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PRODUCTION OF CARDENOLIDES VERSUS SEQUESTRATION OF PYRROLIZIDINE ALKALOIDS IN LARVAE OF *Oreina* SPECIES (Coleoptera, Chrysomelidae)

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Abstract—Adult leaf beetles of the genus *Oreina* are known to be defended either by autogenously produced cardenolides or by pyrrolizidine alkaloids (PAs) sequestered from the food plant, or both. In this paper we analyze larvae of different *Oreina* species and show that the larvae contain the same defensive toxins as the adults in quantities similar to those released in the adults' secretion. Both classes of toxins are found in the body and hemolymph of the larvae, despite their different origins and later distribution in the adults. Larvae of sequestering species differed in their PA patterns, even though they fed on the same food plants. The concentration in first-instar larvae of a PA-sequestering species was similar to that in fourth-instar larvae. In all stages examined, the amount of PAs per larva did not greatly exceed the estimated uptake of one day. Eggs of two oviparous species contained large concentrations of the adult's toxins, while neonates of a sequestering larviparous species had no PAs.

Key Words—*Oreina* spp., Coleoptera, Chrysomelidae, larval defense, cardenolides, pyrrolizidine alkaloids, sequestration.

INTRODUCTION

Leaf beetles show a great diversity of chemical defenses (Pasteels, 1993). In the adults, the toxic or deterrent substances are often associated with conspicuous aposematic coloration. The alpine genus *Oreina* (Chevrolat) (Coleoptera, Chrysomelidae, Chrysomelinae), which combines brilliant metallic coloration with toxic secretions (Pasteels et al., 1989), is a good example of this. The secretion

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of the adult *Oreina* contains either autogenously produced cardenolides (e.g., in *O. bifrons*, *O. gloriosa*, *O. luctuosa*, *O. speciosa*, and *O variabilis*) or pyrrolizidine alkaloid N-oxides (PAs) sequestered from the host plant (e.g., in *O. cacaliae*); both sorts of compounds occur together in some species (e.g., *O. elongata*, *O. intricata*, and *O. speciosissima*). Sequestration of PAs occurs only in species feeding on Senecioneae (Asteraceae), while most species of the genus feed on Apiaceae or Asteraceae devoid of PAs and synthesize cardenolides (Pasteels and Rowell-Rahier, 1991; Rowell-Rahier et al., 1991; Pasteels et al., in preparation). Thus, adult *Oreina* are apparently well defended against predators (summarized in Table 1).

However, the juvenile stages appear to be more vulnerable to predation. The larvae of *Oreina* do not have any mechanical protection and do not possess defensive glands, in contrast to the larvae of other tribes of the Chrysomelidae (Pasteels et al., 1984; Pasteels and Rowell-Rahier, 1991). The lack of defensive glands, however, does not exclude the possibility that the larvae store toxins in their body. In *O. gloriosa* the production of cardenolides starts in the larval instars (Eggenberger and Rowell-Rahier, 1993b). In some species of the related genus *Chrysolina* (Motschulsky), the larvae also produce cardenolides (Daloze and Pasteels, 1979, Hilker et al., 1992), yet in *Chrysolina* (*Dlochrysa*) *fastuosa* the adults do but the larvae do not (Pasteels, personal communication). The PAs that are sequestered by several *Oreina* species are also present in the larval food, since the larvae feed on the same plants as the adults. Whether the larvae are able to store PAs or not has not been investigated previously.

The site of storage of toxins in the larvae poses an interesting problem. In adult *Oreina*, cardenolides are found exclusively in glands producing defensive secretions. The larvae, however, do not possess these glands and the carden-

TABLE 1. DEFENSE CHARACTERISTICS OF ADULTS OF *Oreina* SPECIES

Species	Food plants	Mode of defense	Site of storage
O. bifrons *O. gloriosa* *O. luctuosa* *O. speciosa* *O. variabilis*	Apiaceae or Asteraceae without PA[a]	production of cardenolides	glands only
O. cacaliae	Asteraceae mostly with PA (Senecioneae)	sequestration of PA	body and glands
O. elongata *O. intricata* *O. speciosissima*	Asteraceae with and without PA	sequestration of PA + production of cardenolides	

[a]PA = pyrrolizidine alkaloids.

secretions. The larvae, however, do not possess these glands and the cardenolides must therefore be located elsewhere. In adults of the PA-sequestering species, on the other hand, the defensive compounds are not restricted to the glands but are also found in other parts of the body (Pasteels et al., 1992).

In this paper, we analyze the defensive toxin content of larvae of several *Oreina* species. Each species is tested for the presence of the compounds also found in adults, and the toxin concentration of the body tissue, the gut, and the hemolymph is assessed separately. We compare the content of species whose adults produce cardenolides de novo and species whose adults can sequester PAs from the food plant. Most of the species investigated here are larviparous, but two are oviparous. In the latter two, a transfer of defensive toxins from the female to the eggs should be beneficial for the offspring (Orians and Janzen, 1974). We therefore compare the toxin content of the eggs of the two oviparous species with the neonate of a larviparous species.

METHODS AND MATERIALS

Beetles and Food Plants. Our nomenclature follows the revision of the genus *Oreina* by Bontems (1978, 1981, 1984). Adult beetles were collected in June and July 1991 and 1992 at the following field sites: *Oreina bifrons* near Brülisau (Appenzell, Switzerland, 900 m); *O. cacaliae*, *O. speciosissima*, and *O. variabilis* near Zastler (southern Black Forest, Germany, 900 m); *O. elongata* at the Col du Lautaret (French Alps near Briançon, 1900 m) and at the Mattmark dam (Valais, Switzerland, 2400 m); *O. gloriosa* near Saas Grund (Valais, Switzerland, 1800 m); *O. intricata* and *O. speciosa* near Tschiertschen (Graubünden, Switzerland, 1800 m); and *O. luctuosa* near Hasslach (northern Bavaria, Germany, 500 m). Two populations of *O. elongata* were examined, because they live on different host plants in the field, one containing PA (*Adenostyles alliariae*, Col du Lautaret population) and one devoid of PA (*Cirsium spinosissimum*, Mattmark population). In the laboratory, the offspring of the beetles were raised in groups of approximately 20 individuals at 17°C on the food plants detailed below. Except for neonate and first- and third-instar larvae of *O. cacaliae*, all larvae were fed until the third day after the third (and last) molt, then starved for one day to empty the gut, killed by freezing, and stored at −20°C.

For cardenolide analysis, larvae of species that do not feed on PA-containing plants were raised on their most common field host: *O. bifrons*, *O. speciosa*, and *O. variabilis* on *Chaerophyllum hirsutum*; *O. gloriosa* on *Peucedanum ostruthium*; and *O. luctuosa* on *Centaurea nemorensis*. Larvae normally feeding on PA-containing plants were raised, when possible, on a host with no or low PA content: *O. elongata* on *Cirsium spinosissimum* (no PAs, field host of the Mattmark population), *O. cacaliae* on *Petasites albus* (no PAs), *O. speciosis-*

sima on *Adenostyles alliariae* (with PAs), and *O. intricata* on *Senecio fuchsii* (with PAs).

For PA analysis larvae were raised on *Adenostyles alliariae* to allow a comparison of PA concentrations. Additionally, larvae of *O. cacaliae* and *O. intricata* were raised on *Senecio fuchsii*, because both plants are common hosts in the field but are characterized by different PAs (Rowell-Rahier et al., 1991).

Plants were collected in the field at least once a week (*S. fuchsii*, *A. alliariae*, *P. albus*, *C. hirsutum*, and *C. nemorensis* at the Zastler field site; *C. spinosissimum* and *P. ostruthium* at the Mattmark field site) and kept in the refrigerator until used.

Dissection. Fourth-instar larvae were cut ventrally and the hemolymph collected in capillary tubes and stored in ethanol. The gut was removed and the body washed thoroughly in distilled water to remove remaining gut content and hemolymph. In the samples dissected for analysis of PAs, larvae that had not been starved were used and the guts collected separately. Additionally, exuviae of fourth-instar larvae of *O. cacaliae* were collected. In the samples dissected for cardenolide analysis, the gut and its content were not analyzed separately, as the uptake of cardenolides from the host plant can be excluded (Pasteels and Daloze, 1977; Van Oycke et al., 1987; Pasteels et al., 1992).

Cardenolide Extraction. As the cardenolides in the adult secretion comprise a large polarity range, including rather polar cardenolides, the usual extraction and purification methods based on chloroform extraction (e.g., Duffey and Scudder, 1972, Brower et al., 1982) did not appear appropriate.

Pooled samples of about 20 larvae were lyophilized, weighed, and homogenized in 5 ml methanol. Following centrifugation, the supernatant was evaporated to dryness and redissolved in 400 μl methanol, then 2 ml diethylether was added. Purification was effected using a silica gel column (glass pipet 0.85 cm diameter, 0.5 g silica gel 60 Macherey Nagel 0.04–0.063 mm). The extract was added to the column and eluted first with 15 ml diethylether and secondly with 30 ml diethylether–methanol (2:3). The latter fraction was dried and redissolved in 1 ml methanol for spectrophotometry.

We tested this purification method using the highly polar ouabain and the moderately polar digitoxin as standards that correspond to the polarity range of the cardenolides in the beetles' secretions. One hundred percent of these standards appeared in the diethylether–methanol fraction.

Spectrophotometry of Cardenolides. We modified the spectrophotometric method of Brower et al. (1982), using 3,5-dinitrobenzoic acid (Rowson, 1952) instead of 2,2′,4,4′-tetranitrodiphenyl. The 1-ml sample was divided between two cuvettes, 500 μl of 2% 3,5-dinitrobenzoic acid in methanol was added to the sample cuvette but not to the reference cuvette, and methanol was added to both to give a total volume of 1.7 ml. Then, 300 μl of 3% KOH in methanol was added to both cuvettes, which starts the reaction in the sample cuvette. The

difference in absorption at 535 nm between the two cuvettes was measured after exactly 10 mins, when the absorption is at its peak. With every set of measurements, a series of digitoxin standards (0 to 200 μg) was run. The concentrations of cardenolides in the samples were calculated in digitoxin equivalents, based on a regression equation derived from the standard values. The absorption increases linearly with increasing concentration within the range of 1–500 μg digitoxin. The calculated cardenolide content of twin samples derived from one original sample, that were extracted and measured in parallel, diverged by no more than 10%. Repeated extractions of identical samples gave a precision of 8.21 ± 2.94 μg cardenolides per larva in *O. variabilis* ($N = 6$, SD given) and of 18.11 ± 3.82 μg in *O. luctuosa* ($N = 4$). All twofold replications diverged by less than 10%, with one exception mentioned below.

PA Extraction. We followed the extraction and cleaning procedure described by Mattocks (1986). Pooled samples of larvae (about 20 individuals/sample), eggs, or body parts were lyophilized or cut and oven dried at 60°C, crushed, and weighed. The powder was then extracted with sulfuric acid and zinc dust, which also reduces PA N-oxides to tertiary PAs. After filtering and washing with petroleum ether, the solution was brought to pH 10 with ammonia and the PAs extracted with dichloromethane and evaporated to dryness. The precipitate was redissolved in 300 μl dichloromethane for TLC analysis.

Hemolymph samples were not submitted to the whole extraction process, because they have less interfering substances than the whole larvae and PAs are already in solution. They were centrifuged and the supernatant evaporated and redissolved in 300 μl ethanol for TLC analysis. No reduction of PA N-oxides was made in this case. The color reaction does not discriminate between tertiary PAs and N-oxides.

TLC Analysis. Samples were spotted on silica gel 60 plates (Merck) with a Camag Linomat III TLC Applicator. On each plate, samples of 2, 4, 8, and 16 μg seneciphylline (Roth AG) or monocrotaline (Sigma) were included as standards. The plates were developed and spotted according to the procedure described by Mattocks (1967, 1986). Spot intensity was measured with a Shimadzu CS-930 TLC Scanner. The minimum quantity detectable is 2 μg. For the precision of the method, see Speiser and Rowell-Rahier (1991). All PA concentrations are given in micrograms seneciphylline equivalents, which is the main PA of *Adenostyles alliariae* (Rowell-Rahier et al., 1991). When monocrotaline was used as a standard, seneciphylline equivalents were calculated later using a direct comparison of the absorption of both alkaloids.

RESULTS

Content of Cardenolides. Cardenolides were detected in the larvae of all species in which the adults also produce these compounds (Table 2). These include three species, *O. intricata*, *O. elongata*, and *O. speciosissima*, in which

TABLE 2. CARDENOLIDE CONTENT OF FOURTH INSTAR LARVAE[a]

	Species[b]	N	Cardenolide (μg/g dry wt)	Cardenolide (μg/larva)
I	*O. bifrons*	1	254	4.4
	O. gloriosa	2	565	9.9
	O. luctuosa	4	1840	18.1
	O. speciosa	2	389	5.1
	O. variabilis	6	559	8.2
II	*O. elongata* CL[c]	1	414	3.2
	O. elongata MM[d]	1	569	3.3
	O. intricata	1	1200	12.6
	O. speciosissima	2	621	5.8
III	*O. cacaliae*	1	51	0.6

[a] *N* = number of samples analysed; cardenolide = cardenolide content in digitoxin equivalents.
[b] I: adults produce only cardenolides, II: adults have cardenolides and PAs, III: adults have only PAs.
[c] CL = Col du Lautaret population.
[d] MM = Mattmark population.

the adults have a mixed defensive strategy and produce cardenolides as well as sequester PAs. The cardenolide concentrations of the different species varied by a factor of seven, with two exclusive cardenolide producers, *O. luctuosa* and *O. bifrons*, at both extremes. *O. cacaliae* larvae had only a low concentration of cardenolides. In this species, the adults do not possess cardenolides. The biological significance of this finding remains unclear. Possibly, the detected concentration lies within the background noise of our method.

The analysis of body and hemolymph of fourth-instar larvae of *O. gloriosa* showed that cardenolides were present in the body tissue at a concentration of 997 μg/g dry weight and in the hemolymph at a concentration of 300 μg/ml. Compared to the concentration of 565 μg/g dry weight found in extracts of whole larvae (Table 2), the concentration in the body alone is higher by a third, which indicates that most of the cardenolides are present in tissue consisting of integument, fat body, and muscles.

Sequestration of PAs and PA Pattern in Larvae. In all species sequestering PAs as adults, the larvae contained large amounts (Table 3). However, the concentration found in the larvae varied by more than a factor of 10 between species, or even between larvae of a single species reared on different plants. On the other hand, the two populations of *O. elongata* reared on *Adenostyles alliariae* contained similar concentrations of PAs, although one population (Col du Lautaret) lives on *A. alliariae* in the field while the other one (Mattmark)

TABLE 3. PA CONCENTRATIONS IN FOURTH-INSTAR LARVAE

Species[a]	Food plant	PA (μg/g dry wt)	PA (μg/larva)	Spot[b]
O. cacaliae	*A. alliariae*	4200	61	b
O. cacaliae	*S. fuchsii*	500	5	b
O. elongata CL	*A. alliariae*	1200	10	d
O. elongata MM	*A. alliariae*	1400	8	d
O. intricata	*A. alliariae*	1600	26	a, b
O. intricata	*S. fuchsii*	7300	81	a, b, c, d
O. speciosissima	*A. alliariae*	2400	18	d

[a] MM = Mattmark population, CL = Col du Lautaret population.
[b] Spot refers to the position on the plate: a, apolar spots, traveling shortly behind the solvent front (R_f = 0.69–0.86); b, spot at same height as seneciphylline standard (R_f = 0.45–0.58); c, more polar spot, halfway between seneciphylline and the baseline (R_f = 0.35); d, polar spots in the range of seneciphylline-N-oxide (R_f = 0.14–0.26).

lives on *Cirsium spinosissimum* and does not encounter *A. alliariae*. Similarly, *O. speciosissima* larvae, which in the field mostly feed on *Petasites albus*, a plant without PAs, contained concentrations similar to those found in the well-adapted PA plant feeders *O. cacaliae* and *O. intricata*, when fed on *A. alliariae*. In these latter species, the host plant species seems to influence the PA content of the larvae in a species-specific way: *O. cacaliae* larvae fed on *A. alliariae* had about 10 times higher concentrations of PAs than larvae raised on *Senecio fuchsii*. In *O. intricata*, on the contrary, larvae fed on *S. fuchsii* had four times higher concentrations of PAs than larvae fed on *A. alliariae*. The PA pattern in the two species also differed: extracts of *O. cacaliae* larvae from both plants showed only one spot at the height of the seneciphylline standard. This is the main PA component of *A. alliariae* (Rowell-Rahier et al., 1991) and the only one visible in our TLC of extracts of this plant. *O. intricata* larvae, on the other hand, showed additional components (one less polar spot in larvae from both plants, and a further more polar spot only in larvae from *S. fuchsii*). Seneciphylline was not found in *S. fuchsii* (Rowell-Rahier et al., 1991), and TLC of extracts of this plant gave three spots, all more polar than seneciphylline. Direct comparison of an extract of *O. intricata* larvae and an extract of *S. fuchsii* showed that the most polar spot of the larvae and the least polar spot of the plant extract are at the same height.

The dissection of *O. cacaliae* larvae raised on *Adenostyles alliariae* showed that PAs are found both in the body (at a concentration of 5000 μg PA/g dry weight) and in the hemolymph (940 μg PA/ml). No PAs could be detected in the gut of larvae raised on *A. alliariae*, although the sample of guts from 20 larvae was analyzed and the detection threshold of the method lies at 2 μg PA. Small concentrations, 190 μg PA/g dry weight, were detected in exuviae of

fourth-instar larvae. However, this accounted for only 0.3 μg PA per larva. The concentration in the hemolymph of larvae raised on *S. fuchsii* was similar and amounted to 1000 μg PA/ml. Hemolymph samples of *O. elongata* larvae, on the other hand, differed widely in concentration: in larvae of the Mattmark population we found 700 μg PA/ml, while larvae of the Col du Lautaret population had 1900 μg PA/ml, despite the similar content in extracts of whole larvae of the two populations.

Toxin Content of Eggs and Young Larvae. Eggs of the two oviparous species, *O. elongata* and *O. luctuosa*, contained high concentrations of cardenolides and of PAs in *O. elongata* fed on *A. alliariae* (Table 4). For *O. luctuosa* and *O. elongata* (Mattmark) the cardenolide concentrations in microgram per gram dry weight are in the upper range of the concentrations found in the fourth-instar larvae. For *O. elongata* (Col du Lautaret) the two samples analyzed differed too much to pool them—the cause of this difference is unclear. Nevertheless, both values are smaller than that of the *O. elongata* (Mattmark) population, which has to rely exclusively on cardenolides, as its food plant (*Cirsium spinosissimum*) is devoid of PAs. The eggs of the Col du Lautaret population, on the other hand, contained large amounts of PAs besides the cardenolides.

In the larviparous *O. cacaliae*, we analyzed different life stages (Table 5). No PAs could be detected in the neonate just after birth and before they started feeding (sample of 88 neonate compared to 33 1-day-old larvae). Yet, after one day of feeding on *A. alliariae*, the larvae contained concentrations similar to those of fourth-instar larvae (see Table 3). The mean concentration in *A. alliariae* leaves from the Zastler field site at the end of May is 1130 μg PA/g fresh weight (Speiser, unpublished). The amount detected in the 1-day-old larvae corresponds to a consumed leaf area of 2.9 mm^2, which fits well with the observed feeding capacity of young larvae. The larvae used in this analysis had not been starved, so that a contribution of the gut content to the measured PAs can not be excluded; in fourth-instar larvae, however, no PAs were found in the gut. The PA concentrations found in *O. cacaliae* larvae were highest in the

TABLE 4. CONTENT OF DEFENSIVE TOXINS OF EGGS OF TWO OVIPAROUS SPECIES

Species[a]	Egg weight (mg fresh wt)	Cardenolide		PA	
		μg/g dry wt	μg/egg	μg/g dry wt	μg/egg
O. elongata CL	0.77	500/1400	0.2/0.5	16700	4.5
O. elongata MM	0.70	1700	0.5		
O. luctuosa	1.27	1800	1.0		

[a]MM = Mattmark population, the females had been feeding on *C. spinosissimum*; CL = Col du Lautaret population, the females had been feeding on *A. alliariae*.

TABLE 5. CONTENT IN PA OF DIFFERENT LIFE STAGES OF *O. cacaliae* RAISED ON *Adenostyles alliariae*

Age	PA	
	μg/g dry wt	μg/larva
Neonate	ND	ND[a]
1 day old	4100	0.7
Third instar	6100	20.2

[a]ND = not detectable.

third instar. Expressed as consumed leaf area, the amount per larva would correspond to 76 mm^2 in the third instar and to 230 mm^2 in the fourth instar. As in the case of the 1-day-old larvae, these values roughly agree with the estimated daily consumption.

DISCUSSION

In all species examined, the larvae contain the same toxins as those found in the adult's defensive secretions. The quantities of cardenolides detected in *O. gloriosa* larvae are in agreement with those detected by HPLC (Eggenberger and Rowell-Rahier, 1993b). No differences in larval cardenolide content exist between solely cardenolide producing species and the species in which the adults can also sequester PAs. In *O. cacaliae*, a species in which the adults sequester only PAs, the concentration of cardenolides detected in the fourth-instar larvae is most likely too small to be considered a reliable result. In the species having cardenolides as adults, the amounts present in the last-instar larvae are roughly similar to those released in one adult secretion. Secretions of *O. speciosissima* contain between 2.5 and 6 μg cardenolides (Rowell-Rahier et al., 1991). Adults of *O. gloriosa* release, depending on age and sex, between 4 and 23 μg cardenolides in their pronotal secretions (Eggenberger and Rowell-Rahier, 1993a), and in *O. elongata* 1 μg only (*O. elongata* Mattmark populations; Rowell-Rahier et al., unpublished data). However, in these two species only the secretions released by the glands on the pronotum were quantified. This makes up approximately 50% of the total quantity released on both pronotum and elytra (in *O. gloriosa*, Eggenberger, unpublished). In *O. luctuosa*, in which the highest concentrations and amounts per larvae were detected, the amount of cardenolides in the adult secretion is so far unknown.

In contrast to the species producing cardenolides, the amount of PAs released in the secretion of the sequestering species is only part of the quantity

present in the beetles, as most of the PAs are stored in the body (Rowell-Rahier et al., 1991; Pasteels et al., 1992). Adult *O. cacaliae* collected on *Adenostyles alliariae* released on average 3.4 μg PA/secretion and had additionally 16.6 μg stored in the body. The amount detected in the corresponding larvae was three times higher than the total in the adults. On the other hand, *O. cacaliae* larvae raised on *Senecio fuchsii* and *O. speciosissima* larvae fed on *A. alliariae* contained more PAs than the adult secretion, but less than the adult's body. While adults of *O. cacaliae* and of *O. speciosissima* fed on *A. alliariae* sequestered PAs equally well in the secretion (Rowell-Rahier et al., 1991; Ehmke et al., 1991), the concentrations found in the larvae of *O. speciosissima* fed on *A. alliariae* were only half those detected in *O. cacaliae* on the same plant. This might reflect a difference in intake, since *O. speciosissima* larvae feed and grow less well on *A. alliariae* than *O. cacaliae* larvae and prefer the non-PA plant *P. albus* in the field (Rowell-Rahier et al., 1991, and unpublished observations).

In the adults of *O. cacaliae* and *O. speciosissima*, the concentration of PA in the secretion is dependent on the food plant; only certain PAs—namely the PAs present in *A. alliariae*—can reach the secretory glands, but storage in the body is less specific (Rowell-Rahier et al., 1991). Dependence on the food plant was also observed in the larvae: *O. cacaliae* larvae raised on *A. alliariae* had nearly 10 times the concentration of PAs of larvae fed on *S. fuchsii*. No difference detectable by TLC was seen in the PA pattern of larvae fed on the two plants. *O. intricata* larvae, on the other hand, had about five times higher concentrations on *S. fuchsii* than on *A. alliariae*. The difference in concentration between *O. cacaliae* and *O. intricata* larvae raised on *S. fuchsii* or on *A. alliariae* could be caused by differences in their ability to handle the different PAs typical of these plants. Selective sequestration of plant compounds not detected by TLC of plant extracts, or metabolic transformation of plant PAs, could explain the differences in the PA pattern between plant and larval extracts, which were also observed in *O. elongata* and *O. speciosissima*. In adult *O. cacaliae* no evidence for the metabolism of plant PAs was observed. The PAs found in the body of the beetles mirror the pattern present in the food plant. However, in *O. speciosissima*, a PA not present in the plant was found in the secretion, and metabolism is possible (Rowell-Rahier et al., 1991). Metabolism of plant PAs is the rule rather than the exception in arctiid moths (L'Empereur et al., 1989; Ehmke et al., 1990; Hartmann et al., 1990; Trigo et al., 1993), while an aphid and a coccinellid feeding on the aphid exactly mirrored the PA pattern of the aphid food plant (Witte et al., 1990).

In *O. gloriosa* larvae large proportions of the total toxins were contained in the body (=integument, muscles, and fat body). This agrees with findings in the milkweed bug (Scudder et al., 1986) and in the monarch butterfly (Brower and Glazier, 1975; Brower et al., 1988), both cardenolide-sequestering species, where the integument is the major storage site. Likewise, in ithomiid butterflies

(Brown, 1984) and arctiid moths (Egelhaaf et al., 1990, Ehmke et al., 1990) most of the sequestered PAs are found in the integument. Our data on the distribution of the PAs between body hemolymph are not clearly interpretable. Hemolymph of *O. cacaliae* larvae reared on *A. alliariae* and on *S. fuchsii* had similar concentrations of PAs, although the total concentration in the larvae differed by a factor of 10. Concentration of PAs in the hemolymph of *O. elongata* larvae differed by a factor of three between two populations with otherwise similar total concentrations. Possibly the hemolymph is only involved in transportation of the PAs. Interestingly, in *O. cacaliae* larvae reared on *A. alliariae*, no PAs could be detected in the gut. The uptake of PAs could occur early in the digestion and might be followed by rapid degradation or immobilization of remaining alkaloid in the gut. In adult *O. cacaliae*, radioactively labeled senecionine-N-oxide was found in the feces; however, a large proportion could neither be recovered from the insects nor from the feces (Ehmke et al., 1991) but was apparently transformed into a methanol-insoluble form.

The eggs of *O. elongata* and *O. luctuosa* contained cardenolides, as in the eggs of cardenolide-producing species of the genus *Chrysolina* (Daloze and Pasteels, 1979; Hilker et al., 1992). In the adults of the larviparous species *O. gloriosa*, on the other hand, no cardenolides could be found in the body, but were restricted to the defensive glands (Pasteels et al., 1992). However, the cardenolides are most likely stored in the eggs by maternal transfer. This should be the case in the oviparous *O. elongata* and *O. luctuosa*, whereas in the larviparous *O. gloriosa* this transfer is absent (otherwise cardenolides should be found in the body, which includes the reproductive organ). Similarly, PAs occur in the eggs of *O. elongata* of the population that had been feeding on *A. alliariae* in the field, yet no PAs were found in neonate larvae (before feeding) of the larviparous *O. cacaliae*. In this species, maternal transfer of toxins to the offspring might not be important, since the larvae feed on PA-rich plants immediately after birth and after one day contained concentrations similar to those of fourth-instar larvae. The difference in toxin transfer between ovi- and larviparous species could be explained if the toxins found in the eggs were located in the outer shell, which is reduced to the endochorion in the larviparous species (Bontems, 1989).

The concentrations of both PAs and cardenolides in *Oreina* larvae are far lower than those found in other insects in which a defensive function of the toxins could be shown [e.g., up to 20% dry weight of PAs in Ithomiinae (Brown, 1984); 0.9–3.4% dry weight of cardenolides in the monarch butterfly (Malcolm et al., 1989)]. Nevertheless, the PA and cardenolide quantities present in the larvae are equal to or higher than those in the adult secretion and higher than the amount of PAs stored in the body of *O. cacaliae*. Feeding experiments with adult cardenolide-producing *O. gloriosa* and PA-sequestering *O. cacaliae* and red-winged blackbirds (*Agelaius phoeniceus*) showed that the birds were deterred

from feeding if the beetle's secretion had not been removed. Moreover, adults of *O. cacaliae* from which the secretion had been removed still proved to be deterrent due to the PAs stored in the body (Rowell-Rahier et al., in preparation). The predator spectrum of the larvae certainly differs from that of the adults, and small invertebrate predators such as spiders and ants should be more important predators on the larvae than on the adults. As shown for *O. gloriosa* larvae (Eggenberger and Rowell-Rahier, 1993b), the pattern of cardenolides differs between larvae and adults, and it remains unclear how this affects the larval defense. However, at least in some species, the larvae are aposematic, either uniformly black (*O. luctuosa*, *O. elongata*) or bicolored with head and/or pronotum contrasting with the abdomen (yellow and black in last-instar larvae of *O. cacaliae*, *O. gloriosa*, *O. speciosissima*, and *O. variabilis*, black and white in *O. intricata*). The possible warning function of the coloration in these species and the potential crypsis of other species with sandy colored larvae (*O. speciosa*, *O. bifrons*), await a better understanding but correlate with high and low levels of defensive toxins.

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