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The plastic response of *Manduca sexta* to host and non-host plants

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ABSTRACT

Specialist insect herbivores have evolved efficient ways to adapt to the major defenses of their host plants. Although *Manduca sexta*, specialized on Solanaceous plants, has become a model organism for insect molecular biology, little is known about its adaptive responses to the chemical defenses of its hosts. To study larval performance and transcriptomic responses to host and non-host plants, we conducted developmental assays and replicated RNAseq experiments with *Manduca* larvae fed on different Solanaceous plants as well as on a Brassicaceous non-host plant, *Brassica napus*. *Manduca* larvae developed fastest on *Nicotiana attenuata*, but no significant differences in performance were found on larvae fed on other Solanaceae or the non-host *B. napus*. The RNAseq experiments revealed that *Manduca* larvae display plastic responses at the gene expression level, and transcriptional signatures specific to the challenges of each host- and non-host plant. Our observations are not consistent with expectations that specialist herbivores would perform poorly on non-host plants. Instead, our findings demonstrate the ability of this specialized insect herbivore to efficiently use a larger repertoire of host plants than it utilizes in the field.

1. Introduction

The tobacco hornworm (*Manduca sexta,* henceforth called *Manduca)* has become an important model system in insect science (Baldwin, 2001; Riddiford et al., 2003; Shields and Hildebrand, 2001; Späthe et al., 2013). There are many studies on its biochemistry, behavior and physiology, and many studies on its host plant *Nicotiana*, yet few studies have investigated *Manduca* host-plant interactions at the molecular level. Although *Manduca* larvae specialize on nightshade (Yamamoto and Fraenkel, 1960), they can be reared on artificial diet as well as on non-solanaceous plants, such as *Brassica* spp. (Brassicaceae) under laboratory conditions (Boer and Hanson, 1984). Although such behavior would classify *Manduca* as an oligophagous species rather than a specialist species, the broad host plant range accepted in the lab is not well documented in the field.

In this study, we focused on three typical host plants of *Manduca*: *Nicotiana attenuata* (coyote tobacco), *Solanum lycopersicum* (tomato) and *Datura wrightii* (sacred datura), all belonging to the Solanaceae (nightshade) family, as well as the non-host plant *Brassica napus* (rapeseed). While the solanaceous host plants differ in their secondary metabolites and proteinaceous effectors, *B. napus* also uses a different class of metabolites, glucosinolates, as its major chemical defense (Fahey et al., 2001).

Plants of the nightshade family employ alkaloids, phenylpropanoids, flavonoids, and protease inhibitors to deter herbivores: *Nicotiana,* for example, produces the alkaloid nicotine, as well as trypsin inhibitors. Both of these have been shown to be effective defenses especially against generalist herbivores but can also impact specialist performance (Steppuhn and Baldwin, 2007). *Nicotiana* plants as well as artificial diet containing high nicotine concentrations inhibit the growth of both *Manduca* and the

Abbreviations: AM, antennae and maxillae; G, gut with Malpighian tubules; SG, silk gland (labial gland); W, whole insect; P450, cytochrome P450 monooxygenase; GST, glutathione S-transferase; UGT, UDP-glycosyl transferase; ABC, ATP-binding cassette; IMD, immune deficiency; JAK, janus kinase; STAT, signal transducers and activators of transcription; JNK, c-Jun N-terminal protein kinases; SOCS, suppressor of cytokine signaling; AMP, antimicrobial peptide; WAP, whey acid proteins; AFP, antifungal proteins; MAPK, MAP kinase; OR, olfactory receptor; OBP, odorant binding protein; OSN, olfactory sensory neuron.

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polyphagous *Helicoverpa zea* (Harvey et al., 2007; Voelckel et al., 2001), and trypsin inhibitors from sweet potato have been shown to affect the growth of and therefore confer resistance to *Spodoptera litura* (Yeh et al., 1997). *S. lycopersicum* uses tomatine, chlorogenic acid, polyphenol oxidase, and proteinase inhibitors to deter herbivorous insects (Kennedy, 2003). Tomatine, a glycoalkaloid, has strong negative effects on the growth rate of *Spodoptera exigua* as well as of *H. zea*, both of which are generalist species that naturally occur on tomato (Bloem et al., 1989). *Datura wrightii* synthesizes the alkaloids scopolamine and hyoscyamine (Hare and Walling, 2006; Parr et al., 1990). Scopolamine prolongs the development and enhances the mortality of *Spodoptera frugiperda*, another generalist from the Noctuidae family (Alves et al., 2007).

Unlike the alkaloid-based chemical defenses of Solanaceae, brassicaceous plants use a system that is activated by two components, glucosinolates and myrosinase enzymes, as their major chemical defense (Bruce, 2014). Highly adapted insect herbivores can feed with impunity on their glucosinolate-containing host plants, but most polyphagous herbivores are negatively affected by high levels of glucosinolates (Arany et al., 2008; Kliebenstein et al., 2005; Winde and Wittstock, 2011).

Although transcriptional responses of generalist herbivores to different host plants or isolated toxins and of generalist and specialist herbivores to individual host plants have been analyzed, studies on large-scale transcriptional responses of herbivorous insects to a range of host plants are scarce or focused on aspects other than herbivore-host plant interactions (Zhan et al., 2011). Not only does host plant chemistry have an impact on detoxification-related gene expression, but secondary metabolites can be crucial for continued larval feeding. The chemical perception of the environment provides information on, for example, food sources and mating partners (Hanson and Dethier, 1973). Olfaction is important for larval plant discrimination and differences in host plant chemistry could potentially be reflected at the level of larval gustatory and olfactory gene expression. Likewise, plant secondary metabolites can influence an insect's immune system, resulting in the differential expression of immune-related genes. Immune defense strategies might also vary with the breadth of an organism's diet (Barthel et al., 2014; Lee et al., 2008; Ponton et al., 2013). Similarly, differences in both types and densities of host plant-associated bacteria can have an impact on innate immunity in herbivorous insects (Freitak et al., 2007).

The goal of this study was to compare the performance of larvae feeding on host and non-host plants as well as global changes in the gene expression of *Manduca* larval tissues elicited by feeding on these plants. To investigate these transcriptional responses, we used a replicated RNAseq approach combined with the official *Manduca* Gene Set (OGS2). In our analyses of the transcriptional responses of *Manduca* larvae, we mainly focused on putative detoxification-related, immune-related and olfactory genes. Here we show that *Manduca* larvae grow fastest on *Nicotiana*, one of the main host plants of this specialized insect. However, *Manduca* larvae performed equally well when fed on the non-host plant *Brassica* as when they fed on other host plants. We report specific changes in the expression of genes related to detoxification, immunity and olfaction as a consequence of feeding on different plants, providing insights into the plastic response of an herbivorous insect with a restricted repertoire of host plants.

2. Material and methods

2.1. Biological material and Manduca sexta rearing

Wild types of the following plant species were used for the experiments: *N. attenuata*, *Datura wrightii*, *S. lycopersicum* (cv. Balcony Magic) and *B. napus* (cv. Dwarf Essex). All plants were grown in a greenhouse maintained at 26 °C, 75% humidity, and a 16 h light and 8 h dark cycle.

Manduca larvae were fed on artificial diet (46 g of agar, 144 g of wheat germ, 140 g of corn meal, 76 g of soy flour, 75 g of casein, 24 g of Wesson's salt mixture, 36 g of sugar, 5 g of cholesterol, 12 g of ascorbic acid, 6 g of sorbic acid, 3 g of methyl paraben, 9 mL of linseed oil, 60 mL of 3.7% formalin, 30 mg of nicotinic acid, 15 mg of riboflavin, 7 mg of thiamine, 7 mg of pyridoxine, 7 mg of folic acid, and 0.6 mg of biotin per 1.8 L of water). Insects were kept at 26 °C, 75% humidity, and a 16 h light and 8 h dark cycle.

2.2. Feeding assay

For the feeding assay, larvae were reared on artificial diet up until shortly before reaching the third instar (L3), when they were transferred to one of the four host (*Nicotiana*, *Datura, Solanum*) or non-host (*Brassica*) plants, respectively, or fed the artificial diet. Plants were 5–10 weeks old (flowers had been removed) and larvae were allowed to feed on whole plants or the artificial diet for eight consecutive days (Fig. 1A). Larval weight was recorded every second day. Differences in the development were statistically analyzed using ANOVA (in R). From the average weight per treatment, we calculated the relative growth rate, since it represents the proportional increase in mass per unit time and adjusts for initial size and the nonlinear patterns of growth over time.

Silk (labial) glands (SG), as well as guts together with Malpighian tubules (G), were dissected from L4 non-molting larvae at Zeitgeber time 8–12. Antennae together with maxillae (AM) were collected from L5 non-molting larvae at Zeitgeber time 8–12. Insects were dissected in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5). In addition, whole insects (L4 non-molting larvae) (W) were ground in liquid nitrogen at Zeitgeber time 8–12 for RNA isolation. Dissected tissue was kept at −20 °C in lysis buffer (innuPREP RNA Mini Kit, Analytik Jena, Germany) until being used for RNA isolation.

2.3. RNA isolation and illumina sequencing

RNAseq experiments were carried out with RNA isolated from larvae reared on artificial diet or different host plants. For SG, G, and W samples, three larvae were pooled for each RNA sample (biological replicate). For AM samples, ten larvae were pooled to receive a sufficient amount of RNA. Three replicates were created from each tissue. Total RNA was extracted according to the manufacturer's instructions (innuPREP RNA Mini Kit, Analytik Jena, Germany).

Library construction and sequencing was performed by the Max Planck Genome Center Cologne, Germany (http://mpgc.mpipz.mpg.de/home/). 1 μg of total RNA was used for a TruSeq RNA library and mRNA enrichment was performed. The library was sequenced with an Illumina HiSeq2500 sequencer. Approximately 10 million 100 bp single-end reads per biological replicate, per treatment, and for each of the tissue samples were obtained. Quality control measures, including filtering high-quality reads based on the score given in fastq files, removing reads containing primer/adaptor sequences and trimming read length, were carried out using CLC Genomics Workbench v6.5 (http://www.clcbio.com).

2.4. Gene annotation

The *Manduca* OGS2-predicted gene set was annotated using BLAST, Gene Ontology and InterProScan searches using BLAST2GO PRO v2.6.1 (www.blast2go.de) (Conesa and Götz, 2008). For BLASTX searches against the non-redundant NCBI protein database (NR database) up to 20 best NR hits per transcript were retained, with