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Transcriptional responses to short-term and long-term host plant experience and parasite load in an oligophagous beetle

Caroline Müller[1] 🆔 | Heiko Vogel[2] | David G. Heckel[2]

[1]Department of Chemical Ecology, Bielefeld University, Bielefeld, Germany

[2]Department of Entomology, Max Planck Institute for Chemical Ecology, Jena, Germany

Correspondence
Caroline Müller, Department of Chemical Ecology, Bielefeld University, Bielefeld, Germany.
Email: caroline.mueller@uni-bielefeld.de

Abstract

Oligophagous herbivores must adjust their enzymatic machinery to the different host plant species they consume. If different hosts are used from one generation to the next, adaptation may be highly plastic, while if a single host is used over several generations, there may be a longer-term response due to natural selection. Using an experimental evolutionary approach, we investigated effects of long-term experience vs. short-term responses to different host plants in the oligophagous mustard leaf beetle *Phaedon cochleariae*. After 26 generations of continuous feeding on either *Brassica rapa*, *Nasturtium officinale* or *Sinapis alba*, freshly hatched larvae were kept on these plants or moved to one of the other host plants for ten days. Global transcriptional patterns as shown by microarrays revealed that between 1% and 16.1% of all 25,227 putative genes were differentially expressed in these treatments in comparison with the control line constantly feeding on *B. rapa*. A shift back from *S. alba* to *B. rapa* caused the largest changes in gene transcription and may thus represent the harshest conditions. Infection rates with a gregarine parasite were intermediate in all lines that were constantly kept on one host, but much lower or higher when short-term shifts to other host plants occurred. In conclusion, transcriptional plasticity in genes related to metabolism, digestion and general cellular processes plays a key role in long- and short-term responses of the beetle to changing host plant conditions, whereby the specific conditions also affect the interactions between the beetle host and its gregarine parasite.

KEYWORDS
apicomplexan, detoxification, host plant shift, molecular evolution, *Phaedon cochleariae*, transcriptome

1 | INTRODUCTION

Herbivorous insects show various degrees of host specialization and thus need to have different physiological machineries to deal with the species-specific plant chemistry (Després, David, & Gallet, 2007; Li, Schuler, & Berenbaum, 2007). An important prerequisite to cope with toxic plant allelochemicals is the existence of general detoxifying enzymes that directly metabolize the xenobiotics using phase I enzymes, such as cytochrome P450 monooxygenases (P450s), subsequently conjugate the degradation products by phase II enzymes, such as glutathione transferases (GSTs) or UDP glycosyl-transferases

(UGTs), and finally translocate these metabolites *via* phase III transporters (Li et al., 2007). Apart from such general detoxification steps, more specific proteins are needed by herbivores to deal with the specific plant chemistry of a given plant taxon. For example, to feed on plants containing glucosinolates, characteristic allelochemicals of the Brassicales that are readily hydrolysed by plant myrosinases into toxic metabolites (Fahey, Zalcmann, & Talalay, 2001), specific enzymes have evolved that enable herbivores to use these hosts (Müller, 2009; Winde & Wittstock, 2011). A shift to another host plant species must go along with adjustments in the detoxification machinery and probably implies some costs (García-Robledo &

Horvitz, 2012), which can be detectable as host-specific trade-offs in herbivore performance (Kühnle & Müller, 2011). However, the genetic basis of long-term vs. short-term adaptations to different host plant species is currently only little understood (Ragland et al., 2015; Yu, Fang, Zhang, & Jiggins, 2016).

Physiological adaptations of herbivores to modified plant quality are of particular interest to understand the development of resistance to insecticides (Dermauw et al., 2013; Vogel, Musser, & Celorio-Mancera, 2014) and how herbivores can deal with ecological niche shifts (Yu et al., 2016), but also with regard to speciation following host race formation (Eyres et al., 2016; Ragland et al., 2015). Physiological adjustments to specific diet can occur as an immediate response to the detection of plant allelochemicals or the signalling pathways that induce them (Li, Schuler, & Berenbaum, 2002). Alternatively, they may occur within a few generations (Sezer & Butlin, 1998; Warbrick-Smith, Behmer, Lee, Raubenheimer, & Simpson, 2006). If herbivores feed for several generations on one particular host, stabilizing selection accompanied by a loss of genetic variation could result in a loss of the ability to use other plant species (Carroll, Klassen, & Dingle, 1998; Futuyma, 2005). Apart from studies investigating modifications of target enzymes in response to host plant changes, a few recent studies on short-term responses of the global herbivore transcriptome to novel diets reveal that a substantial proportion of the transcripts show a plastic response to the food environment (Bretschneider, Heckel, & Vogel, 2016; Govind et al., 2010; de Panis et al., 2016; Ragland et al., 2015; Roy et al., 2016). However, less is known about long-term adjustments of the transcriptome (over several generations) of herbivorous arthropods to new host plants (Wybouw et al., 2015). Experimental evolutionary approaches can reveal to what extent altered transcriptional levels associated with (short-term) adaptation are driven by the transcriptional plasticity of the ancestral population (long-term) (Wybouw et al., 2015).

The insect's ability to utilize plant nutrients and to detoxify allelochemicals may be enhanced or diminished by symbiotic or parasitic microorganisms occurring in the gut (Engel & Moran, 2013; Welte et al., 2016). Gut microbiota may assist in digesting and supplementing a low-quality diet (Scully et al., 2014) but could also have detrimental effects caused by nutrient depletion (Gigliolli, Julio, & Conte, 2016). Moreover, the composition of gut microbiota affects the immune responses of herbivores (Tate & Graham, 2015). The specific diet can thus be an important driver of the composition or variation in gut microbiota (Pernice, Simpson, & Ponton, 2014). A pronounced alteration of transcripts associated with microbiota along with a substantial alteration in expression levels of insect transcripts may indicate particularly high challenges when dealing with a certain diet quality.

The mustard leaf beetle, *Phaedon cochleariae* (F.) (Coleoptera: Chrysomelidae), is oligophagous on various species of the Brassicaceae (Reifenrath & Müller, 2009). It can be found at natural sites on *Nasturtium officinale* R. Br. (watercress) but also as pest on crop plants such as cabbage (Müller & Müller, 2016). We established a laboratory colony that was kept for more than 40 generations on *Brassica rapa* L. subsp. *pekinensis* (Chinese cabbage). This colony was then split into three subpopulations that were reared either on

N. officinale or *Sinapis alba* L. (white mustard) or continued to feed on *B. rapa*. After one generation, the beetles reared on the original host, *B. rapa*, attained a higher body mass and developed faster than beetles kept on the two other hosts (Kühnle & Müller, 2011). After ten generations of selection on these three host species, the performance of *P. cochleariae* was influenced to different degrees; females reared on *S. alba* had accelerated their development time to match that of insects kept continuously on *B. rapa*. Females reared on *N. officinale* showed an increased growth rate and pupal body mass in the tenth compared to the second generation on that host. Adjustments within these ten generations may have been due to transgenerational effects, genetic drift, and/or directional selection followed by stabilizing selection (Kühnle & Müller, 2011).

In this study, we used an experimental evolutionary approach to study effects of long-term experience vs. short-term plastic responses of the mustard leaf beetle to these different host species. After 26 generations of exclusive experience with one of the three host plant species, freshly hatched *P. cochleariae* larvae of each subpopulation were distributed to the original host (*B. rapa*) or kept/moved to one of the more recent hosts. Microarray experiments were conducted to compare gene expression profiles unbiased on a global scale. Furthermore, analysis of transcripts enabled us to detect a cryptic infection by an apicomplexan parasite, allowing us to test for complex interactions with the pattern of host plant switches.

2 | MATERIALS AND METHODS

2.1 | Plants and insect rearing

Plants of *B. rapa* ssp. *pekinensis* "Michihili" (Chinese cabbage), *S. alba* cv. Silenda (white mustard) and *N. officinale* (watercress) were reared in a glasshouse at 20°C and a photoperiod of 16 hr:8 hr L:D (seeds from Kiepenkerl, Norken, Germany). Leaves of nonflowering plants at an age of 4–5 weeks were offered as food to the insects. The three species share the same classes of metabolites but differ in the specific composition of glucosinolates, phenolics, terpenoids and carotenoids (Bukovinszky, Gols, Posthumus, Vet, & van Lenteren, 2005; Cartea, Francisco, Soengas, & Velasco, 2011; Fahey et al., 2001; Kolesnikov & Gins, 2001; Kopsell, Barickman, Sams, & McElroy, 2007) and likely other chemical and mechanical plant defence traits. Thus, the metabolic composition as well as the accessibility of nutrients differs between the three plant species.

Beetles of *P. cochleariae* were collected in Germany and had been kept in the laboratory on *B. rapa* for more than 40 generations, with a minimum of 700 beetles per generation distributed over at least four rearing boxes (200 × 200 × 65 mm). They were reared in a climate chamber at 20°C, 70% r.h. and a photoperiod of 16 hr:8 hr L:D. Adults of this original population were distributed in three subpopulations and were kept on either one of the alternative hosts *N. officinale* (*Nasturtium* subpopulation, "N") or *S. alba* (*Sinapis* subpopulation, "S") or reared continuously on *B. rapa* (*Brassica* subpopulation, "B"). Each subpopulation consisted approximately of 200–300 beetles at the start of the experiment and was kept in at least three

rearing boxes for 24 generations and then reunified to one box per subpopulation. After 26 generations (4 years), leaves with eggs were collected and freshly hatched larvae of the offspring generation distributed in the following way: larvae of the B subpopulation were distributed to *B. rapa* (BB line), *N. officinale* (BN) and *S. alba* (BS). Larvae of the N subpopulation were distributed to *N. officinale* (NN) and *B. rapa* (NB), and larvae of the S population were distributed to *S. alba* (SS) and *B. rapa* (SB; Figure 1). The respective larvae were fed on leaves of the identical plant individuals across the lines to reduce variation due to plant source as far as possible. Thus, there was no replication at the individual plant level. Ten days after larval hatch, when larvae were in the middle of the final (third) instar, four replicate samples of 20 larvae of each line were shock-frozen in liquid nitrogen (of the SB and NB lines, only 5–15 larvae could be collected per replicate). The sex of the larvae cannot be determined, but we assumed a more or less equal sex ratio within the larval batches taken for each replicate. The BB, NN and SS line served to investigate long-term responses. The BN and BS lines revealed short-term responses, whereby the NB and SB lines were used to study responses to a switch-back to the "original" host of this laboratory colony and to test whether potentially laboratory-evolved populations (NN and SS) undergo gene expression changes on a new host plant within a short time span.

2.2 | RNA isolation

Total RNA was extracted from the 28 samples (each consisting of up to 20 pooled individuals) using TRIzol Reagent (Invitrogen) according to the manufacturer's protocol. An additional DNase (Turbo DNase, Ambion) treatment was included to eliminate any contaminating DNA. The DNase enzyme was removed, and the RNA was further purified using the RNeasy MinElute Clean up Kit (Qiagen) following the manufacturer's protocol. RNA integrity and quantity were verified on an Agilent 2100 Bioanalyzer using the RNA Nano chips (Agilent Technologies). RNA quantity was determined on a Nanodrop ND-1000 spectrophotometer.

2.3 | Reference transcriptome generation

A dual-approach was taken towards sequencing, combining both Sanger and 454 sequencing of normalized cDNA libraries and RNA-seq of selected samples. Total RNA isolated from *P. cochleariae* (later found to also include parasite RNA) was isolated from all developmental stages, a wide range of tissues and treatments, including larvae and adult beetles exposed to phytochemicals, different host plants (including *N. officinale*, *S. alba* and *B. rapa*), starvation and immune induction experiments using a combination of bacteria and fungi. RNA isolation, library generation and normalization, as well as the combined assembly strategy of the reference transcriptome subsequently used for the custom microarray design, are described in detail in Kirsch et al. (2012). The RNA-seq reads for adult and larval samples were compiled together and assembled using the CLC Genomics Workbench pipeline.

2.4 | Microarrays

An Agilent 4 × 180K G3 gene expression custom microarray was designed based on the eArray platform (Agilent Technologies; https://earray.chem.agilent.com/earray/), containing a total of 144,451 noncontrol custom probes and 1,417 Agilent Technologies
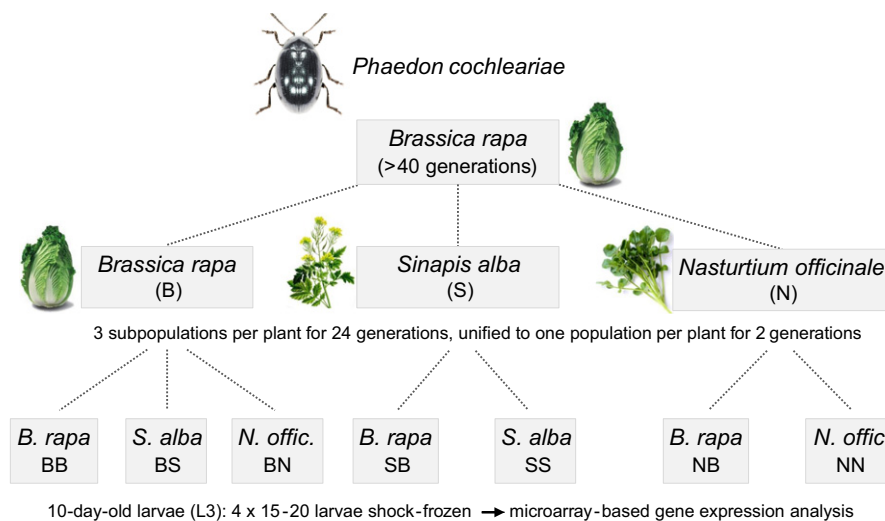
FIGURE 1 Rearing scheme for experimental evolutionary approach. Beetles of *Phaedon cochleariae* were reared on *Brassica rapa* for more than 40 generations and then distributed in three subpopulations that were kept on either one of the alternative hosts *Sinapis alba* (subpopulation "S"), *Nasturtium officinale* ("N'), or reared further on *B. rapa* ("B"). After 26 generations (4 years), freshly hatched larvae were distributed in lines in the following way: larvae of the B subpopulation were distributed to *B. rapa* (BB line, serving as reference line for all comparisons), *S. alba* (BS) and *N. officinale* (BN). Larvae of the S population were distributed on *S. alba* (SS) and *B. rapa* (SB), and larvae of the N subpopulation were distributed on *N. officinale* (NN) and *B. rapa* (NB). Ten days after larval hatch (middle of third instar), 5–20 larvae of each line were pooled per sample for transcriptome analyses [Colour figure can be viewed at wileyonlinelibrary.com]

built-in controls (structural and spike-in). RNA labelling, microarray hybridizations, processing and primary data analysis were essentially done as described (Celorio-Mancera et al., 2011). In brief, Agilent Technologies spike-in RNA was added to 500 ng of total RNA and labelled using the Low RNA Input Linear Amplification kit (Agilent Technologies). RNA samples were labelled with cyanine 3-CTP dyes according to manufacturer instructions following a single dye design. Labelled amplified cRNA samples were purified using Qiagen RNeasy MinElute Cleanup kit and analysed on a Nanodrop ND-1000 spectrophotometer using the microarray function. Amplified cRNA samples were used for microarray hybridization only if the yield was >1,600 ng and the specific activity was >8.0 pmol Cy3 per μg cRNA. 1,600 ng of cyanine 3-labelled cRNA was used for each array and hybridization was carried out at 65°C for 17 hr. Slide processing, data extraction and initial quality assessments were performed as described (Celorio-Mancera et al., 2011).

2.5 | Data analyses

Microarray data were analysed using GeneSpring version 12.5 (Agilent Genomics). Fluorescence signals were $\log_2$-transformed and then subjected to quantile normalization across arrays with default flags (present or marginal in at least one sample) and a threshold of 1.0. A gene-level experiment based on only beetle-derived sequences was created by combining probes derived from the same genes with default flags. Apicomplexan genes were analysed separately as described below.

The de novo assembled transcriptome was annotated using BLAST, Gene Ontology and InterProScan searches with BLAST2GO PRO v3.2 (www.blast2go.de) (Conesa et al., 2005). As we found a number of transcripts that were related to apicomplexans and other nonbeetle species, we filtered the data set by BLAST to beetle transcripts only for subsequent analysis. These data were then filtered for significant changes in expression levels between rearing groups using unpaired t tests with unequal variances ($p \le .05$), corrected with Benjamini Hochberg false discovery rate for multiple comparisons and a fold-change of ≥ 2 or ≤ 0.5 ($\log_2$ difference of >1.0 or <-1.0). All rearing groups were compared to the BB line, which represents the very long-term host association of our laboratory colony. In addition, we compared the gene expression patterns between the NB vs. the NN and the SB vs. the SS line. Furthermore, the differentially expressed genes were tested for Gene Ontology (GO) term enrichment in BLAST2GO version 3.2. Fisher's exact test (implemented in BLAST2GO) was used to identify the over- or underrepresentation of GO terms among lists of differentially expressed genes in the different microarray data sets relative to the complete reference data set. The GO-enriched bar charts were simplified to display only the most specific GO terms by removing parent terms representing existing child terms using the function "Reduce to most specific terms" in BLAST2GO. A GO term was considered significantly enriched if the p-value corrected by FDR was less than 0.05.

The majority of nonbeetle transcripts appeared to originate from a single species of an undescribed gregarine parasite and will be analysed in a separate publication. We used the expression levels of a set of seven gregarine ribosomal protein (Rp) genes as an index of the parasite load. These levels were analysed by a two-way ANOVA on the seven gregarine Rp genes across the seven rearing lines on the different host plants, followed by a Bonferroni–Dunn test. The gregarine ribosomal proteins were found to show a parallel variation across treatments; therefore, the geometric mean was used as an index of gregarine infection based on the fold-change relative to the BB line, which was set equal to 1.0. Then, the average infection index of all lines except the BB line was related to the proportion of differentially regulated genes in each rearing line (relative to BB), using a Spearman rank correlation.

3 | RESULTS

3.1 | Reference transcriptome

Compiling RNA-seq reads for adult and larval samples, the de novo strategy assembled 53,535 contigs or putative genes (called "genes" thereafter for simplicity). Filtering by BLAST hits (NCBI nr database) to beetle entries resulted in 25,227 genes, referred to as "beetle data set". We subsequently focused our microarray data analysis on this beetle data set, including GO term enrichment analysis and functional groups or gene family expression dynamic analysis between treatment groups, always using the BB line as comparison (Figure 1).

3.2 | Modulation of gene expression caused by long-term experiences

In comparison with the BB line, feeding for many generations on *N. officinale* (NN) led to a differential expression in 5.7% of the genes of the beetle data set (1,441 of 25,227 genes), whereas long-term feeding on *S. alba* (SS) led to significant changes in almost twice as many genes (10.6%). In both lines (NN, SS), about half of the significantly modified genes were upregulated, and the other half downregulated. For genes exclusively upregulated in NN larvae, two GO terms were significantly overrepresented, including genes with aspartic-type endopeptidase activity and genes associated with structural constituents of the cuticle (Fig. S1A). In contrast, in genes exclusively upregulated in SS larvae, several GO terms were significantly overrepresented, for example, genes associated with serine-type endopeptidase, oxidoreductase or monooxygenase activity, copper-ion binding and the extracellular region, whereas other more general terms were underrepresented (e.g., intracellular part, nucleic acid binding, nucleic acid metabolic process; Fig. S1B). For genes exclusively downregulated in SS larvae, a large number of GO terms ($N = 26$) were mainly overrepresented, including genes associated with zinc-ion binding, various transaminases, negative regulation of peptidase activity and peptidase inhibitor activity (Fig. S1C). In contrast, only one GO term (associated with the intracellular ribonucleoprotein complex) was underrepresented in genes exclusively downregulated in SS larvae. Within the genes up- or downregulated in both NN and SS compared to BB larvae, no GO term enrichment was found.

Although GO terms generally associated with detoxification were not enriched, a number of detoxification-related genes were differentially expressed between the NN and SS larvae in relation to BB larvae (Figure 3). Of the 170 distinct contigs identified in the transcriptome as part of P450 genes, five were up- and seven downregulated in NN larvae, whereas many more, namely 20 were up- and eight downregulated in SS larvae, with five genes responding in both groups (four up, one down). Of 57 glutathione transferases, five genes were up- and one gene was downregulated in each of the NN and SS larvae, respectively (Figure 3).

With regard to specific detoxification enzymes, interestingly one putative arylsulphatase gene was downregulated in the commonly regulated genes, and another was downregulated in the genes exclusively regulated in SS larvae, of five such genes found in the beetle data set. Regarding cell wall-degrading enzymes, several polygalacturonases were significantly downregulated either in NN larvae ($N = 2$), in SS larvae ($N = 3$) or in both ($N = 2$) compared to BB larvae. Related to immunity, of 16 putative lysozyme genes in the overall data set, four were up- and one was downregulated in SS larvae compared to BB larvae (Fig. S6).

3.3 | Modulation of gene expression caused by short-term plastic responses

A transfer from the original host *B. rapa* to other hosts for 10 days caused the lowest proportion of differentially regulated genes. In comparison with the BB line, feeding for one offspring generation on *N. officinale* (BN) led to differential expression in 2.8% of all genes of the beetle data set, whereas short-term feeding on *S. alba* (BS) led to significant changes in much less genes (1.0%, Figure 2). As for the long-term adaptation, also in the short-term adaptations, about half of the genes were upregulated, and the other half downregulated. For genes exclusively upregulated in BN larvae, 18 GO terms were significantly overrepresented, including genes with different [acyl-carrier-protein] hydrolase activities, oxidation–reduction processes and several genes associated with the cuticle (Fig. S2A). In contrast, six GO terms were underrepresented in this data set, including genes associated with cell communication, single organism signalling and general cellular processes (Fig. S2A). For genes exclusively downregulated in BN larvae, 22 GO terms were overrepresented, including genes associated with polygalacturonase activity and with pectin catabolic processes, relevant in plant cell wall degradation (Fig. S2B). Overrepresentations in downregulated genes were also found in endopeptidase inhibitor activity, sucrose and starch metabolic processes. In contrast, three GO terms were underrepresented in genes downregulated in BN larvae, which were related to general cellular processes (nucleic acid binding, DNA metabolic processes and intracellular organelles; Fig. S2B). In BS larvae, only one GO term, related to innate immune response, was overrepresented in upregulated genes (Fig. S2C). In genes differentially regulated in both BN and BS larvae compared to BB larvae, two GO terms related to vitelline were overrepresented in the upregulated genes (Fig. S2D). Moreover, five P450 genes were significantly changed in

BN larvae (four up- and one downregulated), whereas only one P450 gene was significantly downregulated in BS larvae (Figure 3).

For larvae switched back to their original host *B. rapa* (NB and SB), 8.7% of all genes responded to a switch from *N. officinale* and almost twice as many (16.1%) responded to a switch from *S. alba* back to *B. rapa* compared to the BB line (Figure 2). For genes exclusively upregulated in NB larvae, 21 GO terms were overrepresented, mostly related to rather general metabolic and physiological processes (e.g., aspartic-type endopeptidase activity, oxidoreductase activity, [acyl-carrier-protein] hydrolase activity; Fig. S3A), whereas genes exclusively downregulated in NB larvae revealed four underrepresented GO terms, including one for primary metabolic processes (Fig. S3B). For genes exclusively upregulated in SB larvae, seven GO terms were overrepresented (including very general terms regarding DNA replication, DNA repair, RNA modification and structural constituent of the cuticle; Fig. S3C), whereas one GO term associated with cytoskeletal protein binding was underrepresented. Among 29 over- and four underrepresented GO terms in genes downregulated in SB larvae only, transposase activity and DNA-mediated transposition were overrepresented. Rather general GO terms (related to DNA and RNA metabolic processes and modification etc.) were over- or underrepresented in genes commonly up- and downregulated in both NB and SB larvae. Moreover, several genes related to the cuticle were most pronouncedly upregulated in the SB and NB line (Fig. S7).

For larvae switched from the potentially laboratory-evolved populations on *N. officinale* and *S. alba* back to the original host *B. rapa* for one generation (NB and SB), only 0.67% of the genes were differentially expressed between the NB vs. the NN line, whereas 7.2% were differentially expressed between the SB vs. the SS line (Fig. S5). Just one GO term, related to structural constituents of the cuticle, was overrepresented in genes upregulated in the NB vs. the NN line only, whereas 16 GO terms were overrepresented in downregulated genes, including several sugar transport-related genes (Fig. S4). Many more GO terms (31 each) were overrepresented in genes exclusively up- or downregulated in the SB vs. SS line. These GO terms included again quite general regulatory genes as well as genes related to growth, digestion, monooxygenase activity and innate immune response (the latter four all downregulated). Within the genes up- or downregulated in both the NB vs. NN and the SB vs. the SS line, no GO term enrichment was found.

3.4 | Infection by a gregarine parasite

Most of the nonbeetle transcripts appeared to originate from a single undescribed species of an apicomplexan gregarine parasite (Figure 4b). We chose seven highly expressed ribosomal protein (Rp) genes of this gregarine as a measure of parasite load. A two-way ANOVA revealed significant gregarine Rp effects ($F_{6,147} = 422.9$, $p < .001$) and significant beetle rearing treatment effects ($F_{6,147} = 176.2$, $p < .001$) but no significant interaction effects ($F_{36,147} = 0.687$, $p > .9$); that is, Rp genes expressed at different levels showed parallel responses across beetle treatments. The
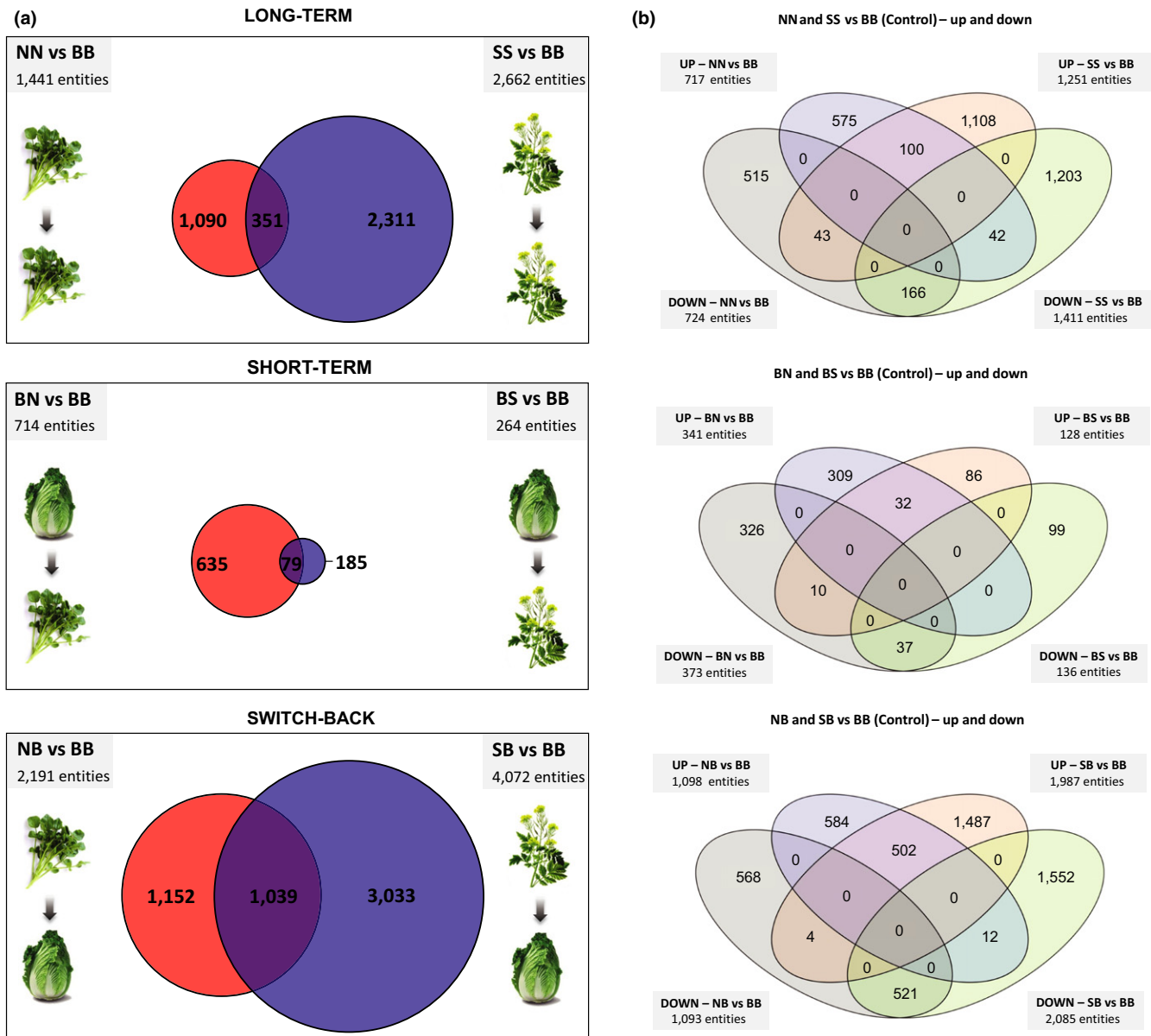
FIGURE 2 Area-proportional Venn diagrams of differentially regulated genes (left) and exclusive vs. shared up- and downregulated genes (right) in *Phaedon cochleariae* larvae with different host plant experience for 26 generations (upper plant symbol) and the target offspring generation (lower plant symbol) (for abbreviations of lines and rearing scheme see Figure 1). Genes were considered as differentially regulated when expression levels between rearing groups differed significantly based on unpaired t tests with unequal variances ($p \leq .05$), corrected with Benjamini Hochberg false discovery rate for multiple comparisons and a fold-change of ≥ 2 or ≤ 0.5. All rearing groups were compared to the BB line, which represents the very long-term host association of our rearing stock. The total number of beetle-derived entities (="genes") was 25,227 [Colour figure can be viewed at wileyonlinelibrary.com]

geometric mean of the gregarine Rp expression level was used to compute an infection index, relative to the BB treatment with an index level of 1.0. All pairwise comparisons among the treatments were significantly different (Bonferroni–Dunn test, $p < .05$), except BB-NN, BB-SS and BN-NB. The infection index of NN larvae and SS larvae did not differ significantly from that of BB larvae (Figure 4a). The infection indices of BN and NB larvae were about half as high as the one of BB larvae. BS larvae showed about twice as many parasites as the BB line and thus the highest infection level, whereas SB larvae only had about one-fifth as many parasites as BB and thus

the lowest level. There was no significant correlation between the infection index and the overall proportion of differentially regulated beetle genes (Spearman rank correlation $p > .05$). Nevertheless, larvae of the SB line with the lowest gregarine infection index had the highest number of regulated genes, whereas larvae of the BS line with the highest infection index had the lowest number of regulated genes.

Variation in the infection index was closely matched by variation in levels of other contigs matching apicomplexans ($N = 1080$), with highest levels in the BS line, and almost no change in the NB line
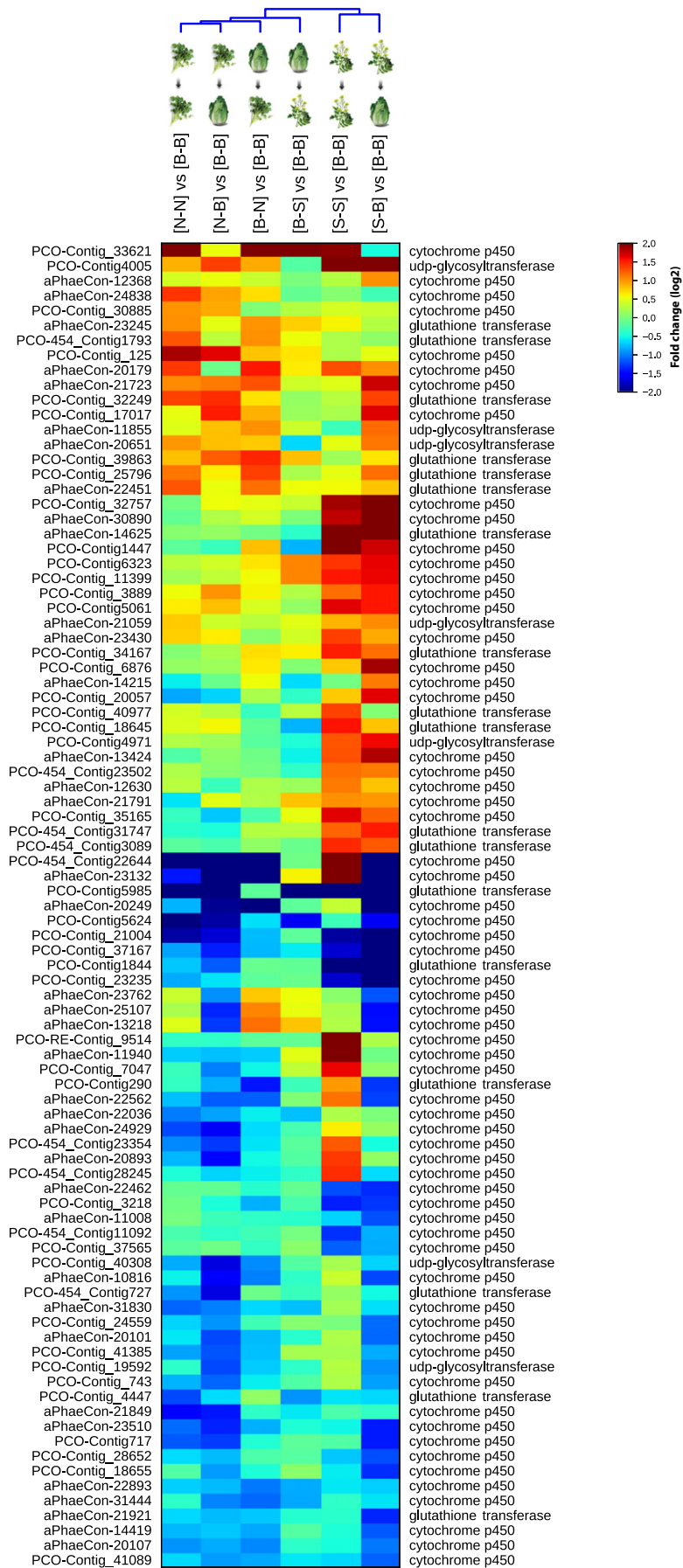
FIGURE 3 Heat map of putative detoxification genes significantly regulated between *Phaedon cochleariae* larvae continuously feeding on *Brassica rapa* plants (BB) and other host plants or host plant switches. Heatmap colours correspond to $\log_2$-transformed fold-change values, with red colours representing induced gene expression levels and blue colours representing downregulated gene expression levels. Two-way clustering of genes and samples was performed applying hierarchical clustering using Euclidean distance metric and the complete linkage method [Colour figure can be viewed at wileyonlinelibrary.com]
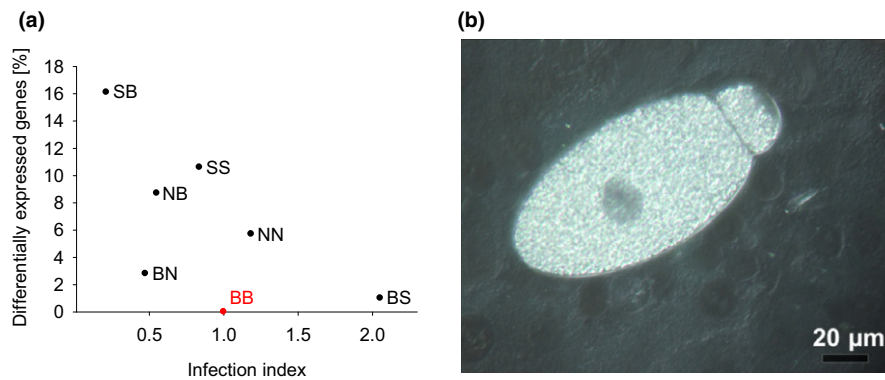
FIGURE 4 (a) Relationship between average gregarine infection index and the proportion of differentially expressed beetle genes in different lines of *Phaedon cochleariae* relative to the line constantly fed on *Brassica rapa* (BB; with an infection index of 1, indicated in red). For treatment abbreviations see Figure 1. The apparent inverse relationship is not statistically significant (Spearman rank *p* > .05). (b) Picture of gregarine dissected from larva of *P. cochleariae*, taken by Svenja Geisler [Colour figure can be viewed at wileyonlinelibrary.com]

(Fig. S8). The lines kept constantly on one host plant showed intermediate levels, whereas the BN line and in particular the SB line had the lowest levels. Similar patterns were also seen in contigs matching bacteria (*N* = 153) and yeasts (*N* = 64; Fig. S9).

4 | DISCUSSION

Global herbivore transcriptional signatures were found to be highly dependent on the long-term vs. short-term host plant experience of *P. cochleariae* larvae (Figure 2). For lines that were maintained on their host species for long-term (SS and NN compared to BB), differences in gene expression levels to the BB line were moderate or intermediate. In contrast, short-term shifts away from *B. rapa* or back from *N. officinale* to *B. rapa* caused the lowest changes in gene expression, indicating a low level of plastic responses (Figure 2; Fig. S5). However, switching from *N. officinale* or *S. alba* back to *B. rapa* led to comparably higher proportions of differentially expressed genes than long-term adaptations to these alternative host plants. Overall, a long-term experience with *S. alba* followed by a switch-back from this host plant species to *B. rapa* for one generation (SB) caused the most distinct gene expression patterns compared to BB larvae and may indicate that dealing with the chemistry and physicomechanical properties of *S. alba* is most challenging for *P. cochleariae*.

Whether the majority of changes in gene expression were adaptive cannot be ultimately determined as we did not analyze the performance of the insects in the 27th generation. Thus, some of the changes may indeed be due to adaptive responses (i.e., adjustments in digestive enzymes and detoxification), whereas others may be simply due to genetic drift and randomly acquired changes. However, by combining three subpopulations per line to a single one after 24 generations, we likely underestimate the effects of drift, by losing information on possible transcriptional divergence of the subpopulations. Most truly random changes in the three replicates would cancel out in the summation of the three. Furthermore,

differential gene expression may have been enhanced if we would have increased the intraspecific variation in plant quality.

4.1 | Long-term adaptations

When being exposed to *N. officinale* for many generations, the GO term aspartic-type endopeptidase was overrepresented in genes exclusively upregulated in NN compared to BB larvae of *P. cochleariae* (Fig. S1A). Endopeptidases are ubiquitously occurring proteolytic enzymes that cleave peptides using an activated water molecule (Barrett & Rawlings, 2007). Aspartic endopeptidases are potentially involved in digesting and killing bacteria in fly maggots (Franta et al., 2016). NN larvae of *P. cochleariae* feeding continuously on *N. officinale* may carry a somewhat higher load of bacteria, as indicated by higher expression levels of the respective genes (Fig. S9). This load may lead to an upregulation of aspartic endopeptidases that could potentially control the microorganisms.

Besides an overrepresentation of aspartic-type endopeptidases, NN larvae showed an overrepresentation of upregulated genes associated with structural constituents of the cuticle (Figs S1A, S7). The insect cuticle provides a physical and chemical barrier at the interface between the individual and its environment. Differential expression of cuticle proteins has likewise been found in aphids in response to insecticides (Puinean, Foster, et al. 2010) and in a polyphagous caterpillar when feeding on a certain diet (Celorio-Mancera et al., 2013). An increased expression of cuticle proteins may thicken the cuticle and thus reduce the penetration of insecticides (Puinean, Foster, et al. 2010). Structural constituents of the cuticle may also stabilize the gut cuticle and thereby prevent or reduce the entry of plant toxins (Celorio-Mancera et al., 2013).

Overall, almost twice as many genes were differentially regulated in SS compared to NN larvae, both compared to the BB line (Figure 2). In contrast to the upregulation of aspartic-type endopeptidases in larvae of the NN line, serine-type endopeptidase activity-related genes were overrepresented in genes exclusively upregulated in the SS line (Fig. S1B). This group of endopeptidases may enable

insect larvae to feed on and adapt to different host plant species, each containing a different set of protease inhibitors (Celorio-Mancera et al., 2013; Chikate, Tamhane, Joshi, Gupta, & Giri, 2013; Hoang, Matzkin, & Bono, 2015; Kuwar, Pauchet, Vogel, & Heckel, 2015). The relatively high number of upregulated genes potentially encoding P450s and several glutathione transferases (Figure 3) and an overrepresentation of GO terms associated with genes encoding monooxygenases and oxidoreductases in SS larvae (Fig. S1B) indicate the induction of a broad metabolic detoxification machinery when feeding long-term on *S. alba*. Oxidoreductases are frequently involved in the degradation of specific groups of plant secondary metabolites (Zhang et al., 2013) but can also be associated with immunity (Sun et al., 2012). Glutathione transferases and P450 monooxygenases are multifunctional enzymes that mediate increased resistance against insecticides (Han, Li, Wan, Zhu, & Meng, 2016; Puinean, Denholm, Millar, Nauen, & Williamson, 2010) and transform xenobiotics (Feyereisen, 2012; Heidel-Fischer & Vogel, 2015; Vogel et al., 2014). Their gene expression can be modulated when insect herbivores face different plant toxins (Celorio-Mancera, Heckel, & Vogel, 2012; Celorio-Mancera et al., 2011; Chandra, Asokan, Manamohan, & Sita, 2016; Koenig et al., 2015; Wybouw et al., 2015), being of major importance for both specialist and generalist herbivores. The majority of differentially expressed transcripts coding for putative detoxification enzymes displayed significantly higher expression levels in both SS and SB larvae (Figure 3). This indicates that previous long-term experience of *P. cochleariae* on *S. alba* plants results in transcriptional signatures that are not easily reversible when larvae are transferred to the original host.

Long-term rearing on *S. alba* was associated with differences in expression levels of genes associated with genetic information processing, such as, for example, nucleic acid binding (Fig. S1B). Differential expression of such genes has likewise been reported in a polyphagous caterpillar species in response to different hosts (Celorio-Mancera et al., 2013). These changes indicate that dealing with different host plants may involve gene expression changes governed by transcription factors. For example, separate cytochromes P450 on different linkage groups were coordinately upregulated when *Helicoverpa armigera* larvae fed on bean and downregulated on both tobacco and cotton (Celorio-Mancera et al., 2013). Furthermore, in genes exclusively downregulated in SS larvae of *P. cochleariae*, several GO terms were overrepresented that are involved in digestion, such as peptidase inhibitor activity, as well as various transaminases, which catalyse the transfer of α-amino groups (Fig. S1C). Such distinct regulation of digestive enzymes depending on the long-term rearing history implies that the nutritional value and digestibility of the three host plant species differ.

Although all three plant species used in this experiment share the same classes of metabolites, in particular glucosinolates, phenolics, terpenoids and carotenoids (Bukovinszky et al., 2005; Cartea et al., 2011; Fahey et al., 2001; Kolesnikov & Gins, 2001; Kopsell et al., 2007), the individual compounds only partly overlap between the species. For example, the leaf glucosinolate composition of the *B. rapa* variety used here consists on average of 17% 2-phenylethyl

glucosinolate, 62% aliphatic and 22% indole glucosinolates (Müller & Müller, 2016), whereas in *N. officinale*, 2-phenylethyl glucosinolate accounts for 85% of the total glucosinolates (Müller & Müller, 2016), and *S. alba* contains mainly *p*-hydroxybenzyl glucosinolate (Reifenrath & Müller, 2007). It is unknown yet, how *P. cochleariae* detoxifies glucosinolates, but the differential expression of several putative arylsulphatase genes in the NN and SS lines compared to the BB line may point to an involvement of sulphatases. Sulphatatases are known to detoxify glucosinolates in the Brassicaceae specialist *Plutella xylostella* (Ratzka, Vogel, Kliebenstein, Mitchell-Olds, & Kroymann, 2002) and the generalist *Schistocerca gregaria* (Falk & Gershenzon, 2007), and potentially also in the whitefly *Bemisia tabaci* (Malka et al., 2016), and the available sequence comparisons indicated that, at least in *P. xylostella*, these have evolved from arylsulphatases.

Polygalacturonases are involved in cleaving the main constituent of pectin in the primary cell wall, which is essential for further degradation of cell wall polysaccharides (Kirsch et al., 2014). In *P. cochleariae*, several polygalacturonase genes have been identified, which could support the herbivorous lifestyle of *P. cochleariae* beetles by breaking down plant cell wall components. These genes were likely acquired by horizontal gene transfer of a polygalacturonase from an ascomycete fungus ~200 million years ago, followed by duplications and replacements, leading to functional diversification (Kirsch et al., 2014). The cell walls of the different Brassicaceae species used in the present experiment likely differ in their composition, making adjustments of gene expression of polygalacturonases necessary, as indicated in the NN and SS line compared to the BB line.

Finally, in larvae of the SS line, several putative lysozyme genes were up- or downregulated in comparison to BB larvae (Fig. S4). In addition to playing some role in digestion, lysozymes are antimicrobial proteins that hydrolyse residues of peptidoglycan, which is the predominant cell wall polymer of bacteria (Fukamizo, 2000). In insects, lysozymes are mainly expressed in the fat body, and after immune challenge also in the hemocytes (Barthel et al., 2014; Beckert et al., 2016; Zhang et al., 2015), but were also shown to play important roles in the gut (Jacobs et al., 2016; Johnston & Rolff, 2015). Specific plant compounds of *S. alba* may act on the insect microbiota of *P. cochleariae*, thereby affecting immune responses particularly in larvae of the SS line. The lack of any commonly over- or underrepresented GO terms among the differentially expressed genes in both NN and SS larvae compared to BB larvae indicates that the long-term association with these three host plant species led to quite distinct adaptations to plant species-specific features but could also be partly explained by genetic drift and random changes.

4.2 | Short-term responses

Short-term responses in gene regulation of *P. cochleariae* larvae to host plant shifts highly depended on the direction in which shifts occurred. When beetles had been reared for tens of generations on *B. rapa*, a shift of the offspring larvae to one of the "alien" plants, *N.*

officinale or *S. alba*, caused only minor differences in gene expression profiles (Figure 2). Differential gene expression on these alternative host plants may become more pronounced with time, that is, with the number of generations reared on these hosts, as seen in the gene expression profiles of NN and SS larvae. Such reinforcement of differences over generations may be a result of transgenerational effects, genetic drift and/or directional selection. These effects could be followed by stabilizing selection, leading to an improved performance of beetles on alternative host plants over several generations, as observed in *P. cochleariae* (Kühnle & Müller, 2011). These findings demonstrate that this beetle can respond to different food environments with sufficient plasticity and is potentially able to adapt to new host plants. Not only the performance but also various behavioural traits of the adults are significantly affected by the host plant quality (Müller & Müller, 2017; Tremmel & Müller, 2013). Interestingly, changes in gene expression compared to the BB line were most pronounced in larvae switched after 26 generations of feeding on *N. officinale* or *S. alba* back onto *B. rapa* (Figure 2). This clearly indicates that a long-term experience with different hosts and thus divergent selection or drift, which causes numerous changes in the insect's physiology, evokes substantial plastic responses once the original host plant species is faced again. Reciprocal cross-rearing experiments, as used in the current study, can help to disentangle such short-term plastic from evolved genetic responses (Ragland et al., 2015; Wybouw et al., 2015). Transcriptional responses may be maladaptive, neutral or beneficial in novel host environments, implying costs and benefits in counter-defences induced in response to the particular diet (Chi et al., 2009).

When larvae were switched from *B. rapa* to *N. officinale*, various genes were exclusively upregulated in this BN line (Fig. S2A). Among these, different [acyl-carrier-protein] hydrolase activities were overrepresented. An upregulation of hydrolases in response to dietary challenges has been also found in a bruchid beetle (Chi et al., 2009). [Acyl-carrier-protein] hydrolases act on thioester bonds and are involved in fatty acid biosynthesis; they have been, however, mainly described for plants (Jones, Davies, & Voelker, 1995). Moreover, as found for NN larvae, also in BN larvae several GO terms related to the cuticle were overrepresented in genes upregulated exclusively in this line (Figs S2A, S4, S7), reinforcing the argument that adjustments of the cuticle structure may be particularly relevant when larvae feed on *N. officinale*.

In contrast to the BN larvae, in BS larvae only very few genes were significantly regulated (Figure 2) and only one GO term associated with innate immune response was overrepresented in genes exclusively upregulated in this line (Fig. S2C). An upregulation of various genes related to immunity was also evident in larvae reared for long-term on *S. alba* (SS line, see above). Thus, feeding on this host plant species may be particularly challenging for immune responses of *P. cochleariae*.

Significant changes in P450 gene expression in BN and BS larvae (Figure 3) suggest differential detoxification potential depending on this short-term host experience, as also found in other herbivores (Feyereisen, 2012; de Panis et al., 2016; Vogel et al., 2014). For example, the expression of several genes encoding P450s is modified in relation to diet high in alkaloid concentrations in the fly *Drosophila buzzatii* (de Panis et al., 2016) or in relation to different terpenoids in the beetle *Dendroctonus armandi* (Dai, Ma, Gao, & Chen, 2016). P450 metabolism of plant secondary metabolites can be considered as a key adaptation to deal with different host plants (Feyereisen, 1999, 2012). Environmentally induced plasticity plays a key role in plant–herbivore interactions. Studies on the generalist mite *Tetranychus urticae* indicate that in polyphagous arthropods that switch more frequently among host plants such large-scale expression responses are likely adaptive (Wybouw et al., 2015). In contrast, in oligophagous insects, such as different *Rhagoletis* species, these plastic responses to novel hosts can also be maladaptive (Ragland et al., 2015).

4.3 | Responses to switch-back

Larvae that were switched from the potentially laboratory-evolved populations on *N. officinale* and *S. alba* back to the original host *B. rapa* for one generation revealed rather large changes in gene expression when compared to the BB line (Figure 2) but much less when compared to the NN or SS line, respectively (Fig. S5). Therefore, the expression levels of the majority of genes had stabilized after 26 generations of experience on the new hosts, and fewer genes exhibited a plastic response when switched back to the original host. The switch from *N. officinale* back to the original host (NB larvae) resulted in differential expression levels in 8.7% of all genes, whereas in SB larvae, almost twice as many genes were differentially regulated compared to the BB line (Figure 2). In genes exclusively upregulated in NB larvae, aspartic-type endopeptidase activity was overrepresented (Fig. S3A), as also found in NN larvae, whereas an overrepresentation of an [acyl-carrier-protein] hydrolase resembled that found for other [acyl-carrier-protein] hydrolases in BN larvae. Whenever *N. officinale* was used as the host over the long- or short-term, genes related to these classes of enzymes seemed to be differentially regulated compared to *P. cochleariae* constantly kept on *B. rapa*. Other GO terms overrepresented in genes upregulated in NB larvae belonged to genetic information processing such as RNA binding, helicase activity and mRNA splicing, indicating that general genetic regulation is affected when larvae are switched back to their original host after a long-term experience with *N. officinale*. Differential expression of genes involved in primary metabolism in response to different food environments, as revealed in the NB larvae (Fig. S3B), has also been found in *Drosophila mettleri* and in *Helicoverpa armigera* (Celorio-Mancera et al., 2012; Hoang et al., 2015). Similar to NB larvae, also for SB larvae genes involved in gene information processing, such as DNA replication, DNA repair, RNA modification and transposition were differentially regulated, highlighting that large-scale transcriptional changes are frequently associated with host plant switches.

Interestingly, also a large number of genes were commonly up- or downregulated when comparing the NB or SB line vs. the BB line. These genes, including several with general regulatory functions, are

thus most likely not affected by genetic drift as they appear independently in the two treatment comparisons. Because the highest proportion of genes was differentially expressed in larvae of the SB line compared to the BB line and likewise numerous GO terms were overrepresented when comparing the genes up- or downregulated in the SB vs. the SS line, a short-term shift away from *S. alba* may represent the harshest conditions for *P. cochleariae*.

4.4 | Differential infection with gregarines and other microorganisms

All lines of *P. cochleariae* investigated in this study were infected with a gregarine species (Figure 4b). Additional RT–PCR analyses of beetles from our laboratory culture and beetles collected in the wild revealed gregarine infections in both cases (data not shown). The primers for these PCR assays were designed specifically for gregarine transcripts picked up in our *Phaedon* data set. Thus, an infection with gregarines is apparently common in this species. Likewise, a gregarine prevalence of 91% was found in field-collected beetles of *Tribolium castaneum* (Tate & Graham, 2015). In this beetle, gregarine infection can drastically increase mortality, likely due to the nutrient depletion caused by the parasite (Gigliolli et al., 2016). In *Phaedon brassicae* populations, infection rates of 100% were found (Kim, Min, Kwon, Choi, & Lee, 2014). Infection of *P. brassicae* larvae with these facultative parasites prolonged the developmental time of each larval instar by half a day, but the overall developmental time did not significantly differ between gregarine-infected and noninfected *P. brassicae* (Kim et al., 2014). Likewise, *P. cochleariae* may well be adapted to this specific gregarine parasite, because we found very low larval mortality rates in our laboratory rearing of this species. When larvae are infected by gregarines, this can evoke an immune priming of the adults. For example, *T. castaneum* adults show a lower parasite load when they have been infected as larvae with gregarines (Thomas & Rudolf, 2010).

Intriguingly, long-term rearing on one host plant led to an intermediate gregarine load that was similar in the BB, NN and SS lines of *P. cochleariae* (Figure 4a), indicating that the parasite may adjust to a kind of homeostatic level under constant feeding on the same host. When insects were reared long-term on *N. officinale* but switched back to the original host for one generation or reared just one generation on *N. officinale*, the gregarine load was reduced by half. Thus, the host plant chemistry of this plant may evoke a suppression of this parasite at least in the short term. On the contrary, a switch from *B. rapa* to *S. alba* caused a twofold increase in gregarine load. However, larvae of this BS line showed the lowest overall differential expression of genes, but an overrepresentation of upregulated immunity-related genes (Fig. S2C). Thus, the heavy gregarine load may trigger immunity but at the same time suppress general transcriptional regulation at least in the short term. In the SS line, the gregarine load was only half as high as in the BS line, indicating that the immune response probably became more effective over time. Remarkably, the switch-back from *S. alba* to *B. rapa* led to the lowest gregarine infestation but at the same time the highest

overall proportion of genes differentially expressed, which mainly belonged to general gene information processing terms (Figure 2; Fig. S2C,D). Further experiments are necessary to study the relationship between host plant chemistry and consequences on the interactions with the gregarine and to identify the gregarine. The differential expression of genes belonging to bacteria and yeasts matched the infection rates with the apicomplexan gregarine, with highest differential expression in the BS and lowest in the SB line (Figs. S8, S9). Whether the gregarine is associated with some of these microorganisms is unknown.

5 | CONCLUSION

This study adds insight to genome-wide transcriptional changes in relation to host plant adaptations in herbivorous insects, a topic that is still only scarcely investigated (Hoang et al., 2015; de Panis et al., 2016; Vogel et al., 2014). Our data revealed that experience of beetles on different host plants over many generations leads to very distinct transcriptional signatures compared to short-term switches to a novel or the original host. Regulation in primary metabolism and digestion is necessary to be able to deal with the host plant environment. However, host plant species can also have consequences on many other cellular processes. The drastically differing gene expression regulation in relation to the gregarine infection presents a new layer of complexity in studies of plant–insect interactions.

DATA ACCESSIBILITY

Microarray data and calculations for the ribosomal proteins are archived in EDMOND (http://dx.doi.org/10.17617/3.z), the Open Access Data Repository of the Max Planck Society.

AUTHOR CONTRIBUTIONS

C.M. provided the insect material and designed the study together with H.V. H.V. performed the molecular laboratory work, generated the reference transcriptome and ran the microarrays. H.V. and D.G.H. performed the gene expression analysis. C.M. drafted a first version of the manuscript and together with the rest of the authors contributed to the interpretation and refinement of the article. All authors have read and approved the submitted version.

ORCID

Caroline Müller (iD) http://orcid.org/0000-0002-8447-534X

REFERENCES

Barrett, A. J., & Rawlings, N. D. (2007). 'Species' of peptidases. *Biological Chemistry*, *388*, 1151–1157.

Barthel, A., Kopka, I., Vogel, H., Zipfel, P., Heckel, D. G., & Groot, A. T. (2014). Immune defence strategies of generalist and specialist insect herbivores. *Proceedings of the Royal Society B-Biological Sciences*, *281*, 20140897.

Beckert, A., Wiesner, J., Schmidtberg, H., Lehmann, R., Baumann, A., Vogel, H., & Vilcinskas, A. (2016). Expression and characterization of a recombinant i-type lysozyme from the harlequin ladybird beetle *Harmonia axyridis*. *Insect Molecular Biology*, *25*, 202–215.

Bretschneider, A., Heckel, D. G., & Vogel, H. (2016). Know your ABCs: Characterization and gene expression dynamics of ABC transporters in the polyphagous herbivore *Helicoverpa armigera*. *Insect Biochemistry and Molecular Biology*, *72*, 1–9.

Bukovinszky, T., Gols, R., Posthumus, M. A., Vet, L. E. M., & van Lenteren, J. C. (2005). Variation in plant volatiles and attraction of the parasitoid *Diadegma semiclausum* (Hellen). *Journal of Chemical Ecology*, *31*, 461–480.

Carroll, S. P., Klassen, S. T. P., & Dingle, H. (1998). Rapidly evolving adaptations to host ecology and nutrition in the soapberry bug. *Evolutionary Ecology*, *12*, 955–968.

Cartea, M. E., Francisco, M., Soengas, P., & Velasco, P. (2011). Phenolic compounds in *Brassica* vegetables. *Molecules*, *16*, 251–280.

Celorio-Mancera, M. D., Ahn, S. J., Vogel, H., & Heckel, D. G. (2011). Transcriptional responses underlying the hormetic and detrimental effects of the plant secondary metabolite gossypol on the generalist herbivore *Helicoverpa armigera*. *BMC Genomics*, *12*, 575.

Celorio-Mancera, M. D., Heckel, D. G., & Vogel, H. (2012). Transcriptional analysis of physiological pathways in a generalist herbivore: Responses to different host plants and plant structures by the cotton bollworm, *Helicoverpa armigera*. *Entomologia Experimentalis et Applicata*, *144*, 123–133.

Celorio-Mancera, M. D., Wheat, C. W., Vogel, H., Soderlind, L., Janz, N., & Nylin, S. (2013). Mechanisms of macroevolution: Polyphagous plasticity in butterfly larvae revealed by RNA-Seq. *Molecular Ecology*, *22*, 4884–4895.

Chandra, G. S., Asokan, R., Manamohan, M., & Sita, T. (2016). Cytochrome P450 isoforms transcriptional, larval growth and development responses to host allelochemicals in the generalist herbivore, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Current Science*, *111*, 901–906.

Chi, Y. H., Salzman, R. A., Balfe, S., Ahn, J. E., Sun, W., Moon, J., ... Zhu-Salzman, K. (2009). Cowpea bruchid midgut transcriptome response to a soybean cystatin - costs and benefits of counter-defence. *Insect Molecular Biology*, *18*, 97–110.

Chikate, Y. R., Tamhane, V. A., Joshi, R. S., Gupta, V. S., & Giri, A. P. (2013). Differential protease activity augments polyphagy in *Helicoverpa armigera*. *Insect Molecular Biology*, *22*, 258–272.

Conesa, A., Gotz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). BLAST2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics*, *21*, 3674–3676.

Dai, L. L., Ma, M. Y., Gao, G. Q., & Chen, H. (2016). *Dendroctonus armandi* (Curculionidae: Scolytinae) cytochrome P450s display tissue specificity and responses to host terpenoids. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, *201*, 1–11.

Dermauw, W., Wybouw, N., Rombauts, S., Menten, B., Vontas, J., Grbić, M., ... Van Leeuwen, T. (2013). A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, E113–E122.

Després, L., David, J. P., & Gallet, C. (2007). The evolutionary ecology of insect resistance to plant chemicals. *Trends in Ecology & Evolution*, *22*, 298–307.

Engel, P., & Moran, N. A. (2013). The gut microbiota of insects - diversity in structure and function. *Fems Microbiology Reviews*, *37*, 699–735.

Eyres, I., Jaquiery, J., Sugio, A., Duvaux, L., Gharbi, K., Zhou, J. J., ... Ferrari, J. (2016). Differential gene expression according to race and host plant in the pea aphid. *Molecular Ecology*, *25*, 4197–4215.

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry*, *56*, 5–51.

Falk, K. L., & Gershenzon, J. (2007). The desert locust, *Schistocerca gregaria*, detoxifies the glucosinolates of *Schouwia purpurea* by desulfation. *Journal of Chemical Ecology*, *33*, 1542–1555.

Feyereisen, R. (1999). Insect P450 enzymes. *Annual Review of Entomology*, *44*, 507–533.

Feyereisen, R. (2012). Insect CYP genes and P450 enzymes. In I. G. Lawrence (Ed.), *Insect molecular biology and biochemistry* (pp. 236–316). San Diego, USA: Academic Press.

Franta, Z., Vogel, H., Lehmann, R., Rupp, O., Goesmann, A., & Vilcinskas, A. (2016). Next generation sequencing identifies five major classes of potentially therapeutic enzymes secreted by *Lucilia sericata* medical maggots. *Biomed Research International*, 8285428.

Fukamizo, T. (2000). Chitinolytic enzymes: Catalysis, substrate binding, and their application. *Current Protein & Peptide Science*, *1*, 105–124.

Futuyma, D. J. (2005). Natural selection and adaptation. In D. J. Futuyma (Ed.), *Evolution* (pp. 247–267). Sunderland, Massachusetts: Sinauer Associates Inc.

García-Robledo, C., & Horvitz, C. C. (2012). Jack of all trades masters novel host plants: Positive genetic correlations in specialist and generalist insect herbivores expanding their diets to novel hosts. *Journal of Evolutionary Biology*, *25*, 38–53.

Gigliolli, A. A. S., Julio, A. H. F., & Conte, H. (2016). The life cycle of *Gregarina cuneata* in the midgut of *Tribolium castaneum* and the effects of parasitism on the development of insects. *Bulletin of Entomological Research*, *106*, 258–267.

Govind, G., Mittapalli, O., Griebel, T., Allmann, S., Bocker, S., & Baldwin, I. T. (2010). Unbiased transcriptional comparisons of generalist and specialist herbivores feeding on progressively defenseless *Nicotiana attenuata* plants. *PLoS ONE*, *5*, e8735.

Han, J. B., Li, G. Q., Wan, P. J., Zhu, T. T., & Meng, Q. W. (2016). Identification of glutathione S-transferase genes in *Leptinotarsa decemlineata* and their expression patterns under stress of three insecticides. *Pesticide Biochemistry and Physiology*, *133*, 26–34.

Heidel-Fischer, H. M., & Vogel, H. (2015). Molecular mechanisms of insect adaptation to plant secondary compounds. *Current Opinion in Insect Science*, *8*, 8–14.

Hoang, K., Matzkin, L. M., & Bono, J. M. (2015). Transcriptional variation associated with cactus host plant adaptation in *Drosophila mettleri* populations. *Molecular Ecology*, *24*, 5186–5199.

Jacobs, C. G. C., Steiger, S., Heckel, D. G., Wielsch, N., Vilcinskas, A., & Vogel, H. (2016). Sex, offspring and carcass determine antimicrobial peptide expression in the burying beetle. *Scientific Reports*, *6*, 25409.

Johnston, P. R., & Rolff, J. (2015). Host and symbiont jointly control gut microbiota during complete metamorphosis. *Plos Pathogens*, *11*, e1005246.

Jones, A., Davies, H. M., & Voelker, T. A. (1995). Palmitoyl-acyl carrier protein (ACP) thioesterase and the evolutionary origin of plant acyl-ACP thioesterases. *Plant Cell*, *7*, 359–371.

Kim, J. I., Min, J. S., Kwon, M., Choi, J. Y., & Lee, S. H. (2014). Morphological and molecular characterizations of the *Gregarina* sp

(Apicomplexa: Protozoa) parasitizing on *Phaedon brassicae* (Coleoptera: Chrysomelidae). *Journal of Asia-Pacific Entomology*, 17, 1–5.

Kirsch, R., Gramzow, L., Theissen, G., Siegfried, B. D., Ffrench-Constant, R. H., Heckel, D. G., & Pauchet, Y. (2014). Horizontal gene transfer and functional diversification of plant cell wall degrading polygalacturonases: Key events in the evolution of herbivory in beetles. *Insect Biochemistry and Molecular Biology*, 52, 33–50.

Kirsch, R., Wielsch, N., Vogel, H., Svatoš, A., Heckel, D. G., & Pauchet, Y. (2012). Combining proteomics and transcriptome sequencing to identify active plant-cell-wall-degrading enzymes in a leaf beetle. *BMC Genomics*, 13, 587.

Koenig, C., Bretschneider, A., Heckel, D. G., Grosse-Wilde, E., Hansson, B. S., & Vogel, H. (2015). The plastic response of *Manduca sexta* to host and non-host plants. *Insect Biochemistry and Molecular Biology*, 63, 72–85.

Kolesnikov, M. P., & Gins, V. K. (2001). Phenolic substances in medicinal plants. *Applied Biochemistry and Microbiology*, 37, 392–399.

Kopsell, D. A., Barickman, T. C., Sams, C. E., & McElroy, J. S. (2007). Influence of nitrogen and sulfur on biomass production and carotenoid and glucosinolate concentrations in watercress (*Nasturtium officinale* R. Br.). *Journal of Agricultural and Food Chemistry*, 55, 10628–10634.

Kühnle, A., & Müller, C. (2011). Responses of an oligophagous beetle species to rearing for several generations on alternative host plant species. *Ecological Entomology*, 36, 125–134.

Kuwar, S. S., Pauchet, Y., Vogel, H., & Heckel, D. G. (2015). Adaptive regulation of digestive serine proteases in the larval midgut of *Helicoverpa armigera* in response to a plant protease inhibitor. *Insect Biochemistry and Molecular Biology*, 59, 18–29.

Li, X. C., Schuler, M. A., & Berenbaum, M. R. (2002). Jasmonate and salicylate induce expression of herbivore cytochrome P450 genes. *Nature*, 419, 712–715.

Li, X. C., Schuler, M. A., & Berenbaum, M. R. (2007). Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology*, 52, 231–253.

Malka, O., Shekhov, A., Reichelt, M., Gershenzon, J., Vassão, D. G. & Morin, S. (2016). Glucosinolate desulfation by the phloem-feeding insect *Bemisia tabaci*. *Journal of Chemical Ecology*, 42, 230–235.

Müller, C. (2009). Interactions between glucosinolate- and myrosinase-containing plants and the sawfly *Athalia rosae*. *Phytochemistry Reviews*, 8, 121–134.

Müller, T., & Müller, C. (2016). Adult beetles compensate for poor larval food conditions. *Journal of Insect Physiology*, 88, 24–32.

Müller, T., & Müller, C. (2017). Host plant effects on the behavioural phenotype of a Chrysomelid. *Ecological Entomology*, 42, 336–344.

de Panis, D. N., Padró, J., Furió-Tarí, P., Tarazona, S., Carmona, P. S. M., Soto, I. M., . . . Hasson, E. (2016). Transcriptome modulation during host shift is driven by secondary metabolites in desert *Drosophila*. *Molecular Ecology*, 25, 4534–4550.

Pernice, M., Simpson, S. J., & Ponton, F. (2014). Towards an integrated understanding of gut microbiota using insects as model systems. *Journal of Insect Physiology*, 69, 12–18.

Puinean, A. M., Denholm, I., Millar, N. S., Nauen, R., & Williamson, M. S. (2010). Characterisation of imidacloprid resistance mechanisms in the brown planthopper, *Nilaparvata lugens* Stal (Hemiptera: Delphacidae). *Pesticide Biochemistry and Physiology*, 97, 129–132.

Puinean, A. M., Foster, S. P., Oliphant, L., Denholm, I., Field, L. M., Millar, N. S., . . . Bass, C. (2010). Amplification of a cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. *Plos Genetics*, 6, e1000999.

Ragland, G. J., Almskaar, K., Vertacnik, K. L., Gough, H. M., Feder, J. L., Hahn, D. A., & Schwarz, D. (2015). Differences in performance and transcriptome-wide gene expression associated with *Rhagoletis* (Diptera: Tephritidae) larvae feeding in alternate host fruit environments. *Molecular Ecology*, 24, 2759–2776.

Ratzka, A., Vogel, H., Kliebenstein, D. J., Mitchell-Olds, T., & Kroymann, J. (2002). Disarming the mustard oil bomb. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 11223–11228.

Reifenrath, K., & Müller, C. (2007). Species-specific and leaf-age dependent effects of ultraviolet radiation on two Brassicaceae. *Phytochemistry*, 68, 875–885.

Reifenrath, K., & Müller, C. (2009). Larval performance of the mustard leaf beetle (*Phaedon cochleariae*, Coleoptera, Chrysomelidae) on white mustard (*Sinapis alba*) and watercress (*Nasturtium officinale*) leaves in dependence of plant exposure to ultraviolet radiation. *Environmental Pollution*, 157, 2053–2060.

Roy, A., Walker, W. B., Vogel, H., Chattington, S., Larsson, M. C., Anderson, P., . . . Schlyter, F. (2016). Diet dependent metabolic responses in three generalist insect herbivores *Spodoptera* spp. *Insect Biochemistry and Molecular Biology*, 71, 91–105.

Scully, E. D., Geib, S. M., Carlson, J. E., Tien, M., McKenna, D., & Hoover, K. (2014). Functional genomics and microbiome profiling of the Asian longhorned beetle (*Anoplophora glabripennis*) reveal insights into the digestive physiology and nutritional ecology of wood feeding beetles. *BMC Genomics*, 15, 1096.

Sezer, M., & Butlin, R. K. (1998). The genetic basis of host plant adaptation in the brown planthopper (*Nilaparvata lugens*). *Heredity*, 80, 499–508.

Sun, W., Shen, Y. H., Yang, W. J., Cao, Y. F., Xiang, Z. H., & Zhang, Z. (2012). Expansion of the silkworm GMC oxidoreductase genes is associated with immunity. *Insect Biochemistry and Molecular Biology*, 42, 935–945.

Tate, A. T., & Graham, A. L. (2015). Trans-generational priming of resistance in wild flour beetles reflects the primed phenotypes of laboratory populations and is inhibited by co-infection with a common parasite. *Functional Ecology*, 29, 1059–1069.

Thomas, A. M., & Rudolf, V. H. W. (2010). Challenges of metamorphosis in invertebrate hosts: Maintaining parasite resistance across life-history stages. *Ecological Entomology*, 35, 200–205.

Tremmel, M., & Müller, C. (2013). Insect personality depends on environmental conditions. *Behavioral Ecology*, 24, 386–392.

Vogel, H., Musser, R. O., & Celorio-Mancera, M. (2014). Transcriptome responses in herbivorous insects towards host plant and toxin feeding. *Annual Plant Reviews*, 47, 197–234.

Warbrick-Smith, J., Behmer, S. T., Lee, K. P., Raubenheimer, D., & Simpson, S. J. (2006). Evolving resistance to obesity in an insect. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 14045–14049.

Welte, C. U., de Graaf, R. M., van den Bosch, T. J. M., Op den Camp, H. J. M., Van Dam, N. M., & Jetten, M. S. M. (2016). Plasmids from the gut microbiome of cabbage root fly larvae encode SaxA that catalyses the conversion of the plant toxin 2-phenylethyl isothiocyanate. *Environmental Microbiology*, 18, 1379–1390.

Winde, I., & Wittstock, U. (2011). Insect herbivore counteradaptations to the plant glucosinolate-myrosinase system. *Phytochemistry*, 72, 1566–1575.

Wybouw, N., Zhurov, V., Martel, C., Bruinsma, K. A., Hendrickx, F., Grbić, V., & Van Leeuwen, T. (2015). Adaptation of a polyphagous herbivore to a novel host plant extensively shapes the transcriptome of herbivore and host. *Molecular Ecology*, 24, 4647–4663.

Yu, Q. Y., Fang, S. M., Zhang, Z., & Jiggins, C. D. (2016). The transcriptome response of *Heliconius melpomene* larvae to a novel host plant. *Molecular Ecology*, 25, 4850–4865.

Zhang, W., Chen, J. H., Keyhani, N. O., Zhang, Z. Y., Li, S., & Xia, Y. X. (2015). Comparative transcriptomic analysis of immune responses of the migratory locust, *Locusta migratoria*, to challenge by the fungal insect pathogen, *Metarhizium acridum*. *BMC Genomics*, 16, 867.

Zhang, M. X., Fang, T. T., Pu, G. L., Sun, X. Q., Zhou, X. G., & & Cai, Q. N. (2013). Xenobiotic metabolism of plant secondary compounds in the English grain aphid, *Sitobion avenue* (F.) (Hemiptera: aphididae). *Pesticide Biochemistry and Physiology*, 107, 44–49.

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