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Molecular mechanisms of insect adaptation to plant secondary compounds

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During feeding, herbivorous insects are exposed to an array of plant defensive compounds. In this review, we examine molecular mechanisms of insect adaptation to these toxic metabolites. We discuss both the importance of evolutionary variation of existing detoxification gene families, as well as the evolution of novel mechanisms through gene recruitment, neofunctionalization and horizontal gene transfer. The ability of insects to cope with the chemical diversity of their host plants and the different mechanisms that insects use to resist these toxins open new avenues for understanding fundamental aspects of insect–plant coevolutionary adaptation.

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Introduction

Because plants are sessile, effective defense systems are necessary to prevent them from being eaten by herbivorous insects. These defenses are most frequently chemical, with an array of plant secondary compounds identified to date [1–3]. Herbivores have to survive exposure to the chemically challenging or toxic environments of their host plants and have in turn evolved various ways to overcome plant defenses or even use them for their own benefit. Plant-derived xenobiotics can be rapidly excreted, metabolized into non-toxic compounds or sequestered by insects for protection against natural enemies [4]. We focus here on the molecular mechanisms that have been shown to enable insects to overcome chemical plant defenses and thus successfully adapt to their host plants.

Detoxification by ubiquitous enzymes

The detoxification and metabolism of most xenobiotics probably occurs via a common set of detoxification-related enzymes, all of which belong to gene families. Phase I enzymes such as cytochrome P450 proteins (P450s) participate in the functionalization step of xenobiotic detoxification, while Phase II enzymes such as UDP-glycosyltransferases (UGTs) convert lipophilic xenobiotics into more hydrophilic compounds to facilitate excretion or sequestration. However, although all insects harbor a large set of these ubiquitous enzymes, in most cases only a small number of detoxifying enzymes can metabolize specific plant compounds.

Cytochrome P450 proteins are encoded by the CYP gene superfamily and are membrane-bound enzymes involved in the metabolism of a variety of molecules such as vitamins and hormones, but are probably best known for their ability to metabolize xenobiotics. Recent research has identified several cytochrome P450 enzymes that play an important role in the adaptation to specific plant defense compounds.

The parsnip webworm (*Depressaria pastinacella*) and the black swallowtail (*Papilio polyxenes*), both specialists on furanocoumarin containing plants, are prominent examples of herbivores that can detoxify these plant compounds using rapid cytochrome P450-mediated metabolism. Although furanocoumarins are capable of interfering with DNA replication, functional characterization of several cytochrome P450 enzymes showed that both insect species harbor specialized CYP6B enzymes that metabolize these toxins with both high specificity and efficiency [5–9]. Allelic variation, gene duplication and the subfunctionalization of CYP6B genes in the furanocoumarin-adapted species produce CYP enzymes that potentially allow the caterpillars to adapt to chemical variation in their host plants [6,7]. The respective CYP6B genes identified in polyphagous insects, such as *Helicoverpa zea*, in turn encode enzymes that metabolize a wide range of furanocoumarins with low levels of activity, which can accept structurally diverse phenolic compounds as substrates [10].

Despite a growing number of descriptive studies on plant toxin-induced P450 transcriptional changes in herbivores, few studies have shown clear evidence of P450-based toxin metabolism. CYP6AE14 in the polyphagous Lepidopteran *Helicoverpa armigera* is another example of the xenobiotics-metabolizing CYP6B subfamily, which was associated with the tolerance of the caterpillars to gossypol, a toxin found in cotton host plants [11]. However, although CYP6AE14 transcript levels are inducible by gossypol, and RNAi-based suppression resulted in a reduction of larval

tolerance to gossypol, conclusive evidence that CYP6AE14 is directly involved in the detoxification (i.e. the modification of gossypol to a less toxic metabolite) is still lacking.

Resistance to a range of xenobiotics can be identified in many pest insects, with the most prominent examples coming from insecticide research. In *H. armigera*, for example, an unequal crossing over event between two CYP337B genes resulted in a chimeric gene that is capable of metabolizing the insecticide fenvalerate (a synthetic version of a chrysanthemum plant toxin) to the non-toxic 4′-hydroxyfenvalerate, which neither of the parental CYP genes is able to do [12•]. The resistance to pyrethroid insecticides arose 6 years after such insecticides were introduced to Australia. A worldwide screening of resistant *H. armigera* populations suggests that the resistance event with the same mechanism involved occurred most probably at least twice independently [13]. Although this example is a special case of metabolic detoxification (i.e. of an insecticide), it shows the vast metabolic capacity and the ability of the CYP gene family to acquire new functions rapidly.

An alternative to the molecular evolution of new functions exemplified above is the over-expression of existing cytochrome P450 genes. In the aphid *Myzus persicae*, the constitutive over-expression of the CYP6CY3 gene allows a tobacco-adapted strain to efficiently detoxify nicotine [14•], while at the same time displaying cross-resistance to neonicotinoid insecticides. The over-expression is caused by the expansion of a dinucleotide microsatellite in the promoter region of the gene and the amplification of the CYP6CY3 gene, with some individuals carrying up to 100 copies in their genome.

In summary, the formation of allelic variants, gene duplications, unequal crossing-over events, over-expression and copy number variation of existing CYP genes enables herbivorous insects to acquire new enzymatic activity or optimize their existing enzymatic activity. CYP genes thus allow these insects to successfully adapt to novel chemical environments.

UDP-glycosyltransferases (UGTs) catalyze the conjugation of lipophilic compounds with sugars, making them more water-soluble and are therefore also important for the detoxification of xenobiotics. UGTs are ubiquitous in insects; so far more than 310 putative UGTs have been identified and classified [15]. Despite the general importance and implied role of UGTs in detoxification processes, detoxification strategies of specific plant xenobiotics, in which UGTs play an important role, have only recently been identified.

Capsaicin, which produces a burning taste when it is ingested by mammals, is also known to be a feeding and oviposition deterrent for insects [16]. The glucosylation of capsacin appears to be an important detoxification strategy in the lepidopteran species *H. armigera* and *Helicoverpa zea*, as well as the in the specialist *Helicoverpa assulta*, that has a higher tolerance toward capsaicin [16,17]. Similarly, *Spodoptera frugiperda* detoxifies maize plant benzoxazinoids (BXDs) by glycosylation. BXDs are indole-derived plant defense compounds, and the corresponding aglucones are known to delay growth and decrease survival in several species of Lepidoptera. The reglucosylation of the BXDs in *S. frugiperda* has been shown to invert the original plant glucosylation, resulting in benzoxazinoids that are no longer substrates for the plant and insect ß-glucosidases [18,19••]. The inverted glucosylation appears to be an elegant mechanism to circumvent the reactivation of substrates and might be a common strategy among insects to circumvent the activity of inert and external ß-glucosidases.

In summary, while glucosylation catalyzed by UGTs appears to be a common detoxification mechanism the specific UGT enzymes and genes responsible for a specific glucosylation event remain to be identified.

Target-site resistance and toxin barriers

Cardenolides are a class of potent plant toxins that block Na,K-ATPases. However, insects across six orders have specialized on cardenolide-containing plants, and it was shown early on that a specific amino acid substitution in the monarch butterfly Na,K-ATPase results in resistance to cardenolide toxicity [20]. A community-wide screening of Na,K-ATPases across four insect orders revealed that different amino acid substitutions in the Na,K-ATPase lead to the target-site insensitivity of the enzyme to cardenolides in all orders; such across-the-board insensitivity points to a convergent molecular mechanism [21•]. However, there are other generalist and specialist insects that harbor cardenolide sensitive Na,K-ATPases, but that are nevertheless able to feed successfully on cardenolide-containing plants. In these, both a passive as well as active mechanisms appear to prevent cardenolides from moving through the insect's perineum and reaching the nervous system, where Na,K-ATPases are predominantly expressed [22–24]. The perineum serves as a diffusion barrier for polar compounds and therefore also acts as a barrier for polar cardenolides. P-glycoprotein-like transporters most probably act as active efflux carriers removing the unpolar cardenolides from the nervous systems. Thus, although the mechanisms of resistance to cardenolides are manifold, the general goal appears to be to prevent cardenolides from reaching their target site. Like the cardenolide-resistance mechanism, a specific organic anion transporting polypeptide (OATP58Db) allows the fruit fly *Drosophila* to efficiently excrete the cardiac glycoside ouabain, making it highly resistant to this toxic compound [25]

Sequestration

In addition to detoxifying plant defensive compounds, specialist insects are also able to sequester cardenolides,