (Castrezana & Bono 2012), and we certainly do not rule out this possibility for *O. littoralis*.

Despite the fact that the vast majority of plastic genes had overlapping reaction norms in the populations, plastic genes were still overrepresented in the overall set of 319 genes exhibiting divergent expression between the populations. We thus sought to further explore the relationship between plasticity and evolutionary divergence. In cases where ancestral plasticity results in the optimal level of expression in the new environment, divergence in the reaction norm may still occur through the process of genetic assimilation, whereby the optimal level of expression becomes fixed in the derived population across all environments, presumably due to costs associated with maintaining plasticity. Although a number of plastic genes exhibited divergence in expression between the populations on saguaro (65), we found little evidence for genetic assimilation, as only four genes had reaction norms consistent with this effect. These results, together with the large number of plastic genes exhibiting no population divergence, suggest minimal costs associated with maintaining plasticity for most genes.

Although plastic genes were not overrepresented in the specific subset of genes that differed in expression on the derived host (coastal prickly pear), a considerable number (121/243) did exhibit divergent expression patterns. We thus analysed these reaction norms for evidence of the Baldwin effect, which results when plasticity is in the same direction as the derived evolutionary change. Presumably this occurs when ancestral plasticity is adaptive in the novel environment, but not yet optimal. More than half of the genes (56%) that were plastic in the Sonoran population and also differed in expression on coastal prickly pear displayed evidence for the Baldwin effect. Furthermore, this set of genes is enriched for terms involved in sugar metabolism, which likely reflects qualitative and quantitative differences in sugar content between saguaro and coastal prickly pear. Almost all of these cases (64/65) involved changes in the vertical position of the reaction norm (as in Fig. 2C) rather than the slope (Fig. 2D), meaning that derived expression levels differed on both hosts. As discussed previously, this may have important evolutionary consequences as it could promote population divergence as a result of performance tradeoffs in derived and ancestral environments. While altogether these results point to a potentially important role of plasticity in facilitating adaptive divergence, we do note that Baldwin effect genes constitute only 27% of the 243 genes with divergent expression between the populations on coastal prickly pear. In fact, almost twice as many genes that differed in expression between the populations on coastal prickly pear were not plastic in the Sonoran population (122 vs. 65), and this set of genes was enriched for several terms relevant to larval–host plant interactions (e.g. drug metabolism, starch/sugar metabolism, sensory perception).

While these results help shed light on the relationship between transcriptional plasticity and evolutionary divergence, we do note some caveats associated with our study. First, these analyses assume that transcription levels in the Sonoran population are a reasonable approximation of the ancestral state for the Santa Catalina Island population. While considerable evidence suggests that ancestors of the Santa Catalina Island population were associated with columnar cacti (e.g. saguaro), we acknowledge that some caution is warranted here, as is true of any study using contemporary populations to estimate ancestral character states. We also note that this study does not yield direct functional links between transcriptional variation and adaptation. Thus, although many of the genes that were differentially regulated in our comparisons belong to functional classes predicted to be involved in host plant adaptation, their involvement has not been verified by functional studies, and some evolutionary changes in gene expression between populations may not be adaptive.

Synthesis with existing studies

Although little attention has been directed towards analysing the importance of transcriptional plasticity during and after colonization of novel environments, recent comparative gene expression studies of *Rhagoletis* flies adapted to different host plants (Ragland *et al.* 2015), killfish adapted to divergent thermal environments (Dayan *et al.* 2015) and spider mites adapting through experimental evolution to an alternative host (Wybouw *et al.* 2015) provide a basis for comparison to our study. All studies found evidence for shared adaptive plasticity between populations that may permit colonization of novel environments and allow populations to persist long enough for subsequent adaptive evolution. However, the relationship between plasticity and subsequent divergence in gene expression appears to be complex

and somewhat variable. Although we found that plastic genes were overrepresented in the set of all genes that diverged in expression between populations, this was not the case for the subset that differed in expression specifically on the derived host. Moreover, reaction norms for only a relatively small proportion of plastic genes were consistent with the idea that plasticity facilitates subsequent adaptive evolution (e.g. through genetic assimilation or the Baldwin effect). These results are in broad agreement with results from the other studies, with Wybouw *et al.* (2015) reporting relatively little evidence for these effects in spider mites, and Ragland *et al.* (2015) and Dayan *et al.* (2015) both finding that the bulk of evolutionary divergence for plastic genes involved selection to counter the initially plastic response rather than enhance it. Furthermore, results from all studies indicate that changes in the expression of genes that were not initially plastic constituted the majority of the evolutionary response to the novel environment (albeit a slim majority in our study). Taken together, these results suggest that transcriptional plasticity likely plays a crucial role during the early stages of colonization of a novel environment, but the relative importance of plasticity in facilitating subsequent adaptive evolution is less clear. Future studies on diverse organisms are thus crucial for understanding the complex relationship between transcriptional plasticity and evolutionary divergence.

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J.M.B. and L.M.M. designed the study; K.H. performed the experiments; K.H. and J.M.B. analysed the data; and K.H., J.M.B. and L.M.M. contributed to writing the manuscript.

Data accessibility

The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and the Short Read Archive and are accessible through GEO Series Accession no. GSE71110. The transcriptome assembly and read count data used for differential expression are also available through Dryad: doi: 10.5061/dryad.61 m60.

Supporting information

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

Table S1 Statistics for the *de novo* transcriptome assembly.

Table S2 Fold change for detoxification genes that were differentially regulated in comparisons within and between populations.

Fig. S1 Functional enrichment analysis for the 1095 genes that were differentially expressed in the Santa Catalina Island population on coastal prickly pear relative to laboratory food.

Fig. S2 Functional enrichment analysis for the 1541 genes that were differentially expressed in the Sonoran population on saguaro relative to laboratory food.

Fig. S3 Functional enrichment analysis for the 211 genes that were differentially expressed in the Santa Catalina Island population relative to the Sonoran population across all three food types (environment-independent genes).

Fig. S4 Functional enrichment analysis for the 32 genes that had a significant population × food interaction and were differentially expressed in the Santa Catalina Island population relative to the Sonoran population specifically on coastal prickly pear.

Fig. S5 Functional enrichment analysis for the 86 genes that that had a significant population × food interaction and were differentially expressed in the Sonoran population relative to the Santa Catalina Island population specifically on saguaro.

Fig. S6 Functional enrichment analysis for the 5803 genes that were plastic and had overlapping reaction norms between the populations.

Fig. S7 Functional enrichment analysis for the 122 genes that diverged in expression between populations, but were not plastic in the Sonoran population.

Fig. S8 Functional enrichment analysis for the four genes that had reaction norms consistent with genetic assimilation.

Fig. S9 Functional enrichment analysis for the 65 genes that that had reaction norms consistent with the Baldwin effect.

Data S1 Differential expression outputs from edgeR and gene lists used in the enrichment analyses.