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Sequestration of plant-derived glycosides by leaf beetles: A model system for evolution and adaptation☆

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Summary Leaf beetles have developed an impressive repertoire of toxins and repellents to defend themselves against predators. Upon attack, the larvae discharge small droplets from glandular reservoirs on their back. The reservoirs are ''bioreactors'' performing the late reactions of the toxin-production from plant-derived or *de novo* synthesised glucosides. The import of the glucosides into the bioreactor relies on a complex transport system. Physiological studies revealed a functional network of transporters guiding the glucosides through the larval body into the defensive system. The first of the involved transporters has been identified and characterised concerning selectivity, tissue distribution, and regulation. The development of a well-tuned transport system, perfectly adjusted to the compounds provided by the food plants, provides the functional basis for the leaf beetle defenses and their local adaptation to their host plants.

Contents

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Introduction

Phylogenetic analyses of butterflies and plants by Ehrlich and Raven (1964) suggested that coevolution is responsible for the tremendous diversification of plant secondary substances, the huge number of angiosperm plants and the many insects that feed on them. A particular tight connection between host/food plants is manifested in the chrysomelids, with *ca.* 36,000 described species in 2500 genera. Especially members of the tribus Chrysomelini (*ca.* 3000 species) are adapted to special host plants on which they feed as monophagous or narrow oligophagous species (Wikipedia, Leaf beetle). To repel their natural enemies, many of these leaf beetles have developed a defensive system consisting of nine pairs of dorsal exocrine glands, which are inserted in the body surface and end in reservoirs containing glandular secretions. In case of predator attack, the larvae compress their glandular reservoirs and present their secretions as small droplets on their back for a few seconds followed by re-import of the precious compounds into the body. The presented toxins vary both in structure and biosynthetic origin. The major components present in the secretion of the ancestral leaf beetle group, covering the taxa Phaedon, Gastrophysa, and Phratora (except *Phratora vitellinae*) are iridoid monoterpenes, produced *de novo via* the acetate-mevalonate pathway (Oldham et al., 1996). Larvae of the more advanced *Chrysomela* spp. and *P. vitellinae* display secretions in which salicylaldehyde is the sole or major component (Pasteels et al., 1982, 1983). When feeding upon willows (Salicaceae), larvae of these species sequester phenolic glycosides (*e.g.*, salicin) from their host plants and use these compounds as precursors to produce salicylaldehyde (Pasteels et al., 1982, 1983; Wallace and Blum, 1969; Gross et al., 2002; Meinwald et al., 1977; Blum et al., 1978; Pasteels and Rowell-Rahier, 1989). Other species are known to import cardenolides (Termonia and Pasteels, 1999), cucurbitacins (Agrawal et al., 2012), and glucosinolates (Eben et al., 1997) from their host plants. They use these compounds as such or after further processing (*e.g.*, removal of the sugars) for their own defense. In many cases, however, biosynthetic intermediates or sequestered precursors constitute of polar glucosides that cannot pass membranes and, hence, require specialised transport systems for uptake and safe guidance of the compounds through the insect body until they reach the final target tissue. In the subtribe *Chrysomelina* the compounds accumulate in a large reservoir that functions as a bioreactor for the terminal modifications of the imported glucoside precursors. The basic principles underlying the import, transport and final modifications in the bioreactor to generate the defensive chemicals will be described.

The defensive system of leaf beetles

Evaluation of the transport characteristics in different leaf beetles

The defensive glands of the juvenile *Chrysomelina* species are arranged in nine pairs on their backs (Fig. 1A and B). According to morphological studies, each defensive gland is composed of a number of enlarged secretory cells, which are in turn connected to a chitin-lined reservoir (Fig. 1C). The secretory cells are always accompanied by two canal cells that form a cuticular canal, which connects the secretory cell with the reservoir (Beran et al., 2014). In *Chrysomelina* larvae, all compounds reaching the glandular reservoir *via* the hemolymph are glucosides that are converted enzymatically into the biologically active form within the reservoir (Noirot and Quennedey, 1974; Pasteels et al., 1990).

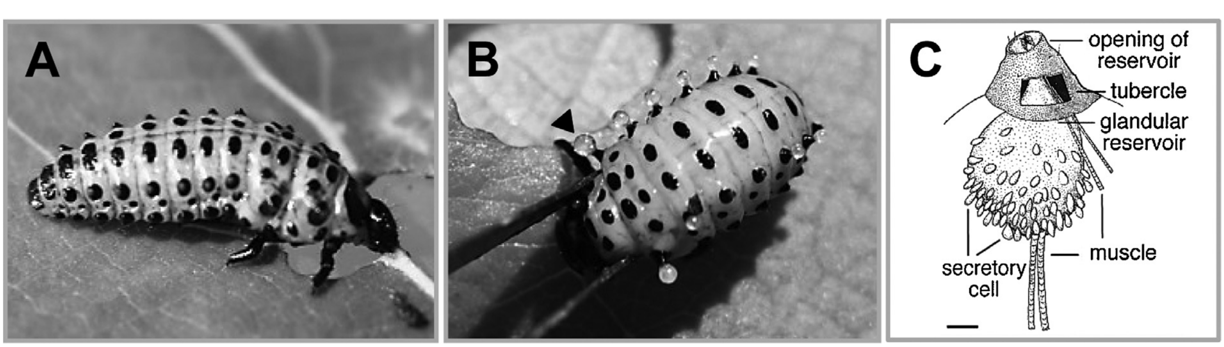

Figure 1 Larval chemical defense of the poplar leaf beetle, *Chrysomela populi*. (A) Non-stimulated larva of the third instar compared to (B), forceps stimulated larva demonstrating the release of droplets of secretions (black triangle) from the everted reservoirs of the defensive glands which are arranged segmentally in nine pairs along the back. (C) Drawing of a dissected defensive gland according to Hinton (1951).