

## Defensive Glands and Secretions as Taxonomical Tools in the Chrysomelidae

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**ABSTRACT.** — Comparative studies of chemical defense in adults and larvae offer new characters for the establishment of natural classification or phylogenies. These characters are especially interesting since chemical defense has evolved independently in larvae and adults, and therefore, convergences are unlikely, if influences of host plants on chemical defense can be discarded.

Data on defensive chemistry are only partly consistent with current classifications. Major divergences were found in the suprageneric classifications of the Chrysomelini: *Phratora* is more related to several genera grouped within the Chrysomelina than is *Colaspidema*. Morphological and chemical results suggest other phylogenetic hypotheses.

Any biologist involved in comparative studies needs a sound natural classification to interpret his results in an evolutionary perspective. Reciprocally, any comparative study can help to refine such classification, since, as nicely stated by Mayr (1969), "a classification is a provisional theory, subject to continuous testing."

In the following, we will assess how our data, from comparative studies of chemical defense in leaf beetles, match the existing classification and phylogenies and in the case of discordance, will examine their value for establishing a better natural classification.

Defensive glands are found in adults as well as larvae. However, glands of these two life stages are morphologically different and within one species, produce compounds of very different chemical nature. It is highly probable that chemical defenses have evolved independently in larvae and adults. Additionally, there is no obligate correspondence between glandular morphology and chemical nature of the secretions. Thus, morphological and chemical data in both larvae and adults offer independent sets of characters. The nomenclature adopted in this paper is that of Seeno and Wilcox (1982), even when the older classification of Weise (1916) is discussed.

**Structure and distribution of defensive glands in adults.** The structure and position of the defensive glands are illustrated in Figs. 1

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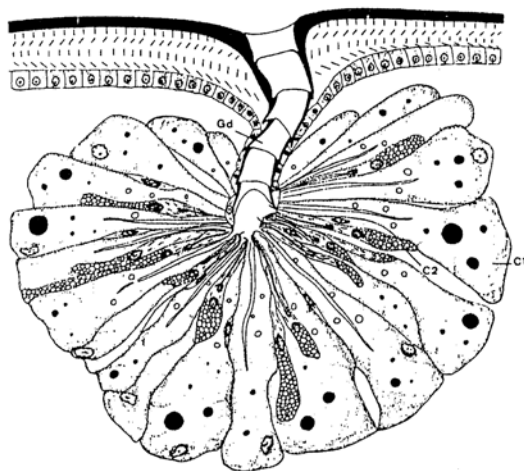


Fig. 1. The general organization of a defensive gland of *Leptinotarsa decemlineata*. Gd: gland duct; c<sub>1</sub> and c<sub>2</sub>: glandular cells (from Deroe and Pasteels, 1977).

& 2 (Deroe and Pasteels, 1977; 1982). Typically, the glands are formed by groups of cells opening into a common duct leading to the surface of the pronotum or elytra. The absence of a common extracellular reservoir in those glands, their large number per individual, and their distribution all over the pronotum and elytra are unusual features for insect defensive glands. These and the fundamentally similar ultrastructural organization of the gland cells in all taxa for four subfamilies, notwithstanding extensive variations on their basic structure, argue for a monophyletic origin of adult defensive glands (Pasteels et al., in press a).

A large survey (Deroe and Pasteels, 1982), mainly of European species, demonstrated that the occurrence of defensive glands is not universal within the family (Table 1). In two subfamilies, the Chrysomelinae and Criocerinae, glands were found in all studied species. In the Alticinae and Galerucinae, the glands were observed in some species only, and when present, they are usually rather small in comparison to those present in the Criocerinae and Chrysomelinae. In the other six subfamilies studied so far, they were never found.

The results suggest that the Criocerinae, Chrysomelinae, Galerucinae, and Alticinae form a monophyletic lineage, sharing a unique apomorphic character, the defensive glands. The small size or even the absence of the glands in some Galerucinae and Alticinae would be a secondary evolutionary event which occurred in insects in which other defensive reactions, other than the emission of exocrine secretions, developed, i.e. reflex bleeding in the galerucinae and escape by jumping in the flea beetles. This conclusion is in accordance with Jolivet (1978)

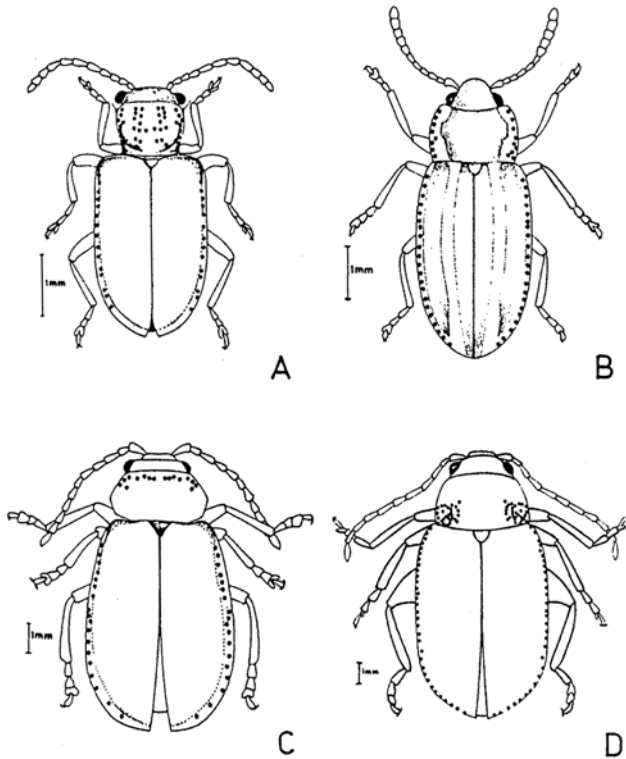


Fig. 2. Distribution of defensive glands in the elytra and pronotum of selected leaf beetles. Each black dot represents one gland. A: *Lema cyanella* (Criocerinae); B: *Prasocuris phellandrii* (Chrysomelinae); C: *Galerucella tenella* (Galerucinae); D: *Altica oleracea* (Alticinae) (from Deroe and Pasteels, 1982).

Table 1. Occurrence of defensive glands in chrysomelid beetles (from Deroe and Pasteels 1982 and unpublished data).

<i>Always present:</i>		<i>Always absent:</i>	
Criocerinae	: 3 genera, 6 spp.	Donaciae	: 1 genus, 2 spp.
Chrysomelinae	: 13 genera, 42 spp.	Zeugophorinae	: 1 genus, 1 sp.
<i>Sometimes present:</i>		Clytrinae	: 3 genera, 3 spp.
Alticinae	{ present : 5 genera, 7 spp. absent : 1 genus, 1 spp.	Cryptocephalinae	: 1 genus, 6 spp.
		Hispininae	: 1 genus, 2 spp.
Galerucirae	{ present : 3 genera, 4 spp. absent : 3 genera, 4 spp.	Cassidinae	: 1 genus, 2 spp.

Table 2. The occurrence of isoxazolinone glucosides and current classifications.

Seeno and Wilcox (1982)	Weise (1916)	Colaspidema	-	}
		<i>Other Chrysomelina :</i>		
		Chrysomela, Linaeidea,		
		Plagioderia, Hydrothassa,	+	
		Phaedon, Gastrophysa,		
		Gastrolina Prasocuris		
		Phratora	+	
		Gonioctena	-	

Brackets on the left side indicate the suprageneric grouping of Weise (1916) or Seeno and Wilcox (1982); that on the right side, the grouping suggested by the present study.

but not with other authors, e.g. Suzuki (1988). The close relationship between the Alticinae and Galerucinae seems to be admitted by most authors, and Chen (1940) recognized that the Chrysomelinae are morphologically related to these two subfamilies. In most classifications, however, the Criocerinae are not considered to be close to the other three subfamilies (for an historical review of the classification of leaf beetles, see Seeno and Wilcox, 1982).

If the available data on the structure and distribution of adult defensive glands seem to support strongly the monophyletic origin of the Criocerinae, Chrysomelinae, Alticinae and Galerucinae, this conclusion must be considered with some precautions. Indeed, the number of taxa examined is limited, neglecting for example most tropical species. Moreover, since a secondary loss seems likely in at least members of two subfamilies, glands offer perhaps less reliable taxonomic characters than their morphological uniqueness suggested at first sight.

**Chemistry of adult secretions.** We will restrict ourself to discussing the chemical nature of the secretions of members of the tribe Chrysomelini, the only tribe where enough species were studied to attempt to draw meaningful taxonomical inferences from comparative chemistry.

Defensive chemistry of the adults was recently reviewed by Pasteels et al. (1988a) and will not be detailed here. Rather, the following discussion will focus on the division of the tribe into subtribes as in the classical classification of Weise (1916) or in the most recent worldwide classification of Seeno and Wilcox (1982).

Only compounds biosynthesized *de novo* by the beetles will be taken into account as useful criteria for establishing taxonomic affinities; the compounds sequestered or derived from host plants will be excluded (for a review of the biosynthetic origin of adult defensive compounds, see Pasteels et al., in press a). Quite evidently, independent evolution in the

use of the same plant precursors is far more likely to occur than the independent evolution of the same peculiar biosynthetic pathway.

Within the tribe Chrysomelini, glands of adults produce an amazing diversity of compounds which belong to at least three chemical classes (Fig. 3), the cardenolides, the dipeptides and the isoxazolinone derivatives, implying the existence of several completely different biosynthetic pathways in these insects. To some extent, chemical data support the current classification in subtribes. For example, cardenolides or other steroids were found so far only in the genera *Chrysolina* and *Oreina* (both in the subtribe Chrysolinina), whereas, a dipeptide characterizes the secretion of *Leptinotarsa decemlineata* (in the subtribe Doryphorina), and isoxazolinone glucosides are found in many genera of the subtribe Chrysomelina. Clearly, defensive chemistry offers useful taxonomic characters at the subtribal level.

An obvious divergence is the distribution of the isoxazolinone glucosides which does not match completely either the subtribal classification of Weise (1916) or that of Seeno and Wilcox (1982) (see Table 2). These compounds are secreted by *Phratora* spp. (subtribe Phyllodectina), but they were not detected in *Colaspidema* (subtribe Chrysomelina) or *Goniocтена* which belongs to the Phyllodectina according to Weise (1916) but not according to Seeno and Wilcox (1982).

Table 3. The occurrence of defensive glands in the larvae of Chrysomelini and current classifications.

Seeno and Wilcox (1982)	Weise (1916)	Colaspidema	9 pairs	]	
		<i>Other Chrysomelina</i>			
		Chrysomela, Linaeidea			
		Plagioderā, Hydrothassa			
		Phaedon, Gastrophysa			
		Gastrolina, Phaedonia			
		Prasocuris			
		Phratora			9 pairs
		Goniocтена			1 pair between tergites 7-8
		Paropsis			1 pair between tergites 7-8
Chrysophtharta					
	Chrysolina	1 pair between tergites 8-9			
	Leptinotarsa	1 pair between tergites 8-9			

Brackets on the left side indicate the suprageneric grouping of Weise (1916) or Seeno and Wilcox (1982); those on the right side, the grouping suggested by the present study.

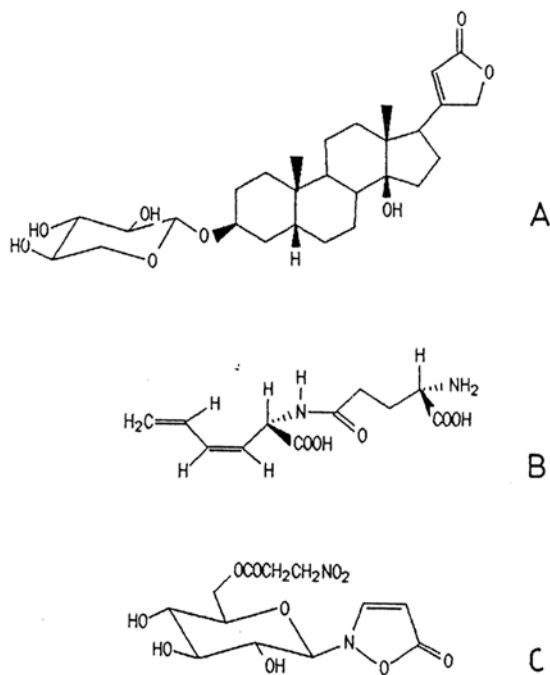


Fig. 3. Selected compounds produced by adults, illustrating the diversity of chemical classes observed in their defensive secretions. **A:** sarmentogenin 3  $\beta$ -xyloside (cardenolide, e.g. in *Chrysolina coeruleans*); **B:**  $\gamma$ -L-glutamyl-L-2-amino-3(Z),5-hexadienoic acid (dipeptide in *leptinotarsa decemlineata*); **C:** [2-6'-(3''-nitropropanoyl)- $\beta$ -D-glucopyranosyl]-3-isoxazolin-5-one (isoxazolinone glucoside, e.g. in *Chrysomela populi*).

**Structure and distribution of defensive glands in larvae.** Defensive glands are known in the larvae of some Chrysomelinae (Chrysomelini and Paropsini). They are exsertile organs. Each gland possesses a cuticular reservoir which is normally invaginated in the body cavity. This reservoir is partly everted when the larvae are disturbed and the defensive secretion released. The larvae of some species possess nine pairs of serial dorsolateral glands, one pair in the meso- and one in the metathorax, as well as one pair in each of the first seven abdominal segments (Fig. 4). In other species, there is only a single pair of more median glands, variously located between the posterior tergites (Fig. 5) (Dettner and Schwinger, 1987).

The distribution of the various types of larval glands in the Chrysomelinae is summarized in Table 3. This distribution correlates only partially with existing classifications. Again, the most striking conflict concerns the position of the genus *Phratora* which shares, with many genera of the Chrysomelina, the nine pairs of serial glands. *Phratora* is

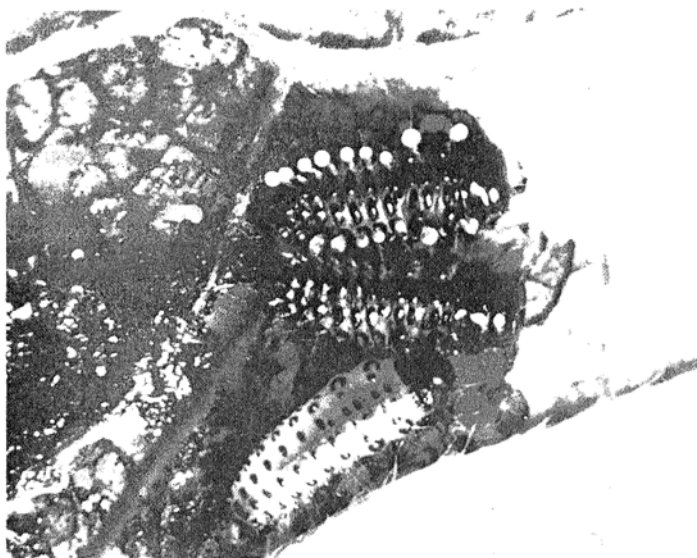


Fig. 4. Defensive reaction of a larva of *Phratora vitellinae*. Drops of secretion at the top of the 9 pairs of exertite glands are clearly visible.

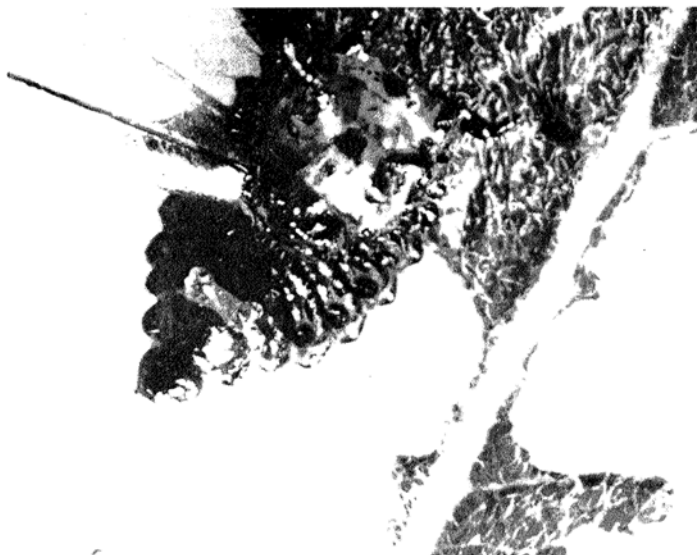


Fig. 5. Everted defensive glands in a larva of *Gonioctena viminalis*.

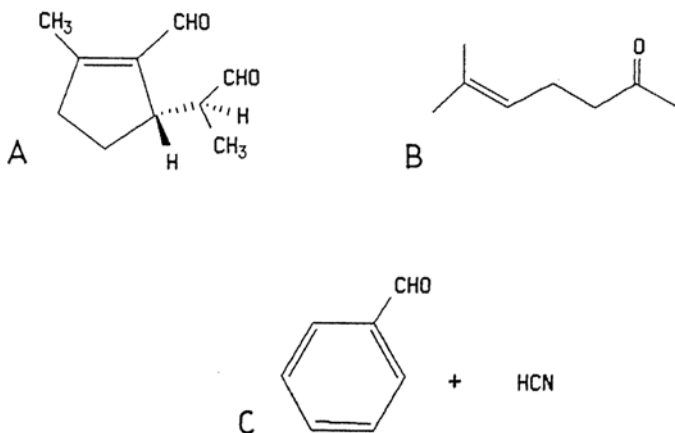


Fig. 6. Selected compounds secreted by the larvae. **A:** chrysolimial (iridoid monoterpene, e.g. in *Gastrophysa viridula*); **B:** 6-methyl-5-hepten-2-one (in *Gonioctena viminalis*); **C:** benzaldehyde and HCN (e.g. in *Paropsis atomaria*).

Table 4. Chemistry of larvae secretions and current classifications.

Seeno and Wilcox (1982)	Weise (1916)	Colaspidema	—	}
		<i>Other Chrysolimelina</i>	iridoid monoterpenes	
		Linaeidea, Plagioderia		
		Hydrothassa, Phaedon		
		Gastrophysa, Prasocuris		
		Phratora	iridoid monoterpenes	
		Gonioctena	aliphatic and aromatic derivatives, terpenes	
		Paropsis	HCN	
		Chrysotharta	benzaldehyde	

Brackets on the left side indicate the suprageneric grouping of Weise (1916) or Seeno and Wilcox (1982); those on the right side, the grouping suggested by the present study.

not related in this respect to *Gonioctena* which possesses a single pair of median glands. Additionally, *Colaspidema*, in which larval glands are totally lacking, does not appear to be related to the other studied genera of the Chrysolimelina.

It is difficult at this stage to infer taxonomic conclusions from the presence of median glands. This grandular character should be used

with caution. Indeed, the structure of those median glands has not yet been studied in detail. In contrast with the serial glands which are always well-developed in all the species examined, the median glands are often reduced and apparently nonfunctional. In the genus *Gonioctena*, some species possess well-developed glands, whereas, in others they are completely missing (Takizawa, 1976).

**Chemistry of larval secretions.** As for the adult secretions, the chemical diversity of larval defensive compounds is quite large (Fig. 6) (for a detailed review, see Pasteels et al., (1988a) and can offer interesting taxonomic characters, if plant-derived compounds are not taken into account (for reviews on the biosynthetic origin of larval defensive compounds, see Pasteels et al., 1988b and in press b).

In Table 4, the main classes of compounds biosynthesized *de novo* by the larvae are listed, as well as their distribution among the Chrysomelinae. Again, the close relationship of *Phratora* with the Chrysomelina is obvious.

## CONCLUSIONS

The morphology of defensive glands and their secretions in both larvae and adults offer very useful taxonomic characters. For example, the close affinity between the genus *Phratora* and the Chrysomelina is strongly suggested, both by the type of defensive glands found in the larvae and by the chemical nature of larval as well as adult secretions. More convincing is that the observed states of morphological and chemical characters are very unusual, and that larval and adult chemical defense evolved independently, excluding convergences. Interestingly, Petitpierre's (1988) investigations of leaf beetle chromosomes also suggest this affinity, which was already pointed out by Cox (1982), Kimoto (1962), Hennig (1938) and Paterson (1931) on the basis of larval morphology.

Undoubtedly, the suprageneric classification of Weise (1916) is in part unnatural, since *Phratora* is grouped with *Gonioctena* in the Phyllodectina and not with the more similar (in terms of defensive glands) genera of another subtribe, the Chrysomelina. *Gonioctena* and *Phratora* are not grouped together by Seenó and Wilcox (1982), but their suprageneric classification is not necessarily more satisfactory for the Chrysomelina and the Phyllodectina. Indeed, *Colaspidema* seems even less related to the other genera of the Chrysomelina than is *Pratora*. Unfortunately, chemical defense remains unknown in too many genera in both subtribes, and it is, therefore, premature at this stage to suggest another suprageneric grouping.

The adult defensive glands provide insight to the relationships between subfamilies. Data available so far suggest not only an affinity between the Alticinae and the Galerucinae but also between those two subfamilies and the Chrysomelinae and perhaps even the Criocerinae.

Though more data are needed before reaching definitive conclusions, we believe that the results obtained so far raise questions on several aspects of the current classification and phylogenies of the leaf beetles and that they should be taken into account in further attempts to improve them.

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