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Know your ABCs: Characterization and gene expression dynamics of ABC transporters in the polyphagous herbivore *Helicoverpa armigera*

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ABSTRACT

Polyphagous insect herbivores are adapted to many different secondary metabolites of their host plants. However, little is known about the role of ATP-binding cassette (ABC) transporters, a multigene family involved in detoxification processes. To study the larval response of the generalist *Helicoverpa armigera* (Lepidoptera) and the putative role of ABC transporters, we performed developmental assays on artificial diet supplemented with secondary metabolites from host plants (atropine-scopolamine, nicotine and tomatine) and non-host plants (taxol) in combination with a replicated RNAseq experiment. A maximum likelihood phylogeny identified the subfamily affiliations of the ABC transporter sequences. Larval performance was equal on the atropine-scopolamine diet and the tomatine diet. For the latter we could identify a treatment-specific upregulation of five ABC transporters in the gut. No significant developmental difference was detected between larvae fed on nicotine or taxol. This was also mirrored in the upregulation of five ABC transporters when fed on either of the two diets. The highest number of differentially expressed genes was recorded in the gut samples in response to feeding on secondary metabolites. Our results are consistent with the expectation of a general detoxification response in a polyphagous herbivore. This is the first study to characterize the multigene family of ABC transporters and identify gene expression changes across different developmental stages and tissues, as well as the impact of secondary metabolites in the agricultural pest *H. armigera*.

1. Introduction

Herbivorous insects face a number of different plant defense mechanisms (Kessler and Baldwin, 2002), but most importantly they encounter secondary metabolites while feeding. These substances act as repellents and antifeedants, or may even be toxic (Heckel, 2014; Ibanez et al., 2012). Known examples are alkaloids, terpenoids and phenolics. Insects have adapted in numerous ways to these metabolites, such as by avoiding, sequestering or converting them into less toxic compounds (Heckel, 2014; Parrott et al., 1983; Willinger and Dobler, 2001). Generalist insect herbivores are thought to possess a range of general detoxifying enzymes to be pre-adapted for feeding on many different host plants (Vogel et al., 2014b), e.g. an expansion of gene families involved in detoxification (Grbić et al., 2011).

The cotton bollworm, *Helicoverpa armigera* (Hübner), is a generalist noctuid moth, whose larval stages feed on more than 60 cultivated plants, such as cotton, tobacco, sunflower and corn (Czepak et al., 2013; Fitt, 1989). Many studies have focused on different life-history aspects of *H. armigera* (Aurade et al., 2010; Joußen et al., 2012; Kuwar et al., 2015; Rajapakse and Walter, 2007), but only a few have focused on host plant adaptation, especially the enzymes involved in the detoxification of plant secondary metabolites (de la Paz Celorio-Mancera et al., 2011; de la Paz Celorio-Mancera et al., 2012; Liu et al., 2006). However, all of these studies focused on cytochrome P450s, UDP-glycosyltransferases, glutathione transferases and esterases.

ATP-binding cassette (ABC) transporters are transmembrane proteins, which can be found in all forms of life, including insects (Dassa and Bouige, 2001; Linton, 2007). Known functions within the cell comprise the transport of lipids, inorganic ions and especially the detoxification of xenobiotics (Holland et al., 2003). In insects ABC transporters have been shown to be involved in

Abbreviations: ABC, ATP-binding cassette (transporter); MT, Malpighian tubules; G, gut; RB, rest body; NBD, nucleotide binding domain; Ha, *Helicoverpa armigera*; Hvir, *Heliothis virescens*; Hsub, *Heliothis subflexa*; Tni, *Trichoplusia ni*; Dple, *Danaus plexippus*.

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glucoside sequestration (Strauss et al., 2013), the transport of eye color pigments (Ewart et al., 1994) and resistance against synthetic insecticides (Aurade et al., 2010; Buss and Callaghan, 2008). ABC transporters also act as targets for *Bacillus thuringiensis* insecticidal toxins (Bt) (Heckel, 2015). For example Bt resistance has been genetically linked to HvABCC2 in *Heliothis virescens* (Gahan et al., 2010) and HaABCC2 in *H. armigera* (Xiao et al., 2014). Just recently ABCA2, a member of the subfamily A, was shown to be involved in the Bt mode of action in *H. armigera* as well (Tay et al., 2015).

A functional ABC transporter consists of four core domains: two membrane spanning domains (transmembrane domain, TM), each built up from six membrane spanning α-helices, alternating with two nucleotide binding domains (NBD) located on the cytosolic side (Fig. 1A) (Linton, 2007). According to their structure and domain organization, these transporters are classified into different subfamilies named from A to H. Interestingly, insects were shown to possess a larger number of ABC transporters than humans (48) (Dean et al., 2001; Dermauw and Van Leeuwen, 2014). The flour beetle *Tribolium castaneum* possesses 73 (Broehan et al., 2013), and 51–53 were identified in the silk moth *Bombyx mori* (Liu et al., 2011; Xie et al., 2012). However, the species with the largest number of transporters is the spider mite *Tetranychus urticae* (103) (Dermauw et al., 2013). This high number of ABC transporter genes might be linked to the extreme polyphagy of this species, especially since it possesses 39 ABC C transporters, a subfamily involved in multidrug resistance (Liu et al., 2011; Migeon and Dorkeld, 2015).

It has been shown before that ABC transporter gene expression differs among tissues and developmental stages as well as after xenobiotic exposure (Dermauw et al., 2013; Fletcher et al., 2010; Labbé et al., 2011; Simmons et al., 2013; Strauss et al., 2014). A recent study on the plastic response of the tobacco hornworm *Manduca sexta* revealed treatment- and tissue-specific expression in larvae. Koenig et al. (2015) show that especially members of subfamilies B and C, both of which are involved in detoxification and multidrug resistance, are upregulated in the gut when larvae were fed on plants. Similar results were observed in *T. urticae,* where a host plant switch induced the expression of ABC transporter genes (Dermauw et al., 2013). Moreover, that study revealed a developmental-stage specific expression between embryos, larvae and adults.

Here we focus on the ABC transporter expression in the polyphagous lepidopteran species *H. armigera*. RNA sequencing (RNAseq) combined with the official *Helicoverpa* Gene Set was performed with different developmental stages as well as larval tissues to investigate when and where these genes are expressed in Lepidoptera. We report developmental- and tissue-specific ABC transporter gene expression. The influence of xenobiotics on the transcriptional response of ABC transporters was assessed by feeding larvae with host and non-host plant derived secondary metabolites prior to RNA collection. Here we show that *H. armigera* larvae developed significantly slower on all diets supplemented with secondary metabolites compared to the control. Furthermore, the xenobiotics elicited complex and compound-specific changes in the ABC transporter expression.

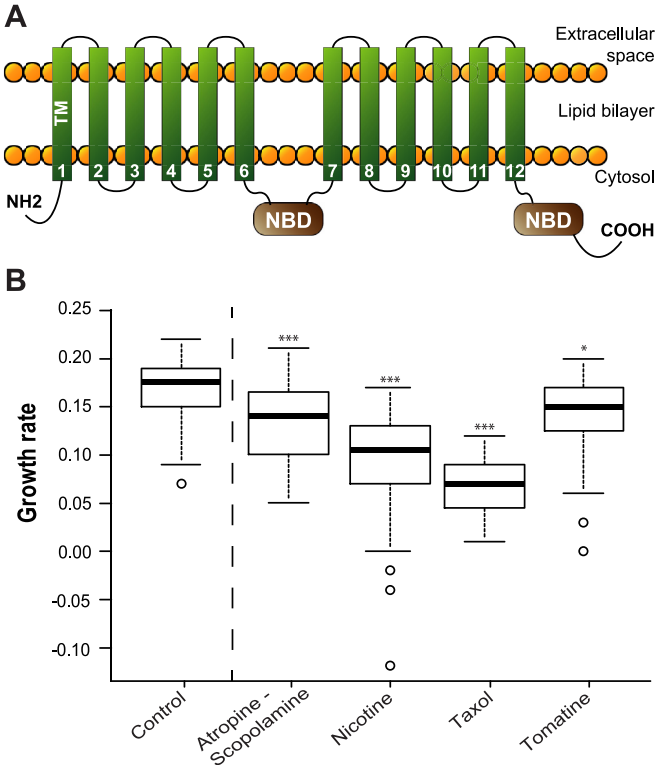

Fig. 1. A) The core structure of a full ABC transporter, containing two transmembrane domains (TMs, composed of 12 membrane-spanning α-helices) alternating with two nucleotide binding domains (NBDs). **B)** Relative growth rate of *H. armigera* larvae on artificial diet supplemented with secondary metabolites after three consecutive days of feeding compared to control diet. The box illustrates the range, in which 50% of the data are situated and the thick line represents the median. The whiskers show 95% of the data points. Nicotine 30.8 mM, tomatine 0.46 mM, taxol 9.4 μM, atropine 10.37 mM, scopolamine 8.24 mM, Tukey HSD: P-value * ≤ 0.05, *** ≤ 0.001; N = 40/diet.

2. Materials and methods

2.1. Insect rearing

The TWB strain of *H. armigera* (Hübner) (Lepidoptera: Noctuidae) originated from the vicinity of Toowoomba, Queensland, Australia, in January 2003 and was transferred to the Max Planck Institute for Chemical Ecology in Jena (Germany) in August 2004. Insects were reared on artificial Bio-Serv diet (General Purpose Lepidoptera). Adults were mated in single pair matings using males and females from different families to minimize inbreeding depression and retain genetic diversity. All life stages were kept under similar conditions in an environmental chamber (55% relative humidity (RH); 26 °C; 16 h light: 8 h dark).

2.2. Feeding assay and tissue collection

Four secondary metabolites, present in host plants of *H. armigera*, were incorporated in artificial Bio-Serv diet: tomatine (0.46 mM, Santa Cruz Biotechnology), nicotine (30.8 mM, Sigma Aldrich), atropine (10.37 mM, Sigma Aldrich) and scopolamine hydrobromide trihydrate (8.24 mM, referred to as scopolamine, Sigma Aldrich). Atropine and scopolamine were mixed together in one diet, since they appear in the same host plant. Furthermore, paclitaxel (9.4 μM, referred to as taxol, Enzo Life Sciences), a secondary metabolite from a non-host plant, was tested. The secondary metabolites were tested in biologically relevant concentrations based on preliminary tests and their availability in the respective plants (Alves et al., 2007; Devitt and Philogene, 1980; Farrar and Kennedy, 1990; Gallardo and Boethel, 1990; Hiraoka et al., 1996; Ketchum et al., 1999).

Freshly molted 5th instar larvae (<240 mg, four different families) were weighed and fed on toxin-incorporated or artificial control Bio-Serv diet for three consecutive days. Afterward, larvae were weighed and dissected in 1x phosphate-buffered saline (PBS, BioRad) into midgut (G), Malpighian tubules (MT) and rest body (RB), which comprises all tissues except the gut, malpighian tubules