The role of horizontally transferred genes in the xenobiotic adaptations of the spider mite Tetranychus urticae

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Adaptation of a Polyphagous Herbivore to a Novel Host Plant Extensively Shapes the Transcriptome of Herbivore and Host

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5.0 Abstract

Generalist arthropod herbivores rapidly adapt to a broad range of host plants. However, the extent of transcriptional reprogramming in the herbivore and its hosts associated with adaptation remains poorly understood. Using the spider mite *Tetranychus urticae* and tomato as models with available genomic resources, we investigated the reciprocal genome-wide transcriptional changes in both spider mite and tomato as a consequence of mite’s adaptation to tomato. We transferred a genetically diverse mite population from bean to tomato where triplicated populations were allowed to propagate for 30 generations. Evolving populations greatly increased their reproductive performance on tomato relative to their progenitors when reared under identical conditions, indicative of genetic adaptation. Analysis of transcriptional changes associated with mite adaptation to tomato revealed two main components. First, adaptation resulted in a set of mite genes that were constitutively down-regulated, independently of the host. These genes were mostly of an unknown function. Second, adapted mites mounted an altered transcriptional response that had greater amplitude of changes when re-exposed to tomato relative to non-adapted mites. This gene set was enriched in genes encoding detoxifying enzymes and xenobiotic transporters. Besides the direct effects on mite gene-expression, adaptation also indirectly affected the tomato transcriptional responses which were attenuated upon feeding of adapted mites, relative to the induced responses by non-adapted mite feeding. Thus, constitutive down-regulation and increased transcriptional plasticity of mite genes play a central role in host adaptation, leading to both a higher detoxification potential and reduced production of plant defense compounds.

5.1. INTRODUCTION

Arthropod herbivory exhibits a broad spectrum of arthropod-plant interactions ranging from herbivores associated exclusively with a single host, to herbivores successfully developing on hundreds of different host plants. Within this spectrum, a difference is traditionally made between herbivore specialists that feed on related plants within a particular family and herbivore generalists that are able to feed and adapt to plants belonging to distant plant families (Schoonhoven et al., 2005; Strong et al., 1984). The spider mite *Tetranychus urticae* is an extremely polyphagous arthropod pest with a host plant range covering over 1,100 plant species, scattered over more than 140 different plant families (Jeppson et al., 1975; Migeon & Dorkeld, 2015), a unique trait amongst phytophagous arthropods. Although the great adaptive potential of *T. urticae* has been previously documented (Agrawal, 2000; Fry, 1989; Gould, 1979; Magalhaes et al., 2009, 2007), the molecular and ecological genetics underlying the mite adaptation mechanisms are largely unknown.

Plants defend themselves against herbivory by physical barriers (which include trichomes, spines and thickened leaves) and/or by synthesizing toxic compounds (or allelochemicals) which, based on their production, can be divided into two categories. Phytoanticipins are plant allelochemicals that are synthesized constitutively and are therefore always present in a plant tissue, while phytoalexins are defense compounds whose production is induced by herbivore attack. To counter the toxic effects of plant defenses, arthropods in turn developed an arsenal of enzymatic mechanisms, of which the use of detoxifying enzymes and xenobiotic transporters are best studied (Despres et al., 2007; Li et al., 2007). The overall detoxification process is traditionally divided into three phases that differentially affect the ingested toxin: by direct metabolism (phase I), conjugation (phase II) and translocation (phase III) (Brattsten, 1988; Despres et al., 2007). Different plant families produce different sets of toxic plant chemicals and anti-nutritional compounds in their defense against herbivores. Krieger et al. (1971) states that polyphagous herbivores adapted to this wide range of plant defense compounds by developing a xenobiotic metabolism that targets a broad range of toxins. Refining Krieger’s hypothesis, molecular studies now indicate that *T. urticae* and other generalists initially survive a host plant transfer by extensively rearranging their xenobiotic metabolism through differential expression of genes that code for enzymes that metabolize, bind or translocate toxins (Celorio-Mancera et al., 2012, 2013; Dermauw et al., 2013b). In addition to detoxification as a major mechanism, arthropods can also survive plant defens-
es by actively attenuating or even suppressing the inducible synthesis of phytoalexins through manipulation of plant induced responses (Alba et al., 2011, 2015; Musser et al., 2002; Sarmento et al., 2011; Zarate et al., 2007; Zhao et al., 2015). Such manipulation of plant defensive physiology by arthropods is thought to be triggered through secreting metabolites, peptides and small proteins, referred to as effectors, via their saliva into their host (Alba et al., 2011; Bos et al., 2010; Hogenhout & Bos, 2011). Suppression of tomato defenses has been shown indirectly for the spider mite *Tetranychus evansi* (Sarmento et al., 2011), but also distinct genotypes of *T. urticae* have been identified that differentially induce anti-herbivore tomato defenses (Alba et al., 2015; Kant et al., 2008). This might suggest that genetic variability in the ability of *T. urticae* to suppress plant defenses might be essential in the adaptation to a novel host, and may play a crucial role in the polyphagous nature of *T. urticae*.

The extent of evolutionary changes as a consequence of host adaptation in a herbivore’s xenobiotic metabolism and in its interaction with the host plant are poorly understood. Using recently available plant and arthropod transcriptomics tools, we can now identify the transcriptional responses of an arthropod generalist to host plant adaptation and its potential indirect effect on the reciprocal genome-wide gene-expression changes of its host. If adaptation affects the arthropod’s transcriptome, it could alter the expression of genes independently of the host plant i.e. a differential constitutive transcription. Additionally, adaptation could also change the degree of responsiveness towards host plant exposure i.e. by evolving an altered transcriptional plasticity. Transcriptional plasticity, or more general, phenotypical plasticity, upon an initial exposure to a novel environment has been proposed as an important mechanism that may greatly facilitate subsequent adaptation (Laland et al., 2014; Pfennig et al., 2010; Schlichting & Wund, 2014; West-Eberhard, 2003; Wray et al., 2014). However, empirical data to test this hypothesis are still scant as this necessitates a proper quantification of plastic regulation in the ancestral population, which is difficult to obtain under natural conditions (Pfennig et al., 2010). Experimental evolutionary studies offer a unique opportunity to test how the altered transcriptional levels associated with adaptation are driven by the transcriptional plasticity of the ancestral population.

In this study, we used *T. urticae* and tomato as models with available genome-wide transcriptomic platforms, and chose a genetically diverse population of *T. urticae* that showed extensive transcriptional plasticity upon transfer to new hosts in previous studies (Dermauw et al., 2013b; Grbic et al., 2011; Wybouw et al., 2014; Zhurov et al., 2014). In an experimental evolutionary
experiment, spider mites were transferred from bean and grown for 30 generations on tomato. Performance tests revealed a strong genetic adaptation. We analyzed the transcriptomes of tomato-adapted vs non-adapted mite populations on tomato and bean plants to uncover to what extent genetic adaptation led to host plant independent (constitutive) versus environmentally induced (plastic) gene-expression changes. We then specifically investigated the transcriptional changes in genes coding for detoxifying enzymes, to determine how the *T. urticae* xenobiotic metabolism evolved. The transcriptomic changes affiliated with adaptation were related to the transcriptional plasticity of the ancestral population upon tomato exposure to study the role of plasticity in subsequent mite adaptation to tomato. Last, we captured the reciprocal tomato transcriptional responses upon feeding of adapted and non-adapted mites to assess the effect of mite adaptation on plant host responses and verify if the ability to interfere with plant defenses is evolvable in *T. urticae*.

### 5.2. Material and Methods

#### 5.2.1. Plants and Spider Mites

All plants were reared in black earth (Structural Professional, pH 5.0-6.5, 20% organic matter; Snebbout NV) at 26 °C with a relative humidity of 60% and a light:dark photoperiod of 16h:8h. The following plant cultivars were used in this study; bean: *Phaseolus vulgaris* L. cv ‘Prelude’, cucumber: *Cucumis sativus* L. cv ‘Tanja’, tomato: *Solanum lycopersicum* L. cv ‘Moneymaker’ and bell pepper: *Capsicum annuum* L. cv ‘California Wonder’.

The ancestral *T. urticae* population was a derived genetically diverse line of the London strain originating from the Vineland region in Ontario, Canada. The genome of the London strain has been sequenced and annotated, and was used to design the custom gene-expression microarray platform (Dermauw et al., 2013b; Grbic et al., 2011; Van Leeuwen et al., 2012). For over 5 years (>100 generations), the London strain has been kept in laboratory conditions on potted bean in high population size (>10,000 individuals) to minimize genetic erosion and drift.

#### 5.2.2. Experimental Evolution

The London strain, reared on bean plants (B), is referred to as the reference line, R (or ancestral line). Three independent tomato-selection lines (adapted lines, A) were generated by transferring 200 randomly chosen adult females of
the R-line from potted bean to three-week-old potted tomato plants (T) on which they were allowed to propagate for 11 months (approximately 30-35 generations). New 3-week-old tomato plants were offered simultaneously to the three selection lines on a biweekly basis. To investigate gene-expression and performance levels that are independent of environmental and maternal effects, mites from the established A-lines were also reared for two generations on bean (common garden) (hereby creating bean-reared A-lines, AB). To investigate transcriptional plasticity, adult female mites from both the bean-reared R-(RB) and the bean reared A-lines (AB) were transferred to tomato for 24 h which resulted in RT and ABT mite populations (FIGURE 5.1A). Hence, this experimental set-up resulted in five mite line–host plant combinations i.e. (1) reference line, R, reared on bean (RB), (2) reference line R, transferred to tomato for 24 h (RT), (3) tomato-adapted lines, A, reared on tomato (AT), (4) A-lines reared for two additional generations on bean (AB) and (5) AB-lines subsequently re-exposed to tomato for 24 h (ABT). For additional performance tests, AB- and RB-mites were also transferred to the additional host plants cucumber and bell pepper.

5.2.3. Performance tests of mites on various host plants

Mites were synchronized in their development by transferring 100 adult females to uninfected bean plants where they were allowed to lay eggs for 36 h. To minimize maternal and environmental effects, 100 first generation females were again transferred to uninfected bean and allowed to lay eggs for 36 h. From the resulting second generation progeny, four replicates per selection line per host plant were established by transferring 35 2-4-day-old females to a fully developed leaf of equal age and size. After 10 days of egg laying and development, the total population sizes (including eggs and mobile stages) were counted and used as a performance estimate. Performance was compared between the three replicate AB-lines (tomato-adapted mites reared for two generations on bean) versus the RB-lines on the four different host plants (bean, cucumber, bell pepper and tomato). By using AB- and RB-mites, host and maternal effects were eliminated from the performance values. Differences in performance between the four host plants, selection regime [reference (R) vs adapted (A)] and their interaction were tested by means of a general linear mixed model, with selection treatment and host plant as fixed effects and selection line and its interaction with the host plant included as a random effects (proc mixed in SAS v. 9.4). A Tukey-adjusted post-hoc test was performed for the pairwise comparisons.
FIGURE 5.1. A: Experimental evolution of *T. urticae* adaptation to tomato. From the reference line grown on bean (RB), mites were transferred to tomato for 24 h (RT). From RB, triplicated adapted lines were created, which were grown on tomato (AT) for 30 generations. Adapted lines grown on bean for two generations (AB) and adapted lines grown on bean for two generations and then re-introduced to tomato for 24 h (ABT) were also included in the experiment. Tomato samples for tomato leaf chlorosis measurements and transcriptome analysis were collected at time points indicated by red background. B: Clustering of all differentially expressed genes in RT-, AT-, AB- and ABT-mites, relative to RB-mites. Based on their expression patterns, the differentially expressed genes were clustered into four distinct groups (1-4) (FDR-corrected *p*-value <0.05 and log2FC>1) (using Pearson Correlation distances). Clusters colored in green showed a stable differential expression and seemed to be determined by the mite selection treatment (A vs R), while the transcript levels of clusters colored in black appeared to be correlated to tomato and bean feeding. The shaded intervals surrounding each full cluster line represent the 95% confidence interval of the transcript levels within that cluster.
Adaptation Shapes Transcriptome of Mite and Host

Additionally, leaf-damage assays were performed on tomato as previously described (Kant et al., 2004) and analyzed using ANOVA followed by a Tukey-adjusted post-hoc test.

5.2.4. MITE TRANSCRIPTOMICS

Of every tomato-selection line in each condition (A, AB and ABT), one RNA sample was collected, while three control samples were taken from the non-selected lines on bean and after 24 h on tomato (RB and RT, respectively). Per RNA sample, 120 female adults were pooled. RNA extraction and labelling was performed with RNeasy Minikit (Qiagen) and with Low Input Quick Amp Labeling Kit (Agilent Technologies), respectively. Cy5-labelling was performed on RNA samples from AT-, AB-, ABT- and of RT-mites. RNA from RB-mites was dyed with Cy3. On every array, a Cy3-labelled RNA sample from RB-mites was hybridized (to serve as a common reference). Hybridization, washing and scanning protocols were identical as in (Dermauw et al., 2013b). The Agilent Feature Extraction software v. 10.5 (Agilent Technologies) provided the data following the GE2_107_SEP09 protocol. Data was analyzed with limma (Smyth, 2004) for final processing and statistical analysis. Background correction was performed by the ‘normexp’-method (offset of 50) (Ritchie et al., 2007). Subsequently the M- and A-values were normalized by within- (global loess) and between-array normalization (‘Aquantile’-method). The quality of the processed data was assessed using arrayQualityMetrics (Kauffmann et al., 2009). Significant differential expression was identified by an empirical Bayes approach with cut-offs for the Benjamini-Hochberg (BH) False Discovery Rate (FDR) corrected p-values and log2 converted fold changes (FC) at 0.05 and 1, respectively (Benjamini & Hochberg, 1997). The NbClust package was used to identify the optimal number of clusters using Pearson Correlation distances with seed set at 1234 (Charrad et al., 2014). The differentially expressed T. urticae genes were annotated using Blast2Go software. BLAST-searches of the T. urticae protein products were performed against the NCBI nr protein database with an e-value cut off of 1e-15. Annotation was further enhanced by InterPro. Applying a cut-off of a FDR-corrected p-value of 0.05, a Fisher’s exact test generated the Biological Process Gene Ontology (GO) annotations for all the differentially expressed T. urticae genes in our experiment. Within specific gene lists that were generated by specific transcriptomic comparisons, Gene Set Analysis (GSA) was performed using the Bioconductor package piano (using Parametric Analysis of Gene set Enrichment, PAGE) to identify significant up- and down-regulation of
Biological Process GO terms (Varemo et al., 2013). The input gene level statistics were obtained from linear modelling in limma.

Biological functions of the *T. urticae* protein families identified in Dermauw et al. (2013b) were evaluated by aligning all encoded proteins of a particular family to a database of curated sequences containing PFAM domains which are representative of certain protein functions/families (Finn et al., 2014). Protein secretion was predicted by SignalP 4.1 (Petersen et al., 2011).

*Tetranychus urticae* transcriptomic data can be retrieved at the Gene Expression Omnibus database using the GSE68708 accession number.

5.2.5. **TOMATO TRANSCRIPTOMICS**

Tomato infestation was performed by transferring 100 adult female mites to terminal leaflets of leaves 3 and 4 on 1-month-old tomato plants (Cazaux et al., 2014). Twenty-four hours post infestation, infested leaves were collected and tissues from two plants were pooled to produce a single biological replicate. Initially, two biological replicates were collected per control (non-treated plants), RB-mites and per individual A-line. As analysis of tomato responses indicated that the individual A-lines per group elicited non-distinguishable responses, A-lines were grouped to derive two conditions: AT and ABT (each containing six replicates). Additional replicates were sampled for control and RT-mites (up to final four replicates each). Total RNA was prepared using the RNeasy Plant RNA extraction kit (Qiagen, Venlo, Netherlands). Analysis was performed using the Bioconductor framework (Gentleman et al., 2004). Initial data quality assessment was conducted using arrayQualityMetrics (Kauffmann et al., 2009). Expression measures were computed using RMA on complete data set (Irizarry et al., 2003). Batch effect was removed using ComBat with non-parametric estimation of priors (Johnson et al., 2007). Detection of differentially expressed genes was performed using limma with BH-corrected p-values at FDR cut-off of 0.05 (Benjamini & Hochberg, 1997; Smyth, 2004).

The annotation of the tomato arrays was based on (Martel et al., 2015). GO analysis was performed using topGO with Fisher’s test statistic and ‘weight01’ algorithm (Alexa et al., 2006) and expanded tomato GO annotation (Martel et al., 2015) to generate a list of top 50 Biological Process GO annotations and annotate lists of genes that were detected as differentially expressed. The lists were further filtered by applying a cut-off of 0.05 to Fisher’s weighted p-values.

GSA was performed using a custom version of Bioconductor package piano (Varemo et al., 2013). Log2FC, p- and t-values obtained using limma were
used as the input gene level statistics for analysis. Following a comparison of available gene set analysis methods, the PAGE algorithm (Kim & Volsky, 2005) was employed. Biological Process Gene Ontology annotation was used to classify genes into sets. We limited analysis to gene sets which had at least five genes associated with them and used BH-corrected p-value cut-off of 0.05 to determine significance of distinct up- or down-regulation of a gene set.

5.3. RESULTS

5.3.1. ADAPTATION OF T. urticae TO TOMATO

Using a genetically diverse T. urticae population (Van Leeuwen et al., 2012), growing on bean (B) for more than 5 years, as a reference line (RB-line), we set up selection lines in three replicates on tomato and allowed the lines to propagate for 30 generations (AT-lines) (M&M, Figure 5.1A). Adaptation is commonly defined as the process whereby gene frequency alterations in a population result in an increased reproductive performance of that population in its environment. Therefore, to validate genetic adaptation in this study, we compared the reproductive performance of the reference line with the selected mite lines on tomato. To minimize maternal and host plant effects (such as tomato induced gene-expression), we grew tomato-selected populations for two generations on bean, creating the AB populations (see M&M and Figure 5.1A for detailed description). Next to the performance on tomato, we also included pepper (as a member of the same Solanaceae plant family as tomato) and cucumber (as a member of a different plant family; Cucurbitaceae) in the experiments to look at the specificity of adaptation.

Performance, quantified as the total population sizes 10 days after the initial inoculation, differed significantly among the four host plants (Figure 5.2A; host plant effect: $F_{3,6} = 65.54; p < 0.0001$), with performance being significantly lower on pepper and tomato across selection lines. However, these differences in performance differed significantly between the two selection regimes (host plant * selection regime interaction: $F_{3,6} = 21.38; p = 0.001$). On tomato, RB-mites had a significantly lower performance compared to AB-mites (Tukey post-hoc; $p < 0.0001$). For the other host plants, no significant differences in performance on the same host plant (including bean) were observed between RB- and AB-mites ($p$ all > 0.3), both when analyzed individually or as a group. This indicates that long-term selection of mites on tomato resulted in adaptation. As performance between RB- and AB-mites did not differ on the other
tested plants, regardless of whether they belonged to the same or different plant family, this adaptation was specific to tomato.

5.3.2. Effect of Genetic Adaptation on Mite Gene-expression

As performance tests revealed that the long term propagation on tomato resulted in adaptation (FIGURE 5.2A), we subsequently sampled the transcriptomes of: (1) the reference (non-selected) line reared on bean (RB), (2) the reference line transferred to tomato for 24 h (RT), (3) the adapted lines reared on tomato (AT), (4) the adapted lines grown for two generations on bean (AB) and (5) finally the adapted lines grown for two generations on bean, but then re-introduced to tomato for 24 h (ABT). Using the transcriptomic profile of RB-mites as a common reference, this dataset captured a total of 1,275 differentially expressed genes in at least one of the other four conditions (FDR-corrected p < 0.05 and log2FC >1). Subsequently, we clustered these 1,275 genes based on their expression across the different conditions using Pearson Correlation distances to get an overview of the transcriptional patterns (FIGURE 5.1B). Differentially expressed genes were grouped into four clusters that identified two overall contrasting patterns: cluster 1 (147 genes) and cluster 3 (48 genes) consisted of genes that were respectively up- and down-regulated on tomato (AT), but not when the adapted lines were growing on bean (AB). Genes in these clusters also responded to the re-introduction to tomato (ABT) and can

**FIGURE 5.2.** A: Total population sizes of AB- and RB-mites on diverse host plant species. Population sizes were counted 10 days after initial inoculation. Letters and asterisks demark significant differences of population sizes between and within host plants, respectively. B: Tomato leaf damage by spider mite feeding for 24 h. Foliar damage was calculated using chlorotic spots (in mm²) as described in Kant et al. (2004).
be considered highly host plant dependent since their expression levels correlated with feeding on either tomato or bean. The majority of these genes were up-regulated. Cluster 2 (376 genes) and cluster 4 (704 genes), representing the vast majority of differentially expressed genes (84.7%), comprised of genes whose expression depended primarily on the selection treatment rather than host plant exposure. The majority of these host plant independent genes were down-regulated.

To identify true host plant independent (or constitutive) differential gene-expression associated with host plant adaptation, we then only considered those genes that were consistently differentially expressed in the same direction in every single contrast of AT, AB and ABT vs RB and subsequently clustered them based on their expression patterns (Figure 5.3A). This criteria showed that long term exposure to tomato resulted in a constitutive change in expression of a total of 318 genes of which the majority (n = 256: 80.50%) was constitutively down-regulated in adapted mites, relative to non-adapted mites on bean. Of these constitutively down-regulated genes, only 2.34% (n = 6) showed a plastic response in the ancestral population, while in the cluster of constitutive up-regulated genes, 6.45% (n = 4) exhibited transcriptional plasticity in the ancestral population when exposed to tomato (RT vs RB) (Figure S5.1A).

In addition, clustering of the overall responses in our experiment also pointed towards an interaction between selection treatment and tomato exposure (clusters 1 and 3 of Figure 5.1B). To determine if a significantly different transcriptional plasticity was selected in adapted mites, we first assessed relative gene-expression levels in adapted mites when they were grown on bean for two generations and were re-introduced to tomato (ABT vs AB). Next, we contrasted these relative expression levels to those in the reference line 24 h on tomato and on bean (RT vs RB). This contrast identified 139 genes that differentially responded to tomato exposure between reference and adapted lines, indicating that tomato adaptation also altered mite transcriptional plasticity (Figure 5.3B). Over 65% of this gene set showed a higher induction upon tomato exposure in the adapted mites compared to the reference line (Figure 5.3B). In addition, by comparing the transcriptomes of the ABT- and RT-lines directly, we show that higher transcriptional plasticity indeed led to increased (43 genes) or decreased (27 genes) absolute transcript levels respectively (with a 2-fold filtering cut-off) (Figure 5.3B, red-white-blue sidebar). Of the 139 differentially responding genes upon tomato exposure, 30.22% (n = 42) already showed a significant transcriptional plasticity in the ancestral population (Figure S5.1B).
In conclusion, adaptation to tomato mainly led to a constitutive down-regulation of a large set of genes, while it also increased the inducible response of a set of host plant dependent and mainly overexpressed genes. Together, both criteria identified a total set of 444 genes that were associated with adaptation to tomato in the polyphagous mite *T. urticae*.

In order to decipher the biological function(s) of the differentially expressed mite genes, we assigned curated Gene Ontology (GO)-terms to the *T. urticae* genome indicative of specific biological processes. Using the four comparisons shown in Figure 5.1B, a total of 40 GO-terms were enriched in the transcriptional differences of the experiment. Typical stress-induced GO-terms were present, such as ‘response to acid’, ‘response to wounding’ and ‘drug metabolic process’ (Table 5.1). Of the 318 constitutively down-regulated genes (Figure 5.3A), only 20.1% was assigned a GO-term and had a homol-

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**Figure 5.3.** A: A gene-expression heatplot of genes with a constitutive (host plant independent) differential expression in adapted vs non-adapted mites. Using our defined criteria, 318 genes showed a constitutive change in expression upon adaptation. Genes were clustered (Euclidean, ward) based on their transcript levels in the AT:RB, AB:RB and ABT:RB comparisons. B: A gene-expression heatplot of genes that respond differently to tomato exposure in adapted vs non-adapted mites. By the (ABT:AB):(RT:RB) comparison, 139 genes showed a differential transcriptional plasticity to tomato exposure as a consequence of adaptation. Genes were clustered (Euclidean, ward) based on their transcript levels in the ABT:AB and RT:RB comparisons. The sidebar labelled with * indicates whether the transcript level of a gene was not (white), twice as high (red) or twice as low (blue) differentially expressed in the ABT- vs RT-populations (FDR-corrected \( p \)-value <0.05). In the sidebar labelled with †, green and orange bars indicate *CYP* and *MFS* genes, respectively.
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</table>

Enriched BP GO-terms in all differentially expressed genes in our experimental evolutionary set-up (FIGURE 5.1A) were identified by a Fisher’s exact test using the complete *T. urticae* genome as a reference and FDR-corrected p-value < 0.05 as cut-off. Column ‘DEG’ indicates the total number of differentially expressed genes linked to each GO-term while column ‘Total’ shows the total number of genes present in the *T. urticae* genome associated with the GO-term. Columns ‘CON’ and ‘PLAS’ show the number of genes linked to the GO-terms that were constitutively differentially expressed and exhibited differential transcriptional plasticity as a consequence of the host plant adaptation, respectively (FIGURE 5.3). In these columns, red and blue backgrounds indicate significant up- and down-regulation, respectively.
ogy to other proteins in the public non-redundant NCBI protein database (e-value cut off of 1e-15). No biological function to this constitutive differential expression could be determined using GO-annotation as none of the associated GO-terms were significantly up- or down-regulated. In contrast, for the gene set showing an altered transcriptional plasticity towards tomato exposure (FIGURE 5.3B), 39.50% was assigned a GO-term of which five terms were either significantly up- or down-regulated (TABLE 5.1). *T. urticae* genes connected to the four up-regulated GO-terms are commonly associated with the xenobiotic metabolism.

As the majority of the gene families associated with detoxification and adaptation to xenobiotics was previously manually annotated in *T. urticae* (Ahn et al., 2014; Dermauw et al., 2013a,b; Grbic et al., 2011), we also specifically investigated the presence of these large multi-gene families in the gene sets showing differential constitutive expression and transcriptional plasticity upon adaptation. Table 5.2 shows that 8.1% of all the detoxification genes were differentially regulated following adaptation. Intradiol ring-cleaving dioxygenases (ID-RCDs), a unique spider mite gene family (Dermauw et al., 2013b), had the highest percentage of differential regulation (28.6%), followed by glutathione-S-transferases (15.6%) and cytochrome P450 monoxygenases (CYPs) (13.3%). These detoxification genes represented 9.9% of the total number of genes affected by host plant adaptation.

As evidenced by Table 5.2, the enhanced transcriptional plasticity of adapted mites upon tomato exposure incorporated more enzymes that transport, bind and metabolize xenobiotics. Furthermore, of these 25 detoxification genes, the higher induction of 13 genes led to twice as high absolute expression levels in adapted vs non-adapted mites 24 h on tomato (ABT vs RT). Of particular notice, of these 13 detoxifying genes, four and five genes coded for CYP enzymes and xenobiotic transporters of the Major Facility Superfamily (MFS), respectively (FIGURE 5.3B).

5.3.3. MITE ADAPTATION TO TOMATO AFFECTS HOST PLANT RESPONSES

To determine if mite adaptation has an effect on plant host physiology, we examined tomato responses to herbivory of RB-, AB- and AT-mites. Initially, we measured the extent of chlorotic area on tomato leaves that resulted from mite feeding, as a proxy for their feeding intensity (FIGURES 5.2B and S5.2). Tomato leaves developed around ten times greater chlorotic surface area upon
feeding of adapted vs non-adapted mites, indicating that adapted mites gained the ability to feed more on tomato leaves. To determine the induced tomato responses associated with an increased feeding intensity of adapted mites, we compared the genome-wide transcriptional responses of tomato plants upon 24 h infestation by RB-, AB- and AT-mites (red coded in FIGURE 5.1A), using non-infested plants as a control. A total of 1,512 differentially expressed genes were detected in at least one of the sample sets after a 24 h infestation (FDR-adjusted p < 0.05) (FIGURE S5.3). Interestingly, despite the strong increase of plant damage produced by AT-mites (FIGURE 5.2B), which was expected to increase the number of differentially expressed tomato genes and the magnitude of the tomato induced responses, the numbers of genes induced by R- and A-mites relative to untreated plants were similar: 909 differentially expressed genes were detected in response to RB-mites, 706 in response to AB-mites and 811 in response to AT-mites. Furthermore, the identity of induced plant responses was similar as seen by the large overlap in differentially expressed genes induced by these mites (with 317 genes differentially expressed in all responses; FIGURE S5.3). However, a part of the induced responses was distinct, as 335 differentially expressed genes were detected between the tomato

<table>
<thead>
<tr>
<th>Phase</th>
<th>Family</th>
<th>Total</th>
<th>Constitutive</th>
<th>Plastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformation</td>
<td>Cytochrome P450 monoxygenases</td>
<td>75</td>
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</tr>
<tr>
<td></td>
<td>Carboxyl/choline esterases</td>
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<td></td>
<td>Intradiol ring-cleaving dioxygenases</td>
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<td>Translocation</td>
<td>MFS transporters*</td>
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<tr>
<td></td>
<td>ABC transporters</td>
<td>96</td>
<td>0</td>
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</tr>
</tbody>
</table>

Overall sum 543 19 25

Eight multi-gene families in T. urticae serving potential detoxification functions are organized according to their phase in the xenobiotic metabolism. The total number of family members was counted present on the array. Gene-expression changes associated with adaptation were divided based on their expression profile across host plants: ‘Constitutive’ and ‘Plastic’. *: Identification of MFS and lipocalin genes was based on significantly assigned PFAM-domains PF07690.11, and PF00061.18 and PF08212.7, respectively (Finn et al., 2014).

### Table 5.2. Effect of host plant adaptation on the transcription of T. urticae multi-gene families that code for enzymes with inferred roles in its xenobiotic metabolism.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Family</th>
<th>Total</th>
<th>Constitutive</th>
<th>Plastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformation</td>
<td>Cytochrome P450 monoxygenases</td>
<td>75</td>
<td>2</td>
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<td>Carboxyl/choline esterases</td>
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<td></td>
<td>Intradiol ring-cleaving dioxygenases</td>
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<td>Conjugation</td>
<td>Glutathione S-transferases</td>
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<td>UDP-glycosyltransferases</td>
<td>78</td>
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<td></td>
<td>Lipocalins*</td>
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<td>Translocation</td>
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<td></td>
<td>ABC transporters</td>
<td>96</td>
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</tbody>
</table>

Overall sum 543 19 25

Eight multi-gene families in T. urticae serving potential detoxification functions are organized according to their phase in the xenobiotic metabolism. The total number of family members was counted present on the array. Gene-expression changes associated with adaptation were divided based on their expression profile across host plants: ‘Constitutive’ and ‘Plastic’. *: Identification of MFS and lipocalin genes was based on significantly assigned PFAM-domains PF07690.11, and PF00061.18 and PF08212.7, respectively (Finn et al., 2014).
response to RB-mites and at least one of the tomato responses to adapted mites (AT- or AB-mites). About \( \frac{2}{3} \) of the 335 genes had attenuated expression in tomato challenged by adapted mites (AB and AT), relative to RB-mites (FIGURE 5.4B). Thus, the adaptation to tomato not only affected mite gene-expression, it also dampened the transcriptional responses of tomato host.

To understand the biological effect of mite adaptation on the induced tomato responses, we analyzed the set of 1,512 differentially expressed tomato genes for biological processes that changed as a result of mite herbivory. Based on the expanded Biological Process Gene Ontology annotation (Martel et al., 2015), 41 gene sets (or categories) were detected as significantly differentially regulated across all conditions. Specifically, in response to non-adapted mites (RB), 39 gene sets showed a differential regulation (FIGURES 5.4A and S5.4). In this network, several up-regulated functional categories were related to herbivore attack (with categories such as ‘response to wounding’, ‘response to stress’, ‘response to endoplasmic reticulum stress’, categories associated with jasmonic acid signaling and biosynthesis and finally categories associated with the activation of proteinase inhibitors, PIs). The down-regulated categories, which represented a substantial proportion of the tomato transcriptional response to RB-mite herbivory (14 of 39), were associated with anabolic processes and growth regulation. In contrast, only 16 and 14 categories were detected as differentially regulated in response to AB- and AT-mites, respectively. Here, core plant responses such as ‘response to wounding’, jasmonic acid associated responses, and activation of PIs were still strongly up-regulated (FIGURES 5.4A and S5.4). However, the distinct down-regulation of anabolism-associated processes observed upon RB-mite feeding was no longer detected as significant. In addition, the activation of programs associated with amino acid metabolism was

**Figure 5.4. A:** Gene set Analysis of biological processes for differentially expressed genes detected in tomato upon herbivory of adapted and non-adapted mites. Nodes and edges represent gene sets and overlap in genes belonging to connected gene sets, respectively. Gene sets: blue – down-regulated, red – up-regulated, grey – not detected as differentially expressed. Node size corresponds to number of genes in a given gene set (5 to 142). Labels are BP GO IDs. Edges: color (grey to red) and width correspond to an overlap size (1 to 23). The associated BP GO category term names are presented in Figure S5.4. **B:** Clustering analysis and heatmap of expression measures of 335 differentially expressed genes detected between tomato responses to adapted and the responses induced by non-adapted mites. Hierarchical clustering of genes and samples was performed using Pearson’s correlation as distance metric with average linkage method.
attenuated in response to AB-mites and not detected as differentially expressed in response to AT-mites (Figures 5.4A and S5.4). Differentially expressed genes represented by these amino acid metabolism associated categories included genes coding for a number of polyphenol oxidases (PPOs), phenylalanine ammonia-lyase (PAL), and genes encoding enzymes in chorismate and shikimate plant pathways. These genes were previously implicated as anti-feedant factors (PPOs) and enzymes involved in production of defensive secondary metabolites (phenylpropanoids) and salicylic acid (SA). Increase of SA concentration and JA-SA pathway cross-talk is a hallmark of the establishment of a sustained (post 24 h) plant defense response (Thaler et al., 2002, 2012), which may be disrupted by herbivory of adapted mites. Two up-regulated programs, ‘cell wall macromolecule catabolism’ and ‘jasmonic acid mediated signaling pathway’ were specifically detected in response to feeding of adapted mites. These programs represent tomato responses that correlate with the increased level of feeding exhibited by adapted mites. Thus, these results indicate that during adaptation, mite populations developed the ability to attenuate a subset of tomato induced defense responses, including the biosynthesis of enzymes that act in the herbivore’s gut to decrease the nutritional value of ingested plant tissue (Thipyapong et al., 1997).

5.4. DISCUSSION

5.4.1. HERBIVORE ADAPTATION

When the reference and adapted populations were reared for two generations on the ancestral host to minimize environmental and maternal effects, performance and foliar feeding intensity on tomato was significantly higher in tomato-selected mites (Figure 5.2). This confirms that genetic adaptation occurs in spider mites as shown by previous studies (Fry, 1989; Gould, 1979). Adaptation of a herbivore to a particular host plant may impact its potential host plant range depending whether adaptation involves no, negative, or positive genetic correlations of fitness across host plants (Savolainen et al., 2013). As negative genetic correlations in fitness lead to host specialization that limits the potential host range, polyphagous herbivores are predicted to develop no or positive correlations since this will result in broadening the potential range of host species they can colonize (Agrawal, 2000; Ehrlich & Raven, 1964; Gould, 1979). Our study shows that adaptation to tomato did not lead to significant fitness costs for feeding on the ancestral bean host. Moreover, bell pepper and cucumber
remained equally challenging for tomato-adapted and tomato non-adapted mites, although they belong to the same (Solanaceae) and a different (Cucurbitaceae) plant family respectively, and produce a different set of phytochemicals and defensive structures (Harborne & Baxter, 1999; Wagner, 1991) (Figure 5.2A). These results indicate that adaptation to tomato effectively increased host plant range and are consistent with other studies showing that spider mite adaptation has a high specificity (Agrawal, 2000; Fry, 1989).

5.4.2. Effect of genetic adaptation on transcriptional regulation in polyphagous herbivores

Polyphagous herbivores like T. urticae modify the expression of genes coding for digestion, detoxification and regulatory proteins on a large scale when feeding on different host plants, most likely to cope with the varying nutritional value (Celorio-Mancera et al., 2013; Dermauw et al., 2013b). However, the role and interaction of genetic versus environmental factors underlying these gene-expression changes in a generalist has not yet been investigated. Here, we unbiasedly showed that the full transcriptomic response of the arthropod generalist T. urticae was not merely the result of direct exposure to a novel host but was also genetically determined and evolvable upon adaptation. About 44.7% (444 out of 994) of the differential expression between the adapted and ancestral mite populations, developing on their respective host, could be attributed to genetic adaptation. Adaptation can alter gene-expression in two ways: by changing its constitutive transcript levels and by altering its transcriptional plasticity. Our study now shows that host plant adaptation in a polyphagous herbivore affects its transcriptome in both ways (Figure 5.3).

5.4.2.1. Effect of host plant adaptation on constitutive gene-expression

The majority of genes with altered expression levels associated with host plant adaptation showed constitutive down-regulation in adapted mites and appeared non-responsive to direct host plant exposure (Figure 5.3A). Down-regulation of genes coding for the transcription and translation machineries as well as for other core metabolic enzymes has previously been implicated in the adaptive responses to environmental stress (Causton et al., 2001; Marden, 2013; Yampolsky et al., 2014). However, no enriched biological functions in the set of the respectively 62 and 256 constitutively up- and down-regulated genes (Figure 5.3A) could be identified. Gene ontology based enrichment studies in arthropods are often difficult due to the large number of proteins without data-
base homology and of unknown function, due to two main reasons. First, despite the increasing number of available arthropod genomes, the phylogenetic distances remain substantial. For instance, in the *T. urticae* genome, over 8,000 genes were identified as mite-specific, lacking homologues in other species (Grbic et al., 2011). Second, functional genomics approaches that identify the biochemical functions of newly discovered genes mainly focus on a few insect model organisms and are not developed for the great majority of arthropods.

A previous study investigated the full transcriptional responses of *T. urticae* to tomato feeding and to acaricide resistance. Although they did not provide information regarding the tomato adaptation status and the associated gene-expression changes, Dermauw et al. (2013b) did identify gene clusters (obtained by ortho-MCL clustering) containing a significant number of genes that are responsive to tomato exposure and acaricide resistance. One of these clusters defines a family of secreted hypothetical proteins (referred to as 10289 in Dermauw et al., 2013b) that is up-regulated in mites reared on tomato, but also in acaricide multi-resistant strains. In our study, five members of this cluster were constitutively up-regulated (TABLE S5.1), and *tetur31g00810* showed the highest average constitutive up-regulation in adapted mites. (Dermauw et al., 2013b) also identified novel *T. urticae* gene families with a predicted regulatory function. These genes coded for proteins with regulative TUDOR-, NYN- or BTB/BACK-domains (Anantharaman & Aravind, 2006; Misra et al., 2011; Ying & Chen, 2012). We found that members of these putative regulatory families were constitutively down-regulated upon genetic adaptation (TABLE S5.2). The BTB/BACK-domain protein families are related to the *Drosophila* Kelch-like domain proteins that coordinate xenobiotic metabolism in *Drosophila melanogaster* (Misra et al., 2011). Therefore, these families might regulate the transcriptional modifications within the *T. urticae* xenobiotic metabolism upon different diets, a crucial trait for polyphagous herbivores.

As in *D. melanogaster*, the xenobiotic metabolism of polyphagous herbivores relies on multi-gene families that interact with dietary toxic and anti-nutritional compounds. Our Gene Set Analysis (GSA) did not reveal a significant enrichment of GO-terms in the list of genes that were constitutively down- or up-regulated upon adaptation. Indeed, only 19 genes coding for proteins with inferred roles in the *T. urticae* xenobiotic metabolism constitutively changed their expression levels (TABLE 5.2).
5.4.2.2. Effect of host plant adaptation on transcriptional plasticity

In addition to constitutive changes, a smaller set of mite genes also showed an altered transcriptional plasticity due to host plant adaptation. GSA identified a significant up-regulation of GO-terms associated with xenobiotic metabolism (‘drug metabolic process’, ‘antibiotic metabolic process’ and ‘lipid modification’) (Table 5.1). Adapted mites recruited more components of their detoxification repertoire when re-exposed to tomato (Table 5.2 and Figure 5.3B). One explanation is that there was selection for more genes to be inducible by tomato exposure, or for regulators such as ‘xenosensors’ to react more rapidly to the tomato chemical blend, resulting in a higher activation of transcription. Indeed, in herbivorous arthropods, induction of CYPs and other enzymes of xenobiotic pathways often determines if a population can feed on a specific plant (Despres et al., 2007; Feyereisen, 1999). Compared to non-adapted mites, adapted mites seemed to respond more rapidly and strongly in CYP expression when confronted with the tomato chemical blend.

Herbivores often handle plant defenses by producing enzymes that translocate plant allelochemicals across membranes (Sorensen & Dearing, 2006). Although multi-drug ABC transporters have traditionally been regarded as key components in this translocation process (Dermauw et al., 2013a), our study did not find any genetic change in their expression. However, five genes coding for transporters of the Major Facilitator Superfamily (MFS), a family of transporters less studied compared to ABCs, showed a higher induction in adapted mites upon tomato exposure (Figure 5.3B). The potential role for MFS transporters in the xenobiotic metabolism of herbivores has only recently been uncovered (Celorio-Mancera et al., 2013; Dermauw et al., 2013b) and finds support in this study.

The set of differentially responding genes to tomato exposure also included ID-RCDs and UDP-glycosyltransferases (UGTs), unique spider mite gene families, as these were horizontally transferred from fungal and bacterial donor species, respectively (Ahn et al., 2014; Dermauw et al., 2013b; Grbic et al., 2011). The genetic change in their transcription upon adaptation further supports the hypothesis that horizontal gene transfer drives adaptive evolution in phytophagous spider mites (Ahn et al., 2014; Dermauw et al., 2013b; Grbic et al., 2011; Wybouw et al., 2014). When looking at the T. urticae families of genes coding for putative regulatory enzymes identified by Dermauw et al. (2013b), nine regulatory F-box-like genes and 19 PAN-domain/HMG-box genes showed increased responsiveness, showing that adaptation not only affected
Adaptation Shapes Transcriptome of Mite and Host

their constitutive gene-expression, but also their transcriptional plasticity upon transfer to tomato (TABLE S5.2).

However, for the whole set of genes with altered transcriptional plasticity, we could not experimentally address whether differences in gene induction in mites also (partially) reflected an altered chemical blend of tomato. Indeed, adapted mites triggered a more dampened plant response and potentially encountered different concentrations of different chemicals than non-adapted mites.

In addition to the plant allelochemical – herbivore detoxification interface, the digestion of plant proteins is also a platform for major antagonistic evolution (Hochuli, 1996; Jongsm & Bolter, 1997). It was shown by (Santamaria et al., 2012) that transgenic Arabidopsis plants over-expressing plant PIs are more resistant to spider mite feeding and affect mite proteinase activity. Tomato defenses that counteract spider mite feeding include the induction of PI expression and activity (Kant et al., 2004; Martel et al., 2015). Indeed, our tomato transcriptome analysis showed that spider mite feeding induced the expression of PIs upon attack by both non-adapted and adapted mites (FIGURE 5.4). However, herbivores can become resistant to these inducible defenses by changes in their ‘digestome’ (Gruden et al., 2004; Jongsm & Bolter, 1997). Here, by changing the expression of 16 proteases (GO:0006508 proteolysis) due to genetic adaptation (TABLE 5.1), adapted mites might be less affected by the tomato PI defenses.

5.4.3. Herbivore adaptation affects plant induced responses

Inducible plant responses are regulated by a set of phytohormones that include jasmonic acid (JA) and lead to the synthesis of a wide range of toxic secondary metabolites (e.g. phenylpropanoids, flavonoids, anthocyanins, alkaloids, terpenoids and glucosinolates), and anti-nutritive enzymes and proteins (e.g. PIs, amino acid catabolizing enzymes, PPOs and peroxidases). However, a number of arthropod herbivores are able to suppress plant defenses (Alba et al., 2011; Elzinga & Jander, 2013; Musser et al., 2002; Mutti et al., 2008). Although both intra- and interspecific genetic variation is demonstrated for this trait in tetranychid mite species, no evidence exists if the ability to suppress or attenuate plant defenses is evolvable by long-term exposure to a certain host (Alba et al., 2015; Kant et al., 2008; Matsushima et al., 2006). Here, we found that mites acquired the ability to manipulate host’s physiology during an adaptation process, resulting in an attenuation of induced responses. The observed attenuation could
result either from the lack of recognition of herbivory (for example by sequestering or down-regulating the elicitors of plant responses upon feeding of adapted mites), or alternatively, adapted mites might actively suppress plant responses through the employment of effectors that will interfere with the proper regulation of plant induced responses. Although our data do not allow us to distinguish between these possibilities, attenuation of only a subset of tomato responses would suggest that herbivory was still recognized by tomato, but partially suppressed. Regardless of the mechanism of response attenuation, it occurs in tomato plants when challenged by both AB- and AT-mites, indicating that changes in mite gene-expression leading to the manipulation of plant responses were dependent on the selection regime and independent of the immediate plant host (thus are part of the constitutive mite response to adaptation). Furthermore, as attenuation of responses are greater when tomato plants were infested by AT- relative to AB-mites, expectation is that mite genes contributing to the manipulation of tomato responses will be constitutive, but showing an enhanced transcriptional change upon feeding on tomato.

The attenuated tomato responses included genes associated with amino acid metabolism and secondary metabolite biosynthesis pathways (that are induced), and genes associated with the anabolic processes (that are repressed). Such attenuated response is expected to result in reduced concentrations of tomato defense compounds and failure to alter resource allocation between attacked leaflets and the rest of the plant. Interestingly, the alteration of tomato responses appeared to be downstream of the highly conserved JA-dependent signaling cascade, affecting only a subset of tomato responses (e.g. activation of PIs is still present) (FIGURE 5.4A). If mite adaptation to tomato resulted in depletion of proteins that elicit plant response, attenuation of only a subset of tomato responses would suggest the existence of independent elicitation pathways. This would contrast patterns observed for plant responses to pathogens, where responses to elicitation through independent receptors converge in the MAP-kinase signal transduction pathway (Jones & Dangl, 2006). If mite adaptation to tomato results in suppression of plant responses, then, effector(s) intercept transcriptional regulation of the tomato responses downstream of the JA-signaling. Suppression of plant defenses by herbivores has been described in several cases (Alba et al., 2015; Elzinga & Jander, 2013; Musser et al., 2002; Mutti et al., 2008), and is typically achieved through saliva-mediated secretion of effectors into the plant tissue where they interfere with plant responses (Bos et al., 2010; Hogenhout & Bos, 2011). In adapted mites,
93 of the 318 constitutive differentially expressed genes and 38 of the 139 genes with differential transcriptional plasticity were predicted to be secreted (using SignalP 4.1, with default settings for eukaryotes) and could be considered in future studies for their effect on plant defenses.

In summary, at least two main processes underlie T. urticae adaptation to tomato: (1) transcriptional responses in the xenobiotic metabolism of mites, responsible for the detoxification of plant defense compounds, and (2) mite transcriptional responses that reduce the production of plant defense compounds by an attenuation of a subset of induced tomato defenses.

5.4.4. TRANSCRIPTIONAL PLASTICITY AS A MEDIATOR FOR GENERALIST ADAPTATION

Environmentally induced responsiveness (or plasticity) plays an integral role in plant–herbivore interactions. Herbivory induces plant defenses that in turn amplify the herbivore’s xenobiotic metabolism (Li et al., 2002; Schuler, 1996). Presently, two views exist to what extent these environmentally induced responses facilitate subsequent genetic adaptation.

In a first view, the expression of a phenotypic (or here: transcriptional; Renn & Schumer, 2013) trait is considered to be relatively independent of the environment and adaptation primarily occurs by changing the constitutive expression of genes. In second alternative view, exposure to a new environment induces an expression response and adaptation acts on this in two main processes (Pfennig et al., 2010). Through natural selection, the induced expression response may either change in a constitutive up- or down-regulation, a process referred to as genetic assimilation (Pigliucci et al., 2006; Waddington, 1953) or in the degree and pattern of plasticity, a process called genetic accommodation (Schlichting & Wund, 2014; Suzuki & Nijhout, 2006; West-Eberhard, 2003). In the processes of genetic accommodation and assimilation, natural selection thus mainly acts on the genes showing initial transcriptional plasticity and, in contrast to the view where genes are mainly constitutively regulated, responding and non-responding transcripts are not equally affected.

It is clear that assessing the role of transcriptional plasticity in adaptation necessitates a proper quantification of gene-expression responses before the onset of selection. Much of the current debate emerges from the fact that most studies focus on the evolutionary endpoints, yielding little information on ancestral state plasticity. Our study of experimental evolution of mite adaptation is now able to examine ancestral transcriptional plasticity by comparing the
reference line 24 h on tomato (RT) with the reference line reared on bean, the ancestral host (RB) (FIGURE 5.1A) and therefore represents a unique opportunity to investigate the role of ancestral plasticity in evolution. Comparing the transcriptional plasticity of non-adapted mites with the transcriptional changes affiliated with adaptation, we uncovered only 10 and 42 genes that qualified for genetic assimilation and genetic accommodation, respectively (FIGURE S5.1). Interestingly, 5/42 of the genetically accommodated genes coded for CYP and MFS enzymes (FIGURE S5.1). Our study shows that although 27.7% of the ancestral transcriptional plasticity was involved in genetic adaptation, the adaptation of *T. urticae* to tomato mainly involved genes that did not show an initial expression response to tomato. These results show that the great adaptive potential of *T. urticae* is mainly due to the standing genetic variation in constitutive expression within a population and not cryptic variation uncovered by its highly inducible transcript regulation. These results also indicate that even though the transcriptional plasticity of *T. urticae* might increase its short-term reproductive performance upon an initial exposure to a novel plant, it is most likely not a major factor in subsequent adaptation.

5.5. REFERENCES


Chapter 5


Adaptation Shapes Transcriptome of Mite and Host


Adaptation Shapes Transcriptome of Mite and Host


**Supplementary material**

**Figure S5.1.** A: A gene-expression heatplot of genes that showed transcriptional plasticity in the ancestral, non-selected population and exhibited differential constitutive expression upon tomato adaptation. Genes were clustered (Euclidean, ward) based on their transcription in the RT:RB, AT:RB, AB:RB and ABT:RB comparisons. B: A gene-expression heatplot of genes that showed transcriptional plasticity in the ancestral, non-selected population and exhibited a differential transcriptional plasticity upon tomato adaptation. Genes were clustered (Euclidean, ward) based on their transcription in the ABT:AB and RT:RB comparison. In the sidebar labelled with †, green and orange bars indicate CYP and MFS genes, respectively.
### Table S5.1. Constitutive differential gene-expression associated with host plant adaptation within a novel secreted hypothetical protein family in *Tetranychus urticae*.

<table>
<thead>
<tr>
<th>TeturID</th>
<th>AT vs RB</th>
<th>AB vs RB</th>
<th>ABT vs RB</th>
</tr>
</thead>
<tbody>
<tr>
<td>tetur31g00810</td>
<td>3.01</td>
<td>4.50</td>
<td>6.04</td>
</tr>
<tr>
<td>tetur31g00690</td>
<td>2.89</td>
<td>3.34</td>
<td>4.55</td>
</tr>
<tr>
<td>tetur31g00830</td>
<td>2.42</td>
<td>3.92</td>
<td>5.25</td>
</tr>
<tr>
<td>tetur14g02720</td>
<td>2.33</td>
<td>1.60</td>
<td>2.71</td>
</tr>
<tr>
<td>tetur31g01870</td>
<td>2.30</td>
<td>2.30</td>
<td>3.79</td>
</tr>
</tbody>
</table>

Identification of the protein family was based on an ortho-MCL clustering algorithm performed by Dermauw et al. (2013b). For each gene, the log2FC in every comparison is given, using the transcriptome of RB-mites as a common reference. *T. urticae* gene IDs can be accessed at [http://bioinformatics.psb.ugent.be/orcae/overview/Tetur](http://bioinformatics.psb.ugent.be/orcae/overview/Tetur).

### Figure S5.2. Tomato leaf damage by adapted and non-adapted mites. A: Damage caused by non-adapted mites (RB). B: Damage caused by bean-reared adapted mites (AB). C: Damage caused by tomato-reared adapted mites (AT). All mites were allowed to feed for 24 h.

### Figure S5.3. Venn diagram of differentially expressed tomato genes after a 24 h infestation of adapted (AT and AB) and non-adapted (RB) mites, relative to non-infected control plants (FDR-corrected p-value < 0.05) (see Material & Methods for more details of infestation procedures).
Adaptation Shapes Transcriptome of Mite and Host

Table S5.2. Differential gene-expression as a consequence of host plant adaptation in novel *T. urticae* regulatory gene families.

<table>
<thead>
<tr>
<th>Family description</th>
<th>Family nr</th>
<th>Total</th>
<th>Constitutive</th>
<th>Plastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTB/BACK-domain proteins</td>
<td>10254</td>
<td>17</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>BTB/BACK-domain proteins</td>
<td>10034</td>
<td>57</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>NYN domain proteins</td>
<td>10040</td>
<td>54</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>PAN-domain/HMG-box proteins</td>
<td>10010</td>
<td>124</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>F-box-like proteins</td>
<td>10003</td>
<td>234</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Identification of the protein families was based on an ortho MCL clustering algorithm performed by Dermauw et al. (2013b). Dermauw et al. (2013b) identified these clusters by the numbers shown in the ‘Family nr’ column. The total number of cluster members was counted present on the mite array. Columns ‘Constitutive’ and ‘Plastic’ show the number of mite genes that exhibited constitutive differential expression and differential transcriptional plasticity as a consequence of host plant adaptation, respectively (FIGURE 5.3).

**Figure S5.4.** Reference PAGE network based on BP GO annotation with significantly enriched up- and down-regulated gene sets detected in tomato plants upon herbivory of adapted and non-adapted mites. Nodes and edges represent gene sets and overlap in genes belonging to connected gene sets, respectively. Node size corresponds to number of genes in a given gene set (5 to 142). Node labels are BP GO category terms. Edges: color (grey to red) and width correspond to an overlap size (1 to 23).