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CYANOGENESIS—A GENERAL PHENOMENON IN THE LEPIDOPTERA?

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Abstract—There are two different pathways known to be used for the detoxification of hydrocyanic acid in insects, viz., rhodanese and β-cyano-L-alanine synthase. We consider the latter to be indicative for cyanogenesis, while rhodanese might, in general, play a more important role in sulfur transfer for protein synthesis. This paper reports on the distribution of β-cyano-L-alanine (BCA) in the Lepidoptera. First reports of cyanogenesis are presented for the following families: Papilionidae, Pieridae, Lycaenidae, Hesperiidae, Lymantriidae, Arctiidae, Notodontidae, Megalopygidae, Limacodidae, Cymatophoridae, Noctuidae, Geometridae, and Yponomeutidae. New and old records for three other families, the Nymphalidae, Zygaenidae, and Heterogynidae, are included to complete the present state of knowledge. Special emphasis has been laid on the Nymphalidae, where BCA has been detected in eight subfamilies. Taxonomic, geographic, and seasonal variation has been found in a number of cases. In all cases observed so far, the source of cyanogenesis in the Lepidoptera is most probably the cyanoglucosides linamarin and lotaustralin, although cyanogenesis based on mustard oil glucosides and cyclopentenoid glucosides might occur as well. BCA has been found in both cryptic and aposematic species, including taxa such as the Pieridae, Danainae, Ithomiinae, and Arctiidae, where the defensive biology is believed to be linked with other compounds, like mustard oil glucosides, cardenolides, or pyrrolizidine alkaloids. The ecological interaction and significance of such secondary compounds is not yet understood.

Key Words—Lepidoptera, chemical defense, cyanogenesis, cyanoglucosides, β-cyano-L-alanine, rhodanese, phytophagous insects.

INTRODUCTION

According to recent records, cyanogenesis in animals is restricted to a few cases in the Arthropoda, where examples are known from the Myriapoda, the Diplo-

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poda, and the orders Heteroptera, Coleoptera, and Lepidoptera in the Insecta (Davis and Nahrstedt, 1984). In the Myriapoda, Diplopoda, and Coleoptera (Cicindellinae and Chrysomelidae), HCN and benzaldehyde are produced by catabolic decomposition of mandelonitrile (benzaldehyde-α-hydroxynitrile) (Blum et al., 1981). In other insects, i.e., in *Acraea* and *Heliconius* butterflies (Nymphalidae) and in *Zygaena* moths (Zygaenidae), the source of cyanogenesis has been detected as the cyanoglucosides linamarin (2-β-D-glucopyranosyloxy-2-methylpropionitrile) and lotaustralin (2-β-D-glucopyranosyloxy-2-methyl-2*R*-butyronitrile) (Davis and Nahrstedt, 1979; Nahrstedt and Davis, 1983).

Most larval food plants of *Zygaena* moths and *Acraea* and *Heliconius* butterflies are themselves cyanogenic, but the source of cyanogenesis is different: in certain species of *Zygaena* the cyanoglucosides of the larval food plants (Fabaceae) and those of the insects are chemically identical, while the food plants of *Acraea* and *Heliconius* (Passifloraceae) usually contain cyanoglucosides with a cyclopentenoid aglycon (Davis and Nahrstedt, 1984). This indicates that larvae of *Zygaena*, *Heliconius*, and *Acraea* biosynthesize their cyanoglucosides independently from larval ingestion of cyanogenic food plants. In fact, Wray et al. (1983) have demonstrated that *Heliconius* and *Zygaena* use the same biosynthesis as plants that contain linamarin and lotaustralin. This synthesis starts from valine for linamarin and isoleucine for lotaustralin (Conn, 1979) (see Figure 1). The high amounts of cyanogenic compounds in these insects are generally thought to be linked with distastefulness to predators, which again is believed to be connected with the aposematic patterns of both the larvae and imagines.

Two different detoxification pathways for hydrocyanic acid have so far been demonstrated in insects (Figure 1).

1. The more commonly known, which had been favored especially in the older literature (Jones et al., 1962), is that found in vertebrates which produces thiocyanate (rhodanid) via the enzyme rhodanese (EC 2.8.1.1). The presence of this enzyme has been demonstrated by Beesley et al. (1985) to occur in a considerable number of phytophagous insects.

2. In other insects the catabolic reaction leads to β-cyano-L-alanine (BCA) via BCA synthase (EC 4.4.1.9). The presence of BCA in insects was first demonstrated by Duffey (1981) in larvae of *Heliothis zea* and *Spodoptera exigua* (Lepidoptera, Noctuidae) and in *Oncopeltus fasciatus* (Heteroptera, Lygaeidae). BCA has also been detected in the defensive secretion and tissues of *Zygaena* larvae, in *Zygaena* moths and in dried specimens of *Acraea* and *Heliconius* (Witthohn and Naumann, 1984a,b).

None of these detoxification systems is necessarily bound to the cyanoglucosides linamarin and lotaustralin; both could well be able to detoxify hydrocyanic acid released from any other cyanogenic precursor as well. It has also been argued that the role of rhodanese as a sulfur donor in protein synthesis

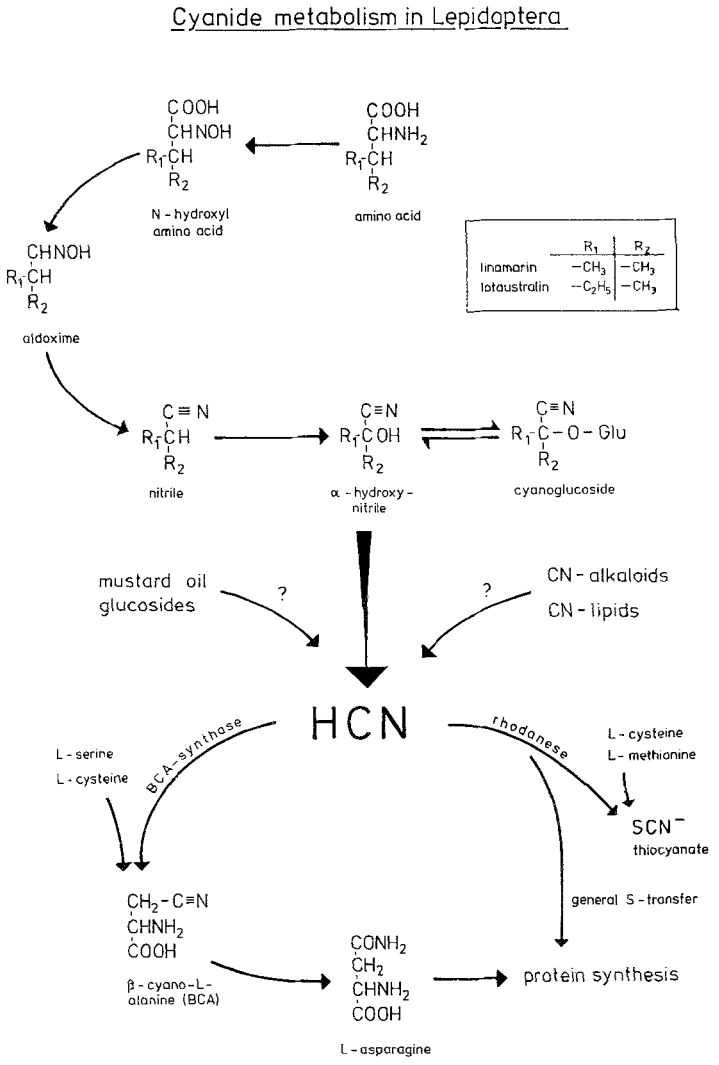

FIG. 1. Metabolic pathways for cyanoglucoside synthesis (modified, after Conn, 1979) and detoxification of hydrocyanic acid.

might be more important than its detoxifying properties (Volini and Alexander, 1981). If so, the presence of rhodanese does not necessarily imply that an organism is cyanogenic, but the evidence of BCA in animal tissues can generally be regarded as indicative for the presence of cyanogenic compounds. It is in this sense that we have used the test for BCA as an indicator for cyanogenesis in Lepidoptera in the present paper. Of course, the absence of BCA does not necessarily mean that the animal concerned is acyanogenic.

It has been shown that nonaposematic species of the Zygaenidae, especially those of the Zygaeninae, also contain the cyanoglucosides linamarin and lotaustralin (Davis and Nahrstedt, 1984; Witthohn and Naumann, 1984b), although like some aposematic species, they live on the same noncyanogenic food plants, the alkaloid-rich Celastraceae. Furthermore, we have previously demonstrated that cryptic species of the Zygaeninae detoxify hydrocyanic acid via BCA synthase, using the same method as their aposematic relatives. Similarly BCA has been demonstrated in some nymphalid genera unrelated to *Acraea* and *Heliconius* (viz., *Vanessa*, *Cynthia*, and *Maniola*). Of these, at least *Maniola* must be regarded as cryptic, although females of this species have been found to contain a histamine-like compound, leading to their rejection by a shama thrush (*Kittacincla malabaricus*) (Rothschild, 1985). Neither *Vanessa*, *Cynthia*, nor male *Maniola* butterflies have hitherto been thought to be chemically protected. Finally BCA has even been detected in the danaine butterfly *Danaus chrysippus*, a species which has been thought to be associated with cardioglucosides and pyrrolizidine alkaloids exclusively (Witthohn and Naumann, 1984b).

Following these results, we decided to investigate other families of butterflies for the presence of BCA and, having found it to be present in an astonishing number of cases, to extend the study to other lepidopterous families as well. The results of our research are presented in this paper.

METHODS AND MATERIALS

The tests were preferably carried out with fresh specimens which were either bred in captivity or caught in the wild. When such material was not available, specimens were either obtained from private collectors or from commercial dealers (Fa. Lörcher, Urach, F.R.G.). All tests on dried material reported here were done with specimens less than 5 years old, as we have found that BCA decomposes with time and its presence may be difficult to prove in older specimens, even of highly cyanogenic species. Thus, we could not demonstrate BCA in dried specimens of *Morpho* (Nymphalidae, Morphinae), while examples freshly emerged from imported pupae proved positive. The specimens were crushed and mixed with an aliquot solution of methanol–conc. aqueous ammonia (80:20), of which 10 μl were used for the tests. BCA was detected by two-dimensional thin-layer chromatography (TLC sheets 5 × 5 cm, Merck, silica gel 60, 0.2 mm), eluants: (1) chloroform, methanol, conc. aqueous ammonia (5:5:1); and (2) *n*-butanol, acetic acid, water (5:1:2). The sheet was sprayed with ninhydrin solution after drying. BCA could be identified by its characteristic blue color and its retention times: $R(F1) = 0.55$, $R(F2) = 0.19$. In a number of cases, the presence of the cyanoglucosides linamarin and lotaustralin was also examined by thin-layer chromatography with available lin-

amarin (Calbiochem) as reference; eluant ethylacetate–acetone–water (4 : 5 : 1), and spraying with anisaldehyde (Stahl and Kaltenbach, 1961).

Enzymatic activity, which must be responsible for the presence of BCA, was demonstrated following Henrickson and Conn (1969).

RESULTS

The results are given in Table 1. We have also provided information on the geographic origin of the specimens, the larval food-plant family, and whether cyanogenesis has been recorded in these food plants. These data have been taken from various literature sources.

According to the strength of the reaction, we have classified the concentration (c) of BCA into three groups: +: $c \leq 2$ μg/insect; ++: $2 \leq c \leq 20$ μg/insect; +++: $c \geq 20$ μg (175 nmol)/insect. The presence of the cyanoglucosides linamarin/lotaustralin has not been detected in all cases where BCA was found to be present, but they usually are present, when the reaction for BCA is strong (+++) and sometimes even when it is medium (++). Thus, the results are influenced by the fact that the test for the cyanoglucosides is less sensitive than that for BCA.

DISCUSSION

One might argue that the amounts of BCA detected in the different Lepidoptera could be due to postmortem microbial action and not to actual biosynthesis of the living insect. We have therefore compared the strength of the ninhydrin reaction for BCA in fresh and dried specimens of a number of species, including *Clossiana euphrosyne* (Nymphalidae), a number of *Zygaena* species and *Aglaope infausta* (Zygaenidae), and *Heterogynis penella* (Heterogynidae). There was no difference, and we therefore feel justified to presume that the amount of BCA contained in dried insects is, in fact, derived from the insect itself. We have furthermore demonstrated the presence of BCA synthase in *Clossiana euphrosyne* (Nymphalidae); *Pryeria sinica*, *Orna nebulosa*, *Zygaena trifolii*, *Rhagades pruni*, and *Aglaope infausta* (Zygaenidae); and *Heterogynis penella* (Heterogynidae). Of these, only *Zygaena trifolii* had been known to possess BCA synthase (Witthohn and Naumann, 1984a). These results suggest that BCA is actually biosynthesized by the insects themselves and is neither accumulated after larval or imaginal ingestion of food plants nor a result of microbial tissue decomposition.

Our data include the first records of cyanogenesis for another 14 lepidopterous families, i.e., the Papilionidae, Pieridae, Lycaenidae, Hesperiidae, Lymantriidae, Arctiidae, Notodontidae, Megalopygidae, Limacodidae, Cymato-

TABLE 1. DISTRIBUTION OF β-CYANO-L-ALANINE (BCA) IN LEPIDOPTERA[a]

Species	Origin	Stage	Preservation	BCA	LIN/ LOT	Larval food plant[b]
Papilionidae						
Trogonoptera brookiana Wall.	12	I	dr	–	–	Aristolochiaceae
Iphiclides podalirius L.	18	I	dr	+	–	Rosaceae
Zerynthia polyxena D. & Sch.	3	I	dr	–	–	Aristolochiaceae
Parnassius apollo L.	18	I	dr	+	–	Crassulaceae
	3	I	dr	–	–	
Pieridae						
Aporia crataegi L.[c]	18	I	dr	+	–	Rosaceae
[c]	7	I	fr	–	–	
Pieris brassicae L.[c]	7	L	fr	–	–	Brassicaceae
(gen.vern.)[c]	7	I	dr	++	–	
(gen.aest.)[c]	7	I	fr	–	–	
Pieris rapae L.	7	L	fr	–	–	Brassicaceae
	7	I	fr	–	–	
Pieris napi L.						
(gen.vern.)[c]	7	I	dr	++	–	Brassicaceae
(gen.aest.)[c]	18	I	dr	++	–	
Anthocharis euphenoides Stgr.	18	I	dr	–	–	Brassicaceae
Colias phicomone Esp.	10	I	dr	+	–	Fabaceae
Colias chrysotheme Esp.	8	I	dr	–	–	Fabaceae
Colias crocea Fourcr.[c]	18	I	dr	+	–	Fabaceae
[c]	7	I	dr	–	–	
Colias australis Vrty.	18	I	dr	+	–	Fabaceae
Gonepteryx rhamni L.	18	I	dr	+	–	Rhamnaceae
Gonepteryx cleopatra L.	18	I	dr	–	–	Rhamnaceae
Leptidea sinapis L.	18	I	dr	+	–	Fabaceae

Nymphalidae						
Apaturinae						
Apatura ilia L.[d]	7	I	dr	–	–	Salicaceae
Apatura ilia f. *clythie* D. & Sch.[d]	7	I	dr	–	–	Salicaceae
Limenitis reducta Stgr.	18	I	dr	+	+	Caprifoliaceae
Limenitis camilla L.	7	I	fr	–	–	Caprifoliaceae
	7	I	dr	–	–	
Nymphalinae						
Nymphalis polychlorus L.[c,d]	7	I	dr	–	–	Salicaceae
Inachis io L.[c]	18	I	dr	–	–	Urticaceae
	10	I	dr	–	–	
[c]	7	I	fr	–		
[c,d]	7	I	dr	–	–	
Vanessa atalanta L.[c,d]	18	I	dr	+	–	Urticaceae,
[c]	10	I	dr	+	–	Asteraceae
[c]	7	I	fr	–	–	
[c]	7	I	dr	–	–	
Cynthia cardui L.[c]	18	I	dr	+	–	Urticaceae,
[c]	7	I	dr	–	–	Asteraceae
Aglais urticae L.[c,d]	7	I	dr	–	–	Urticaceae
Polygonia c-album L.[c]	18	I	dr	+	–	Urticaceae
[d]	7	I	dr	–	–	
Araschnia levana L.[c,d]	7	I	dr	–	–	Urticaceae
Argynnis paphia L.[c]	18	I	dr	+	–	Violaceae
Mesoacidalia aglaia L.[c]	18	I	dr	++	–	Violaceae
Fabriciana adippe D. & Sch.[c]	18	I	dr	++	+	Violaceae
[c]	6	I	dr	+	–	
Fabriciana niobe L.[c]	18	I	dr	++	+	Violaceae
[c]	6	I	dr	+	–	
Issoria lathonia L.[c]	7	I	dr	+++	+	Violaceae, Fabaceae
Brenthis daphne D. & Sch.[c]	18	I	dr	++	+	Violaceae, Rosaceae
Brenthis ino Rott.[c]	18	I	dr	++	+	Rosaceae

TABLE 1. CONTINUED

Species	Origin	Stage	Preservation	BCA	LIN/ LOT	Larval food plant[b]
Clossiana selene D. & Sch.[c]	6	I	dr	+	–	Violaceae
Clossiana euphrosyne L.[c]	7	I	fr	+++	+	Violaceae
[c]	7	I	dr	+++	+	
Clossiana dia L.[c]	18	I	dr	+	+	Violaceae
Mellicta phoebe D. & Sch.[c]	18	I	dr	+	–	Asteraceae, Plantaginaceae
Mellicta dictynna Rott.[c]	18	I	dr	+	–	Valerianaceae, Plantaginaceae
Mellicta parthenoides Kef.[c]	18	I	dr	+	–	Dipsacaceae, Plantaginaceae
Danainae						
Danaus chrysippus L.[d]	17	I	dr	++	–	Asclepidiaceae
Ithomiinae						
Eutresis hypereia D. & Hew.	13	I	dr	–	–	Passifloraceae
Titorea harmonia Cr.	16	I	dr	+	–	Apocynaceae
Mechanitis cf. *polymnia* L.	16	I	dr	++	–	Solanaceae
Godyris zavaleta Hew.	16	I	dr	++	–	Solanaceae
Dircenna dero Hbn.	16	I	dr	++	–	Solanaceae
Dircenna cf. *loreta* Haensch	16	I	dr	+	–	Apocynaceae
Acraeinae						
Acraea horta L.[d]	4	I	dr	+++	+	Passifloraceae, Compositae
Acraea encedon L.	4	I	dr	++	+	Passifloraceae,
	17	I	dr	++	+	Compositae
Acraea igola Trimen	4	I	dr	+	+	Passifloraceae, Compositae
Acraea caldarena Hew.	17	I	dr	+	–	Passifloraceae

Acraea eponina Cr.	17	I	dr	++	–	Passifloraceae
Acraea natalica Bsd.	17	I	dr	++	–	Passifloraceae
Pardopsis punctatissima Bsd.	17	I	dr	+	+	Passifloraceae
Heliconiinae						
Philaetria dido L.	16	I	dr	–	–	Passifloraceae
Dryadula phaetusa L.	13	I	dr	++	+	Passifloraceae
Agraulis vanillae L.[d]	13	I	dr	++	+	Passifloraceae
	16	I	dr	++	+	
Podotriche telesiphe Hew.	16	I	dr	++	–	Passifloraceae
Eueides isabella Cr.	13	I	dr	++	+	Passifloraceae
Heliconius doris L.[d]	20	I	dr	+++	+	Passifloraceae
Heliconius charitonia L.[d]	20	I	dr	+++	+	Passifloraceae
Morphinae						
Morpho caternarius Per.	5	I	fr	+	–	Poaceae
Satyrinae						
Melanargia g. galathea L.[c]	7	I	dr	–	–	Poaceae
Melanargia g. lachesis Hb.[c]	18	I	dr	++	–	Poaceae
Hipparchia alcyone D. & Sch.	18	I	dr	+	–	Poaceae
Hipparchia semele L.	18	I	dr	+	–	Poaceae
	6	I	dr	+	–	
Satyrus ferula F.	10	I	dr	+	–	Poaceae
Brintesia circe F.	18	I	dr	++	–	Poaceae, Rosaceae
Erebia medusa D. & Sch.	7	I	dr	–	–	Poaceae
Erebia meolans Prun.	18	I	dr	++	–	Poaceae
Maniola jurtina L.[c]	18	I	dr	++	–	Poaceae
[c]	6	I	dr	+	–	
Aphantopus hyperantus L.	6	I	dr	+	–	Poaceae
Pyronia bathseba F.	18	I	dr	+	–	Poaceae
Coenonympha pamphilus L.[c]	6	I	dr	–	–	Poaceae
[c]	7	I	dr	–	–	
Coenonympha dorus Esp.	18	I	dr	+	–	Poaceae
Coenonympha arcania L.	18	I	dr	++	–	Poaceae
Lasiommata maera L.	18	I	dr	+	–	Poaceae

TABLE 1. CONTINUED

Species	Origin	Stage	Preservation	BCA	LIN/ LOT	Larval food plant[b]
Lycaenidae						
Nordmannia ilicis Esp.	7	I	dr	–	–	Fagaceae
Nordmannia esculi Hbn.	18	I	dr	+	–	Fagaceae
Lycaena phlaeas L.	7	I	dr	–	–	Polygonaceae
Heodes alciphron Rott.	18	I	dr	–	–	Polygonaceae
Palaeochrysophanus hippothoe L.	18	I	dr	+	–	Polygonaceae
	6	I	dr	+	–	
Cupido minimus Fuessl.	6	I	dr	+	–	Fabaceae
Plebejus argus L.	18	I	dr	+	–	Fabaceae
Aricia artaxerxes F.	6	I	dr	+	–	Geraniaceae, Rosaceae
Agriades pyrenaicus B.	18	I	dr	–	–	Fagaceae
Plebicula escheri Hbn.	18	I	dr	+	–	Fabaceae
Plebicula dorylas D. & Sch.	18	I	dr	–	–	Fabaceae, Lamiaceae
Plebicula amanda Schn.[c]	18	I	dr	–	–	Fabaceae
[c]	6	I	dr	–	–	
Lysandra sp.	18	I	dr	–	–	Fabaceae
Lysandra hispana H.Sch.	18	I	dr	–	–	Fabaceae
Polyommatus icarus Rott.[c]	7	I	dr	–	–	Fabaceae
[c]	6	I	dr	–	–	
Polyommatus eros O.	18	I	dr	+	–	Fabaceae
Hesperiidae						
Pyrgus sp.	18	I	dr	++	–	Rosaceae, Malvaceae

Erynnis tages L.	18	I	dr	++	–	Fabaceae, Apiaceae
Thymelicus actaeon Rott.	18	I	dr	++	–	Poaceae
Thymelicus lineola O.	18	I	dr	++	–	Poaceae
Thymelicus sylvestris P.[c]	18	I	dr	+	–	Poaceae
[c]	6	I	dr	–	–	
[c]	7	I	dr	–	–	
Ochlodes venatus Br. & Gr.	18	I	dr	++	–	Poaceae
Lymantriidae						
Lymantria monacha L.	7	I	fr	++	–	polyphagous
Arctiidae						
Phragmatobia fuliginosa L.[c]	7	I	dr	–	–	polyphagous
Parasemia plantaginis L.	18	I	dr	++	–	polyphagous
Spilosoma lutea Hfn.[c]	18	I	dr	++	–	polyphagous
[c]	7	I	fr	–	–	
Syntomidae						
Syntomis phegea L.	7	I	dr	–	–	polyphagous
Syntomis mogadorensis Blach.	14	I	fr	–	–	polyphagous
Notodontidae						
Stauropus fagi L.	18	I	dr	+	–	Fagaceae
Pheosia tremula Cl.	7	I	dr	–	–	Salicaceae
Pterostoma palpinum L.	18	I	dr	–	–	Salicaceae
Zygaenidae						
Procridinae						
Rhagades pruni D. & Sch.[d]	7	I	fr	+++	+	Ericaceae, Fagaceae
Adscita mannii Led.[d]	7	I	dr	+++	+	Cistaceae
Chalcosiinae						
Campylotes histrionicus Koll.[d]	9	I	dr	+++	+	Symplacoceae, Ericaceae
Agalope bifasciata Moore[d]	15	I	dr	+++	+	Aquifoliaceae, Rosaceae

TABLE 1. CONTINUED

Species	Origin	Stage	Preservation	BCA	LIN/ LOT	Larval food plant[b]
Elcysma westwoodi Voll.	11	L	dr	+++	+	Rosaceae
Aglaope infausta L.[d]	7	I	fr	+++	+	Rosaceae
	7	I	dr	+++	+	
Zygaeninae						
Pryeria sinica Moore[d]	11	I	dr	+++	+	Celastraceae
[d]	11	L	fr	+++	+	
Orna nebulosa Guerin[d]	17	I	dr	+++	+	Celastraceae
[d]	17	L	fr	+++	+	
Neurosymploca caffra L. (*auctorun*)[d]	17	I	dr	+++	+	Celastraceae
Praezygaena agria Dist.	17	I	fr	+++	+	Celastraceae
Reissita simonyi Rbl.[d]	21	I	dr	+++	+	Celastraceae
Epizygaenella cashmirensis Kll.[d]	1	I	dr	+++	+	Celastraceae
Zygaena orana Dup.[d]	2	I	dr	+++	+	Fabaceae
Z. carniolica Scop.[d]	7	I	dr	+++	+	Fabaceae
[d]	7	L	fr	+++	+	
Z. fausta L.[d]	7	I	dr	+++	+	Fabaceae
Z. viciae D. & Sch.[d]	7	I	dr	+++	+	Fabaceae
Z. doryenii O.[d]	19	I	fr	+++	+	Fabaceae
Z. hippocrepidis Hb.[d]	18	I	dr	+++	+	Fabaceae
Z. transalpina Esp.[d]	7	I	dr	+++	+	Fabaceae
[d]	7	L	fr	+++	+	
Z. trifolii Esp.[d]	7	I	dr	+++	+	Fabaceae
[d]	7	L	fr	+++	+	
Z. lonicerae Sch.[d]	7	I	fr	+++	+	Fabaceae
Z. corsica Bsd.[d]	10	I	dr	+++	+	Asteraceae
Z. punctum O.[d]	10	I	dr	+++	+	Apiaceae

Anomoeotinae						
Anomoeotis levis Fld. & Fld.	17	I	dr	+++	+	Caesalpiniaceae
Heterogynidae						
Heterogynis penella Hbn.[d]	10	I	fr	+++	+	Fabaceae
	10	I	dr	+++	+	
Megalopygidae						
Trosia dimas	13	I	dr	++	+	polyphagous
Norape ovina Sepp	13	I	dr	++	+	polyphagous
Psycharium pellucens H.S.	17	L	dr	+++	+	Pinaceae
Limacodidae						
Tortricidia testacea Pack.	20	I	dr	++	–	–
Sphingidae						
Mimas tiliae L.	7	I	dr	–	–	Tiliaceae
Laothoe populi L.	7	I	dr	–	–	Salicaceae
Acherontia atropos L.	18	I	fr	–	–	Solanaceae
Cymatophoridae						
Thyatira batis L.	18	I	dr	+	–	Rosaceae
Palimpsestris ocularis L.	18	I	dr	+	–	Salicaceae
Drepanidae						
Drepana lacertinaria L.	7	I	dr	–	–	Betulaceae
Lasiocampidae						
Malacosoma neustria L.	18	I	dr	+	–	polyphagous, Rosaceae
Lasiocampa quercus L.	7	I	fr	–	–	polyphagous
Sesiidae						
Sesia apiformis Cl.	7	I	dr	–	–	Salicaceae
Noctuidae						
Scotia exclamationis L.	18	I	dr	–	–	polyphagous
Ochropleura plecta L.	18	I	dr	+	–	polyphagous
Noctua pronuba L.[c]	18	I	dr	++	–	polyphagous
[c]	7	I	dr	–	–	
Polia hepatica Cl.	18	I	dr	–	–	polyphagous
Polia nebulosa Hufn.	18	I	dr	+	–	polyphagous

TABLE 1. CONTINUED

Species	Origin	Stage	Preservation	BCA	LIN/ LOT	Larval food plant[b]
Phlogophora meticulosa L.	7	I	dr	–	–	polyphagous
Cosmia diffinis L.	18	I	dr	+	–	Ulmaceae
Apamaea monoglypha Hufn.	18	I	dr	+	–	Poaceae
Apamaea remissa Hbn.	7	I	dr	–	–	polyphagous
Oligia strigilis L.	7	I	dr	–	–	Poaceae
Meristis trigrammica Hufn.	7	I	fr	–	–	polyphagous
Autographa gamma L.	18	I	dr	–	–	polyphagous
Ectypa glyphica L.	18	I	dr	–	–	Fabacae
Hypena proboscidalis L.	7	I	dr	–	–	polyphagous
Geometridae						
Geometra papilionaria L.	7	I	dr	–	–	Betulaceae
Euphyia bilineata L.	18	I	dr	+	–	polyphagous
Eupithecia sp.	7	I	dr	–	–	–
Campaea margaritata L.	7	I	dr	–	–	Fagaceae
Bapta temerata D. & Sch.	18	I	dr	+++	–	Rosaceae, Fagaceae
Apeira syringaria L.	7	I	dr	–	–	polyphagous
Opistograptis luteolata L.	18	I	dr	–	–	polyphagous
Pseudopanthera macularia L.	18	I	dr	–	–	polyphagous
Macaria alternaria Hbn.	18	I	dr	–	–	polyphagous
Chiasmia clathrata L.	18	I	dr	+	–	Fabaceae
	7	I	dr	–	–	
Biston betularia L.	7	I	dr	–	–	polyphagous
Peribatodes rhomboidaria D. & Sch.	18	I	dr	+	–	polyphagous
Alcis repandata L.	18	I	dr	+	–	polyphagous
Ascotis selenaria D. & Sch.	18	I	dr	–	–	polyphagous

Boarmia sp.	18	I	dr	+	–	Ericaceae
Ematurga atomaria L.	18	I	dr	+	–	polyphagous
Pyralidae						
Pyrausta cingulata L.	7	I	dr	–	–	Poaceae
Crambus sp.	7	I	dr	–	–	Poaceae
Tortricidae						
Tortrix sp.	7	I	fr	–	–	Fabaceae
Yponomeutidae						
Yponomeuta padellus L.	7	I	dr	–	–	Salicaceae
Gymnogramma rufiventris Wlk.[e]	17	I	fr	+++	+	Anacardiaceae
	17	L	fr	+++	+	
Adelidae						
Nemophora degeerella L.	7	I	dr	–	–	–

[a] Origin: 1 Afghanistan, 2 Algeria, 3 Austria, 4 Botswana, 5 Brasil, 6 Denmark, 7 Germany, 8 Hungary, 9 India, 10 Italy, 11 Japan, 12 Malaysia, 13 Mexico, 14 Morocco, 15 Nepal, 16 Peru, 17 South Africa, 18 Spain, 19 Turkey, 20 USA, 21 Yemen. dr = air-dried specimens; fr = fresh specimens, usually bred. BCA concentration: – not detected; +: $c < 2$ µg/insect, ++: $2 < c < 20$ µg/insect, +++: $c > 20$ µg (175 mmol)/insect. LIN/LOT (linamarin/lotaustralin) test: – not detected, + present.

[b] Cyanogenesis has been recorded for the following plant families: Apiaceae (1 sp.), Asclepidiaceae (2 sp.), Asteraceae, Caprifoliaceae, Crassulaceae, Brassicaceae, Ericaceae, Fabaceae, Poaceae, Passifloraceae, Rosaceae, Tiliaceae, Ulmaceae (Hegnauer, 1962–1973; Conn, 1981a,b).

[c] 3–6 specimens examined for each population.

[d] Previously recorded in Witthohn and Naumann (1984b).

[e] Probably to be transferred to Zygaenoidea (Kyrki, personal communication).

phoridae, Lasiocampidae, Noctuidae, Geometridae, and Yponomeutidae. Hitherto, cyanogenesis had only been known to occur in three families, viz., the Nymphalidae, Zygaenidae, and Heterogynidae (Witthohn and Naumann, 1984b, and references therein). The specimens used in the Heliconiinae and Acraeinae (Nymphalidae) had obviously lost some of their original amounts of BCA, as could be seen from the generally poor representation of amino acids in TLC. We expect very strong reactions (+++) for all species when fresh specimens are available. This will presumably apply also to those species of the Heliconiinae and Acraeinae which were negative for BCA.

The cyanogenic species *Gymnogramma rufiventris* will be excluded from the Yponomeutidae in the near future. It might be placed in the cyanogenic Zygaenoidea, but not in the Zygaenidae proper (Kyrki, 1984, personal communication). Larvae of this highly cyanogenic species possess lateral abdominal glands which can be extruded and are probably used to store or compartmentalize toxic—possibly cyanogenic—compounds (Naumann, unpublished). A similar, but not homologous, larval defensive system has been found in the Zygaeninae (Povolny and Weyda, 1981; Franzl and Naumann, 1984, 1985).

The number of species actually detoxifying cyanide via BCA synthase might still be greater than our results show: firstly, because the greatest need to detoxify cyanide might occur in the larval instars, which might lead to a reduction of the amount of stored BCA in the imagines, and secondly because an insect might detoxify HCN via BCA synthase but might not store the product of the reaction.

Based on these results, cyanogenesis can no longer be considered a phenomenon restricted to some aposematic species, such as *Zygaena*, *Acraea*, *Heliconius*, and their close relatives. However, there is still a general trend for some groups to show stronger reactions than others. A rather singular case is found in the geometrid moth *Bapta temerata*, a cryptic and mainly night-active species. Since the larval food-plant spectrum includes some cyanogenic Rosaceae, there may exist a connection between cyanogenesis of the larval food plant and that of the moth itself.

The degree of cyanogenesis as evident from the amount of stored BCA varies in a number of respects:

Variation within Lepidoptera. While certain taxa such as the Zygaenidae, Acraeinae, and Heliconiinae must be considered as highly cyanogenic at the family or subfamily level, other taxa such as the Nymphalinae and most other butterfly families show considerable variation which seems to be only partially connected with cyanogenesis of larval food plants. Thus, in the Nymphalidae, the subfamily Satyrinae are confined to the Poaceae, which are known to be cyanogenic. The imagines of this subfamily are cyanogenic at a rather low level. In the Nymphalinae, those species which live on the Violaceae, a plant family not known to be cyanogenic, show comparatively high concentrations of BCA,

as judged from the strength of the ninhydrin reaction. *Clossiana euphrosyne*, a species where BCA synthase was proved in a separate experiment, belongs to these species which are bound to the noncyanogenic Violaceae. Species of the same subfamily living on the acyanogenic Urticaceae or Asteraceae only occasionally contain BCA. It cannot be excluded, of course, that the genera *Vanessa*, *Cynthia*, and *Polygonia* do in fact accumulate cyanogenics from their larval food plants, but at very low concentrations, so that the amount of BCA cannot be detected by the usual analytical technique.

Geographic Variation. Geographic variation in the amount of allelochemicals is certainly not new and was demonstrated for the pyrrolizidine alkoloids in the monarch butterfly, *Danaus plexippus* as early as 1972 (Brower et al., 1972). In the present study an additional geographic pattern is apparent in *Parnassius apollo*, *Aporia crataegi*, *Colias crocea*, *Vanessa atalanta*, *Cynthia cardui*, *Polygonia c-album*, *Melanargia galathea*, *Thymelicus sylvestris*, *Spilosoma lutea*, and *Noctua pronuba*: central European specimens of all these species do not contain BCA, while Mediterranean ones do. In most cases we have examined several specimens of each species. An explanation for such a general pattern is still lacking.

Seasonal Variation. In *Pieris brassicae* our data suggest seasonal variation: members of the spring generation contain BCA, which is absent in the summer generation.

These three different types of variation, which were just discernible in the present study, will necessitate further research on an individual, population, seasonal, and geographic basis, preferably with respect to the larval and imaginal food plants of the various individuals tested.

As discussed above the presence of the cyanoglucosides linamarin/lotaustralin could only be demonstrated in a limited number of cases, because the TLC anisaldehyde reaction is much less sensitive than the TLC ninhydrin reaction used as a test for BCA. Nevertheless, we believe it to be most likely that these cyanoglucosides are the source of cyanogenesis, at least in all cases of the Rhopalocera and Zygaenoidea tested so far. This is indicated by the data on Zygaenidae, Acraeinae, and Heliconiinae supplied by Davis and Nahrstedt (1984), and by the fact that in the present paper the cyanoglucosides have been detected in some cases of related but cryptic species also storing BCA. One might even suppose that in the Lepidoptera the two mentioned cyanoglucosides are usually detoxified as in plants, i.e., via BCA synthase.

At present we have no evidence of an insect that would make use of both detoxification pathways, rhodanese *and* BCA-synthase, to some biologically significant extent. On the contrary, the Zygaenidae seem to use the BCA pathway exclusively, since Beesley et al. (1985) found practically no rhodanese in this group. The same seems to apply to *Erynnis tages* (Hesperiidae). Conversely, rhodanese has been demonstrated in sufficient amounts in the blue but-

terfly *Polyommatus icarus* (Parsons and Rothschild, 1964; Beesley et al., 1985), while BCA is obviously not stored.

Jones (1979) has argued that there might be a general need for phytophagous insects to detoxify HCN, because small amounts of cyanoglucosides seem to be present in nearly all angiosperms. Larvae of the southern army worm, *Spodoptera eridiana*, even use hydrocyanic acid as a feeding stimulant (Brattsten et al., 1983). It has been shown that rhodanese occurs in a considerable range of insects, but often in very small amounts only (Beesley et al., 1985). We suggested that BCA might be used as a stored product for the biosynthesis of asparagine and other amino acids (Witthohn and Naumann, 1984a). Similarly Volini and Alexander (1981) have stressed the possible importance of rhodanese for the biosynthesis of sulfur-containing amino acids. If these assumptions prove correct, then the products will be reintroduced into the primary metabolic process in both reactions.

With regard to the role of the cyanoglucosides linamarin and lotaustralin in the ecology of *Zygaena*, it is important to note that the parasitoid braconid wasp *Apanteles zygaenarum*, which lives almost exclusively in *Zygaena* species, does not use BCA synthase to detoxify linamarin, which is obviously acquired from the host's tissues (Davis and Nahrstedt, 1984). The same applies to the parasitic fly *Zenillia*, which also lives in *Zygaena* species and uses the rhodanese pathway (Jones et al., 1962).

Recently Davis and Nahrstedt (1984) have stressed the importance of delimiting the distribution of the cyanoglucosides in the Nymphalidae and suggested that cyanogenesis might be a taxonomically important character for the Zygaenidae. The same was proposed by Naumann (1985). It is now clear that cyanogenesis is not restricted to the Nymphalidae but occurs in all families of butterflies, where it is always based on the two cyanoglucosides linamarin and lotaustralin. It will now be of great importance to delimit the amount of cyanogenics acquired or synthesized by butterflies with respect to their geographic origin, seasonality, and the cyanoglucoside content of their food plants. In the Zygaenoidea strong cyanogenesis extends far beyond the family level and has now been shown to occur in the Limacodidae and Megalopygidae which, at present, are believed to belong to the Cossoidea, but which have also been associated with the Zygaenoidea in the past (Brock, 1971). As the Cossidae themselves are good candidates for cyanogenesis (because they are cyanide-resistant as well), the use of this character for taxonomic and phylogenetic purposes diminishes. On the other hand, the larval storage system for cyanogenic secretion might provide a useful character to delimit at least one major monophyletic entity within the Zygaenidae (Naumann et al., in preparation).

Thus we conclude that cyanogenesis is a widespread phenomenon in the Lepidoptera. It seems to be based mainly or possibly exclusively on the cyanoglucosides linamarin and lotaustralin, and the main detoxification pathway of

the Lepidoptera seems to be BCA synthase. We suggest cyanogenesis to be a basic characteristic of the Lepidoptera, but at present it is impossible to say whether this could, at the same time, be a synapomorphy of the taxon. The use of BCA synthase to detoxify HCN originating from mandelonitrile in the Chrysomelidae (Coleoptera) has been proposed by Blum et al. (1981). It can thus be assumed that a wide distribution of BCA could be found in other phytophagous insect orders as well. The presence of BCA and BCA synthase has recently been demonstrated in the chrysomelid beetle *Paropsis atomaria*, which uses mandelonitrile and prunasin as sources for hydrocyanic acid (Nahrstedt and Davis, 1986).

Rothschild (1985) has recently listed allelochemicals that have been recorded from various British Macrolepidoptera. When compared with the list presented above, it becomes obvious how little is known at present about the cooccurrence of several allelochemicals in nonglandular tissues and their possible ecological interaction. Rothschild rightly points out that one of the major deficiencies of this branch of insect chemical ecology is the present concentration of research on one or two major compounds, mainly cardenolides and pyrrolizidine alkoloids (Boppré, 1986), while very little is known about the ecological importance of other compounds, which sometimes cooccur. Thus, Muhtasib and Evans (1987) have demonstrated synergism in the defensive properties of linamarin and histamine. As for the cyanoglucosides, we shall report elsewhere on the reaction of various predators to the cyanogenic compounds linamarin and β-cyanoalanine.

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