Pyrrolizidine Alkaloids in *Danaus plexippus* L. and *Danaus chrysippus* L.

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Summary. Danaus plexippus L. and *Danaus chrysippus* L. have been found to store pyrrolizidine alkaloids obtained from adult food plants and it is suggested that the alkaloids contribute to the unpalatability of the butterflies to potential predators.

The association of male danaine butterflies with plants containing pyrrolizidine alkaloids has recently been the subject of considerable study[2-13]. By feeding on these plants the adult males of many danaine species, including *D. chrysippus*, acquire the alkaloid precursors of dihydropyrrolizines which they secrete on pheromone-disseminating brushes (hairpencils)[8,13]. The hairpencils are used during courtship[14] and deficiency in the appropriate dihydropyrrolizine ketone has been shown in one species (*Danaus gilippus berenice* Kramer) to markedly reduce courtship success[15]. Unlike many other danaines male *D. plexippus* do not appear to secrete dihydropyrrolizines, or other pyrrolizidine alkaloid-derived substances, on their hairpencils. Nor does 'hairpencilling' play an important part in *D. plexippus* courtship[16], yet in some localities[8] male *D. plexippus* are strongly attracted to and are avid feeders on pyrrolizidine alkaloid-containing plants suggesting that the plants' constituents have another role to play in this species.

We report here the finding that *D. plexippus* and *D. chrysippus*, when captured in the field, contain unmodified pyrrolizidine alkaloids and that *D. plexippus* is capable of retaining alkaloids for extended periods. On the basis of these results and the demonstration by Eisner et al.[17] that pyrrolizidine alkaloids are distasteful to some insect predators, we suggest that these alkaloids may contribute to the unpalatability of *D. plexippus* and *D. chrysippus*.

A preliminary alkaloid analysis was carried out on 4 male, 20-month-old museum specimens of *D. plexippus* captured at Brown Hill Creek (site A) near Adelaide, South Australia. Freshly caught butterflies from 4 localities in South Australia were subsequently examined. Three of the collection sites – A, B (Waterfall gully) and C (Morialta Falls) in the foothills of the Mount Lofty ranges near Adelaide – are within 10 km of each other while the 4th, D (Rapid Bay), is 100 km south of Adelaide. In each locality larvae were found feeding on *Asclepias rotundifolia* Mill. No other larval food plant was found. The main sources of pyrrolizidine alkaloids at sites A, B and C were *Senecio pterophorus* DC. (Compositae) and *Echium plantagineum* L. (Boraginaceae)[18]. No pyrrolizidine alkaloid-containing plant was observed in the vicinity of site D.

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Table I. Pyrrolizidine alkaloids in *D. plexippus* and *D. chrysippus*

Species	Site[a]	Number	Sex	Principal alkaloids identified[b] (μg/butterfly)[c]
D. plexippus	A	4	♂	Seneciphylline/senecionine[d] (40) Rosmarinine (20)
D. plexippus	A	5	♂	Seneciphylline/senecionine[d] (40) Rosmarinine (30)
D. plexippus	B	5	♂	Seneciphylline/senecionine[d] (30) Rosmarinine (10)
D. plexippus	C	4	♂	Seneciphylline/senecionine[d] (40) Rosmarinine (40)
D. plexippus	D	7	♂	–
D. plexippus	D	5	♀	–
D. chrysippus	A	5	♂	Seneciphylline/senecionine (40) Rosmarinine (50)
D. chrysippus	C	2	♂	Seneciphylline/senecionine (130) Rosmarinine (130)

[a] See text. [b] Identified by comparison of their gas chromatographic (GC) retention times, mass spectra (MS) and thin layer chromatographic (TLC) behaviour with those of authentic samples. GC/MS was performed on a Varian MAT 111 GC/MS using a 1.5 m × 3 mm glass lined stainless steel column packed with 2.7% SE 30 on Chromosorb P, mesh size 80–100 with temperature programming from 180° to 230 °C at 6 °C/min and carrier gas (helium) flowing at 15 ml/min. TLC plates (silica gel G made with *N*/10 NaOH) were run using chloroform, methanol, conc. aq. NH_3 (84.5:14.5:0.5) as solvent. Alkaloids were made visible with iodine vapour followed by Ehrlich's reagent to give characteristically coloured spots. [c] Estimated from GC peak heights using the authentic alkaloids for calibration. [d] GC peaks unresolved.

Butterflies captured at sites A, B and C contained pyrrolizidine alkaloids (Table I), the main alkaloids identified being those present in *S. pterophorus* (see Table II). This plant therefore seems to be a major source of pyrrolizidine alkaloids for both species of butterfly, although none were seen actually feeding on *S. pterophorus* while specimens were being collected for the present investigation. In fact, on only one of several previous visits to these sites have we observed *D. plexippus* (3 males) apparently feeding on the surface of a dead branch of this plant. In contrast however, all but one of the *D. chrysippus* examined were captured while feeding on dead stems of *E. plantagineum* and traces of echimidine and other alkaloids of *E. plantagineum* were detected (by thin layer chromatography) in their extracts as well as in the extracts of *D. plexippus* from sites A, B and C. Unfortunately the quantity of these alkaloids could not be determined accurately because of their instability in the gas chromatograph.

The ability of *D. plexippus* to retain ingested alkaloids for extended periods was demonstrated by feeding 4 newly emerged, insectary-reared butterflies (2 males and 2 females) for 2 days on a sucrose solution containing 2 μg/μl of a pure pyrrolizidine alkaloid (rinderine) which had been neutralized with carbon dioxide. They were then fed a sucrose solution free of alkaloid for 7 days before being analyzed. The results obtained are reported in Table III. Control butterflies (1 male, 2 female), fed for the same period a sucrose solution only, were found to be free of alkaloid.

In a second experiment, *D. plexippus* were allowed to feed for 2 days on a sucrose solution containing 1 μg/μl of the alkaloid monocrotaline and an equal concentration of the alkaloid heliotrine. They were then kept on a diet free of alkaloid for either 4 or 9 days before being analyzed. The analysis results (Table IV) establish that the butterflies are able to store monocrotaline and heliotrine. Comparison of the alkaloid content of the male butterflies after 4 and 9 days indicated a tendency for them to lose monocrotaline more readily than heliotine. This may reflect the fact that monocrotaline, an alkaloid of tropical *Crotalaria* species, is not normally encountered by butterflies of southern Australia and as a result binding sites for monocrotaline may not be well developed. The converse may occur with *D. plexippus* inhabiting tropical areas where *Crotalaria* species are found.

The unpalatability of *D. plexippus, D. chrysippus* and other Danainae is well recognized. Their distastefulness to potential predators has previously been ascribed to emetic and toxic cardenolides obtained from their larval food plants[19,20]. However some *D. plexippus* and *D. chrysippus* larval food plants are deficient in cardenolides and consequently there are butterflies which lack these protective phytochemicals[21–25]. Protection of butterflies deficient in cardenolides is thought to depend on their being visually indistinguishable from those containing higher levels of these compounds[21–25] and the term automimicry has been used to describe this phenomenon[23].

The presence of unpalatable, toxic pyrrolizidine alkaloids in natural populations of *D. plexippus* and *D. chrysippus* and presumably in other Danainae, may provide an alternative predator deterrent. This could explain some findings of danaine unpalability that are not satisfactorily explained on the basis of cardenolide storage alone. ROTHSCHILD et al.[26] and BROWER et al.[27] have

Table II. Identification of pyrrolizidine alkaloids in the larval food plant and the pyrrolizidine alkaloid-containing plants found at butterfly collection sites

Species	Alkaloids present[a]
S. pterophorus[b]	Seneciphylline, senecionine, rosmarinine[c] and acetylseneciphylline[d]
E. plantagineum[b]	Echiumine, echimidine
A. rotundifolia	None

[a] Identified as described under Table I (note [b]). [b] See ref. [18] for earlier analyses of these species. [c] Previously unknown from an Australian source and not found in earlier studies of this species [18]. [d] A new alkaloid; characterization to be described fully elsewhere.

Table III. Analysis of four *D. plexippus* given access to the pyrrolizidine alkaloid rinderine then maintained on a diet free of rinderine for 7 days

Specimen	Sex	Rinderine content (μg)
1	♂	80
2	♂	20
3	♀	60
4	♀	70

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Table IV. Analysis of *D. plexippus* fed for 2 days on a sucrose solution containing 1 μg/μl of the pyrrolizidine alkaloid monocrotaline and an equal concentration of the alkaloid heliotrine, then given a diet free of alkaloid for a period before analysis

No. of butterflies	Sex	Alkaloid-free period	Alkaloids found[a] (μg/butterfly)
2	♀	4 days	Heliotrine (38) Monocrotaline[b] (34)
3	♂	4 days	Heliotrine (44) Monocrotaline[b] (32)
2	♂	9 days	Heliotrine (36) Monocrotaline[b] (18)

[a] Using GC/MS and TLC systems described under Table I. [b] GC/MS performed on butylboronate derivative.

shown that West African *D. chrysippus* are poor storers of cardenolides compared with *D. plexippus* and East African *D. chrysippus*. These workers demonstrated a genetic element in the relative failure of *D. chrysippus* to store cardenolides establishing that this was not merely a reflection of the cardenolide content of their food plants. In view of the particularly poor cardenolide storage capacity of West African *D. chrysippus*, ROTHSCHILD et al.[26] were led to wonder how these butterflies retain their aposematic life-style and they suggested that some other deterrent factor, apart from cardenolide storage in the adult butterfly, is involved. This other deterrent may be the pyrrolizidine alkaloids stored primarily by male butterflies.

Our results (Tables III and IV) indicate, at least in the case of *D. plexippus*, that female danaines are capable of storing pyrrolizidine alkaloids. However females rarely feed on pyrrolizidine alkaloid-containing plants so that, in areas where the butterflies lack cardenolides and require pyrrolizidine alkaloids for distastefulness, they may depend on their similarity to the males for protection from predators. In these areas a scarcity of Batesian mimics might be expected since the unpalatable males would be less able to support a population of palatable mimics as well as conspecific females. This may help to explain the apparent scarcity of *D. chrysippus* mimics in West Africa[26, 27].

The relative importance of pyrrolizidine alkaloids and cardenolides in conferring distastefulness on the Danainae remains to be assessed. Genetic selection favoring pyrrolizidine storage (and perhaps female feeding) may occur in areas where a high proportion of larvae feed on plants lacking cardenolides. In these regions the alkaloids may contribute significantly to the unpalatability of the butterflies while in others they may serve only to augment that conferred by cardenolides.

Investigations on the Toxic Effects of Bayer 73 (Bayluscid WP) on Eggs and Yolk-Sac Larvae of *Tilapia leucosticta* (*Cichlidae*)

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Summary. The toxic effect of Bayer 73 on larvae of *Tilapia leucosticta* shows a sharp increase from the day of hatching to the end of the yolk-sac stage. Different degrees of deformity appear in the course of development.

In many tropical waters, Bayer 73 is used to combat Bilharziosis. It kills the intermediate hosts (snails, snail-spawn) and also the parasite in its developmental stages. The preparation is introduced directly into the water (concentration 1 ppm[2, 3] and 4–8 ppm · h[4]). Unfortunately it also effects the fish populations, an important food supply for the people[5–7]. Since the effect of molluscicides on fry is not yet known, I have examined the toxic effect of Bayer 73 on eggs and various developmental stages of *Tilapia*. For this purpose, the fertilized eggs were removed from the mouth of the brooding female and artificially incubated (25 °C). Thus the development of more than 2000 eggs and yolk-sac larvae could be tested. As early as the 5th day after the fertilization, the embryos hatch. On the 14th day the yolk-sac larvae become alevins. Between the 3rd and 14th day batches of 20–40 eggs or yolk-sac larvae were dipped for 60 min into well-oxygenated glass tubes containing different concentrations of Bayer 73 (0.5–15 ppm), then rinsed with distilled water and put back into the incubator. There the further development was observed for 15 days and the dead and

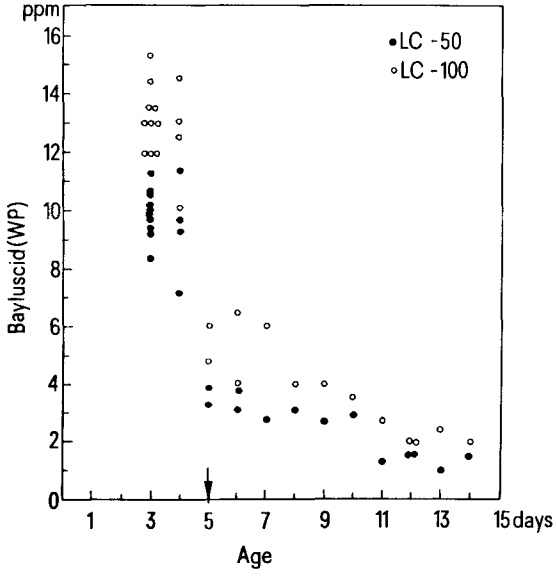

Fig. 1. LC-50 and LC-100 values of the dipping tests with eggs and yolk-sac larvae of *Tilapia leucosticta*. Arrow = hatching day.

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