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Molecules of Interest

Cyanogenesis in plants and arthropods

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ABSTRACT

Cyanogenic glucosides are phytoanticipins known to be present in more than 2500 plant species. They are regarded as having an important role in plant defense against herbivores due to bitter taste and release of toxic hydrogen cyanide upon tissue disruption, but recent investigations demonstrate additional roles as storage compounds of reduced nitrogen and sugar that may be mobilized when demanded for use in primary metabolism. Some specialized herbivores, especially insects, preferentially feed on cyanogenic plants. Such herbivores have acquired the ability to metabolize cyanogenic glucosides or to sequester them for use in their own defense against predators. A few species of arthropods (within diplopods, chilopods and insects) are able to *de novo* biosynthesize cyanogenic glucosides and some are able to sequester cyanogenic glucosides from their food plant as well. This applies to larvae of *Zygaena* (Zygaenidae). The ratio and content of cyanogenic glucosides is tightly regulated in *Zygaena filipendulae*, and these compounds play several important roles in addition to defense in the life cycle of *Zygaena*. The transfer of a nuptial gift of cyanogenic glucosides during mating of *Zygaena* has been demonstrated as well as the involvement of hydrogen cyanide in male attraction and nitrogen metabolism. As more plant and arthropod species are examined, it is likely that cyanogenic glucosides are found to be more widespread than formerly thought and that cyanogenic glucosides are intricately involved in many key processes in the life cycle of plants and arthropods.

1. Introduction

Plants, herbivores and pathogens have co-evolved in a constant chemical warfare for about 430 million years. Plants are known to produce more than 300,000 different secondary metabolites (bioactive natural products) including the group of cyanogenic glucosides (CNglcs). CNglcs are widely distributed in the plant kingdom. More than 60 different CNglcs are known and they are present in more than 2500 plant species including ferns, gymnosperms and angiosperms (Bak et al., 2006; Conn, 1980; Møller and Seigler, 1999; Poulton, 1990).

CNglcs belong to those secondary metabolites which are classified as phytoanticipins. When a plant tissue containing CNglcs is disrupted, *e.g.* by herbivore attack, the CNglcs are brought into contact with degrading enzymes which cause release of toxic hydrogen cyanide (HCN), an aldehyde or ketone and glucose (Nahrstedt, 1996; Saunders and Conn, 1978; Vetter, 2000) (Fig. 1). This binary system provides plants with an immediate chemical defense response to herbivores and pathogens that cause tissue damage. Natural populations of plants vary greatly in their content of CNglcs (Zagrobelny et al., 2007a; Bjarnholt et al., 2008) and variation is also pronounced with respect to leaf age and environmental conditions (Miller et al., 2004; Goodger et al., 2004; Gleadow and Woodrow, 2002). Biosynthesis of CNglcs primarily occur in young and developing tissues (Halkier and Møller, 1989) and the CNglc levels found in older plant parts decrease because *de novo* biosynthesis typically proceeds at a lower rate than catabolic turnover or cannot keep up with the net gain in total biomass (Busk and Møller, 2002). This signifies, that a plant which is harmless to an herbivore under one set of conditions could be toxic or lethal under another, exemplified in *Sorghum* where young plants are highly toxic to grazing live stock, but becomes suitable for pasture as the plants mature (Boyd et al., 1938) and for Eucalyptus where the trait for cyanogenesis shows quantitative polymorphism (Neilson et al., 2006). In plants, CNglcs may also serve as storage compounds of sugar and reduced nitrogen (Sánchez-Pérez et al., 2008; Selmar et al., 1988) and remobilization does not necessarily involve the release of HCN (Jenrich et al., 2007). In agreement with a storage function, in some plant species the level of CNglcs increases with high nitrogen levels (Busk and

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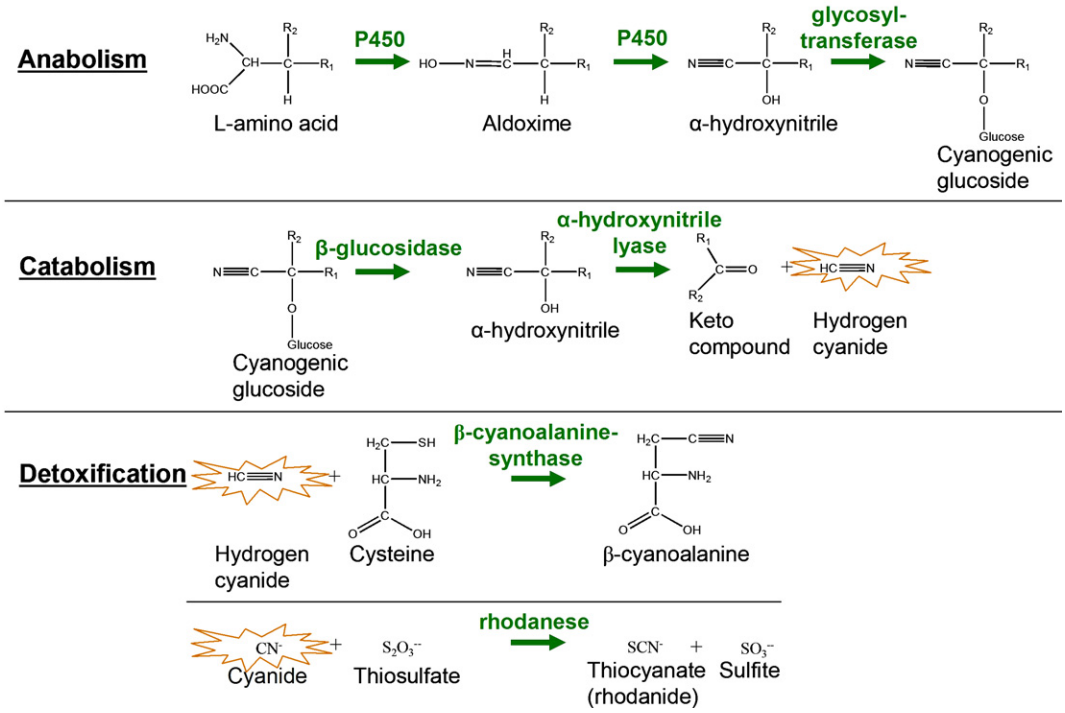


Fig. 1. Anabolism, catabolism and detoxification of CNglcs in plants, insects and animals. Enzymes involved are shown in green and HCN is highlighted in orange.

Møller, 2002). Cyanide is a toxic substance, mainly due to its affinity for the terminal cytochrome oxidase in the mitochondrial respiratory pathway (Brattsten et al., 1983). The lethal dose of cyanide for vertebrates lies in the range of 35–150 μmol/kg, if applied in a single dose. Much higher amounts of HCN can be tolerated if consumed or administered over a longer period, *e.g.* by the grazing of live stock (Davis and Nahrstedt, 1985).

CNglcs can act as feeding deterrents as well as phagostimulants towards herbivores, depending on the herbivore species feeding on the plant containing CNglcs. It appears that the prime deterrents are the carbonyl compounds that are released in equimolar amounts to HCN during cyanogenesis rather than HCN itself (Jones, 1988) (Fig. 1). Cyanogenesis is most effective against polyphagous herbivores (especially mollusks and some mammals) which casually encounter and make an attempt to eat cyanogenic plants. These herbivores can do enormous damage to plants in years when they are common (Jones, 1988). The co-evolution between plants, herbivores and pathogens have obviously allowed some insects and fungi to overcome the defense system based on CNglcs, either by their ability to transform the compounds into non-toxic constituents or by sequestration and further use in their own defense.

2. Metabolism of cyanogenic glucosides in plants

CNglcs are β-glucosides of α-hydroxynitriles derived from the aliphatic protein amino acids L-valine, L-isoleucine and L-leucine, from the aromatic amino acids L-phenylalanine and L-tyrosine or from the aliphatic non-protein amino acid 2-(2′-cyclopentenyl)-glycine. The biosynthesis of CNglcs has been investigated in several plant species and shown to follow a general pattern (Bak et al., 2006; Møller and Poulton, 1993; Møller and Seigler, 1999; Møller and Conn, 1980) (Fig. 1). The first committed enzyme in CNglc biosynthesis is a cytochrome P450 (Sibbesen et al., 1994), that catalyzes two sequential N-hydroxylations followed by a dehydration, a decarboxylation reaction and an isomerization reaction to produce the corresponding *Z*-aldoxime (Sibbesen et al., 1995; Halkier and Møller, 1990; Halkier et al., 1989). The *Z*-aldoxime is subsequently converted to an α-hydroxynitrile through an NADPH dependent dehydration reaction and C-hydroxylation catalyzed by a second cytochrome P450 (Bak et al., 1998; Kahn et al., 1997, 1999). The final step in CNglc biosynthesis is glycosylation of the cyanohydrin moiety and is catalyzed by a UDPG-dependent glycosyltransferase (Jones et al., 1999; Hansen et al., 2003; Thorsøe et al., 2005).

In plants, the biosynthesis of CNglcs was elucidated using *Sorghum bicolor* as a model system. *S. bicolor* contains the tyrosine-derived, aromatic CNglc dhurrin (Fig. 2) and its biosynthesis involves the P450s CYP79A1 and CYP71E1 and the UDPG-glycosyltransferase UGT85B1 (Bak et al., 1998; Jones et al., 1999; Sibbesen et al., 1994). Typically, the first committed step in a biosynthetic pathway is catalyzed by an enzyme with high substrate specificity (Kahn et al., 1999). This serves to limit the number of available substrates for subsequent enzymes in the same pathway and these enzymes may thus possess a wider substrate specificity that

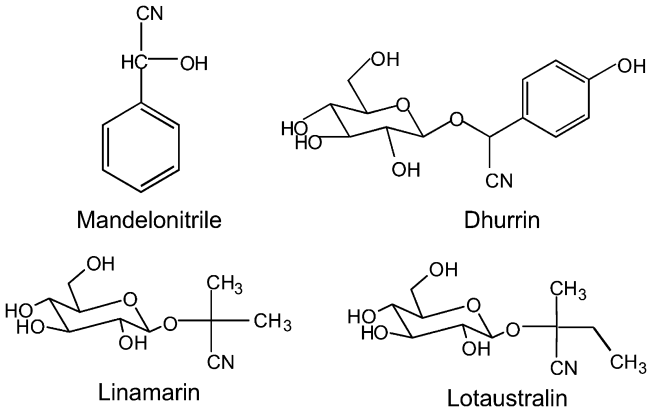


Fig. 2. Structures of the four most prominent aromatic and aliphatic cyanogenic constituents found in arthropods.

provides overall metabolic flexibility, yet desired specificity with a limited number of genes (Vogt and Jones, 2000). As predicted by this scheme, the CYP79A1 enzyme has been found to determine the substrate specificity of the CNglc metabolism in *S. bicolor* (Kahn et al., 1999). Phylogenetic analysis of CYP79 sequences from angiosperms show that the first committed step in CNglc biosynthesis in plants predates the segregation of angiosperms and gymnosperms (Bak et al., 2006; Werck-Reichhart and Feyereisen, 2000). In plants containing the aliphatic CNglcs linamarin and lotaustralin (Fig. 2), the relative amounts of the two CNglcs is largely determined by the preference of the CYP79 enzyme for either valine or isoleucine (the parent amino acids) as substrates. This is illustrated by the high linamarin/lotaustralin ratio in cassava (Andersen et al., 2000) and the low ratio in *Lotus japonicus* (Forslund et al., 2004). The Cassava CYP79D2 exhibit similar *in vitro* catalytic efficiencies towards valine and isoleucine while the Lotus ortholog CYP79D3 has a 6-fold higher affinity for isoleucine than for valine *in vitro* (Forslund et al., 2004).

The final step in the biosynthesis of CNglcs in plants is catalyzed by a Family 1 glycosyltransferase (Jones et al., 1999; Vogt and Jones, 2000). Family 1 glycosyltransferases are soluble proteins which utilize UDP-activated sugar moieties as the donor molecule to glycosylate acceptor molecules. Glycosyltransferases generally exhibit a low degree of overall sequence similarity, and are often regioselective or regiospecific rather than highly substrate specific (Hansen et al., 2003; Jones et al., 1999). Similar to cytochromes P450, Family 1 glycosyltransferases are encoded by a multigene family and are ubiquitously found in plants, animals, fungi, bacteria and viruses (Paquette et al., 2003).

The production of the CNglc dhurrin has been shown to be highly channeled in *Sorghum bicolor* (Møller and Conn, 1980) and evidence supports that the three enzymes involved in the pathway do indeed form a metabolon (Jørgensen et al., 2005b; Nielsen et al., 2008; Winkel, 2004). Metabolon formation serves to co-localize the enzymes, increases the concentrations of their substrates, facilitates swift delivery of the intermediates from one active site to the next, avoids escape of toxic intermediates, and reduces the risk of undesired metabolic cross-talk. Metabolon formation may also ensure a quick and precise response to environmental challenges (Jørgensen et al., 2005b; Nielsen et al., 2008; Hückelhoven, 2007). Metabolon formation was also observed after transfer of the pathway to *Arabidopsis thaliana*, a plant species which does not normally produce CNglcs but rather glucosinolates (Kristensen et al., 2005; Tattersall et al., 2001). The possibility of redirecting L-tyrosine into the glucosinolate or CNglc pathways without loss of plant fitness demonstrates the existence of routes for the transport and storage of new classes of natural products. This shows that plants contain an ability to redirect and optimize the flux of intermediates to counteract imbalances in primary and secondary metabolism (Jørgensen et al., 2005a; Tattersall et al., 2001).

Catabolism of CNglcs is initiated by enzymatic hydrolysis by a β-glucosidase to provide the corresponding α-hydroxynitrile, which spontaneously dissociates into a sugar, a keto compound, and HCN at pH values above 6 (Fig. 1). At lower pH values, the dissociation reaction is catalyzed by an α-hydroxynitrile lyase. β-Glucosidases generally have an acidic pH optima (pH 5–6) and an absolute specificity towards β-glucosides (Esen, 1993). Plant β-glucosidases involved in cleavage of CNglcs exhibit a high specificity towards the aglycone moiety of CNglcs present in the same plant species (Hösel et al., 1987; Hösel and Conn, 1982; Nahrstedt, 1985; Morant et al., 2008). α-Hydroxynitrile lyases have been characterized from a few plant species (Hu and Poulton, 1997, 1999; Wajant and Pfizenmaier, 1996). They appear to be located in the same tissues as the CNglc degrading β-glucosidases although their activity appear to be confined to protein bodies (Swain et al., 1992) instead of to chloroplasts or the apoplastic space as typical for β-glucosidases (Hickel et al., 1996).

HCN is detoxified by two main reactions (Conn, 1980). The first route involves the formation of the amino acid β-cyanoalanine from cysteine or serine and is catalyzed by β-cyanoalanine-synthase. β-Cyanoalanine synthase activity is generally found in plants (Miller and Conn, 1980) and plays a central role in detoxification of HCN released as a result of cleavage of CNglcs or formed in stoichiometric amounts with the plant hormone ethylene (Yip and Yang, 1988). β-Cyanoalanine synthase activity in plants is primarily located in mitochondria, the organelle that is most vulnerable to HCN toxicity (Meyers and Ahmad, 1991; Watanabe et al., 2008). In plants, β-cyanoalanine synthase has pyridoxal phosphate as a cofactor (Ikegami et al., 1988) and is a member of an ancestral family of β-substituted alanine synthases that also includes cysteine synthase (Ikegami and Murakoshi, 1994; Watanabe et al., 2008). Cysteine synthase also possesses β-cyanoalanine synthase activity and *vise versa*. β-Cyanoalanine is a potent neurotoxin and its accumulation in some plants may serve to deter predators (Ressler et al., 1969). β-Cyanoalanine is subsequently converted into asparagine or aspartate and ammonia by the action of nitrilases (Miller and Conn, 1980) In dicotyledonous plants, the nitrilases involved are homomers of the A type (Piotrowski and Volmer, 2006). In grasses, the nitrilases are 4A/4B type heteromers with the active site of the nitrilase complex located on the 4A isoform (Jenrich et al., 2007) The second route for cyanide detoxification proceeds by conversion of HCN into thiocyanate and is catalyzed by rhodanese (Lang, 1933) (Fig. 1). In contrast to β-cyanoalanine synthase, rhodanese is not universally present in plants and its *in vivo* function is not well understood. In species of higher animals, plants and insects where rhodanese is present, it plays a role in cyanide detoxification (Beesley et al., 1985). In plants, this assignment is supported by high levels of rhodanese activity in 3-day-old etiolated *Sorghum bicolor* seedlings (Miller and Conn, 1980), which has an exceptionally high cyanide potential (Halkier and Møller, 1989). Recent studies on catabolism of dhurrin in *S. bicolor* have provided evidence for the operation of an endogenous route that enable the plant to recover nitrogen stored in the CNglc without any prior release of HCN. The initial step in this proposed pathway is the conversion of dhurrin into *p*-hydroxyphenylacetonitrile. This involves modulation of the β-glucosidase involved in a similar manner as the myrosinase enzyme involved in glucosinolate degradation is modified by an epithio specifier protein (ESP) (Wittstock and Burow, 2007). Nitrilase heteromers composed of the NIT4A and NIT4B2 isoforms were shown to efficiently convert *p*-hydroxyphenylacetonitrile into *p*-hydroxyphenyl acetic acid and ammonia and the *p*-hydroxyphenyl acetic glucoside was shown to be present in etiolated *Sorghum* seedlings which actively degraded dhurrin (Halkier and Møller, 1989; Jenrich et al., 2007). This study provides evidence that CNglcs may serve as storage reservoirs for reduced nitrogen. Studies in almonds which produce the CNglc prunasin and the cyanogenic diglucoside amygdalin point in the same direction (Sánchez-Pérez et al., 2008). In the bitter almond genotypes, prunasin synthesized in the tegument is transported into the cotyledon *via* the transfer cells and converted into amygdalin in the developing almond seed giving rise to a bitter almond kernel. In the sweet genotypes, amygdalin formation is prevented because the prunasin is degraded upon passage of a β-glucosidase rich cell layer in the inner epidermis of the tegument. The prunasin turn-over may offer a buffer supply of ammonia, aspartic acid, and asparagine enabling the plants to balance the supply of nitrogen to the developing cotyledons. The bitter almond genotypes accumulating amygdalin in the cotyledons profit from the protection offered by this secondary metabolite towards herbivores and pests and retain the advantage of using the secondary metabolite as a buffer for primary metabolism. But in contrast to the situation in the sweet variety, this opportunity is

exploited when the amygdalin containing seed is ready to germinate and more than 80% of the amygdalin stored in the cotyledons is turned-over within a three week period (Swain and Poulton, 1994). The distinction between secondary and primary metabolism clearly vanishes when we begin to understand the intimate roles secondary metabolites play in plants.

3. Metabolism of cyanogenic glucosides in arthropods

In plants and insects, cytochromes P450 are encoded by some of the largest multigene families known. Their primary amino acid sequences are highly variant, while the tertiary and secondary structures of P450s are more well conserved (Paquette et al., 2000; Werck-Reichhart and Feyereisen, 2000; Morant et al., 2003). In insects, cytochromes P450 play crucial roles in defense against natural products that insects have to fend off in order to be able to feed on otherwise toxic plants. The ability of an insect cytochrome P450 to metabolize a specific natural product is often the key to the adaptation of insect herbivores to their food plants (Feyereisen, 1999). Insect genomes carry around a hundred P450s which fall into four groups (Feyereisen, 2005). CYP6, CYP9, CYP28, CYP308-310 and CYP321 constitute the first group, and many of the genes encoding the P450s belonging to this group may be characterized as environmental response genes. The genes are highly diverse, have proliferated by duplication events, show rapid rates of evolution, occur in gene clusters and have tissue- or developmental-specific expression. CYP4, CYP311-313, CYP316 and CYP325 belong to the second group of insect P450s, which again is highly diverse, with some of the genes encoding these P450s being induced by xenobiotics. The third group comprises the mitochondrial clade, CYP12. Some of the P450s in this group are linked to the ecdysteroid pathway. CYP15, CYP18 and CYP303-307 fall into the fourth group of insect P450s, some of which are involved in essential physiological functions like juvenile hormone biosynthesis and ecdysteroid catabolism. The functional evolution of insect P450s remains obscure. No clades are devoted to conversion of a single or a few types of substrate classes, and even closely related P450s may exhibit very different specificities. Currently, it is not possible to assign a function to an insect P450 gene based solely on phylogenetic analysis (Feyereisen, 2006). No glucosyltransferase involved in CNglc formation has yet been isolated from arthropods. Likewise, it is not known whether the biosynthetic pathway for CNglc biosynthesis is organized within a metabolon in insects. Metabolons have been demonstrated to be involved in melanin synthesis in insects (Sugumaran, 2002), the involvement of metabolon formation in arthropod cyanogenesis may thus also be likely.

In contrast to the well characterized β-glucosidases involved in CNglc catabolism in plants (Cicek et al., 2000; Cicek and Esen, 1998; Czjzek et al., 2000), only little is known about the insect β-glucosidases and their substrate specificity. A β-glucosidase with strong activity towards linamarin and lotaustralin was purified from the haemolymph of *Zygaena trifolii* (Zygaenoidea). The enzyme probably functions as a dimer and it has characteristics similar to plant β-glucosidases (Franzl et al., 1989). No α-hydroxynitrile lyases have been characterized in detail in insects. A partly purified α-hydroxynitrile lyase was reported from the haemolymph of *Z. trifolii* (Müller and Nahrstedt, 1990). Haemolymph is also the site of β-glucosidase activity in *Zygaena*, so the distribution of CNglc degrading enzymes is somewhat similar to plants. The enzyme is supposedly a dimer probably containing a flavin group and with high substrate affinity. However, the purification and characterization of the *Z. trifolii* α-hydroxynitrile lyase is not well documented.

β-Cyanoalanine synthase is present in many cyanogenic insects including Zygaenidae and is primarily located in the mitochondria. In *Heliconius melpomone* (Papilionoidea), β-cyanoalanine synthase is only present in feeding larval stages, corresponding to a theory of presence when in demand (Davis and Nahrstedt, 1985; Witthohn and Naumann, 1987). In insects, rhodanese cannot be directly linked to cyanide detoxification, because enzyme activity is absent from many insects including cyanogenic species. But a complete absence of rhodanese in insects would be unlikely, since rhodanese enzymes serve a variety of functions other than cyanide detoxification, the most important of which is to donate sulfur to proteins (Beesley et al., 1985; Bordo and Bork, 2002). The lability of rhodanese in crude insect homogenate suggests that some insect species examined would contain rhodanese although tested negative. Accordingly, the enzyme may also be present in Zygaenidae although previous attempts to demonstrate this were negative (Davis and Nahrstedt, 1985). The high toxicity of cyanide in vertebrates infer that rhodanese, which is present in almost all vertebrates, is not a very effective detoxification system for cyanide (Davis and Nahrstedt, 1985). The use of β-cyanoalanine synthase for cyanide detoxification in insect species may explain the proficiency in cyanide detoxification observed in many insects.

4. Presence and evolution of cyanogenic glucosides in arthropods

The presence of CNglcs in animals appear to be restricted to Arthropoda (Duffey, 1981), although many animal species produce or sequester other toxic secondary metabolites. As in plants, species of arthropods which contain CNglcs are thought to use the compounds for defense. CNglcs are found in species within Diplopoda (millipedes), Chilopoda (centipedes) and particularly within Insecta (Davis and Nahrstedt, 1985) (Fig. 3). Within Insecta, CNglcs have hitherto only been found in Coleoptera (beetles), Heteroptera (true bugs) as well as in Lepidoptera (butterflies and moths) (Nahrstedt, 1988). Diplopoda, Chilopoda and Coleoptera contain aromatic CNglcs while Lepidoptera contain aliphatic CNglcs (Fig. 3).

Contrary to plants, most of the data available on cyanogenic arthropods were obtained before 1980 using much less precise methods compared to those available today. Often the chemical structure of the constituent thought to give rise to cyanide formation was not determined. Furthermore, several extraction methods included the use of 5% NaOH (Casnati et al., 1963), *i.e.* experimental conditions where for example mandelonitrile (Fig. 2) would decompose, or drying at 105 °C for 2 h (Pallares, 1946) which would result in major loss or decomposition of most other constituents involved in cyanogenesis. In many cases it is also difficult to ascertain the factors contributing to cyanogenesis, because the cyanogenic secretions from the examined arthropods contain a mixture of HCN, benzaldehyde, mandelonitrile, mandelonitrile benzoate, benzoic acid, benzoyl cyanide, phenol, guaiacol and fatty acids (Conner et al., 1977; Duffey et al., 1977; Jones et al., 1976). Therefore, the entire field of cyanogenesis in arthropods would gain much from a reinvestigation of cyanogenic species using state-of-the-art methods. With these experimental limitations in mind, an overview of the present knowledge on cyanogenesis in arthropods is presented below.

4.1. Diplopoda

The production of HCN from polydesmoid millipedes has been known for more than 120 years. The HCN arises from the degradation of mandelonitrile (Guldensteeden-egeling, 1882; Weber, 1882). All polydesmoid millipedes so far examined are cyanogenic, and the best known is *Harpaphe haydeniana*. *H. haydeniana* stores mandelonitrile in oily droplets in dorsal storage chambers. When the millipede is alarmed, it increases the hydrostatic pressure in its haemolymph to squeeze mandelonitrile from the storage chambers through a reaction chamber, where it is mixed with degrading

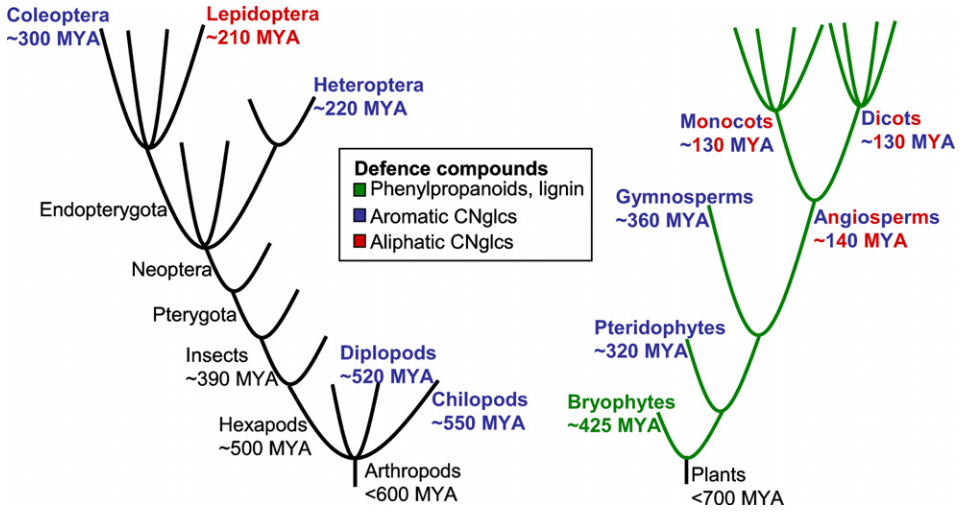


Fig. 3. Evolutionary trees depicting the evolution of arthropods and plants. Classes of defense compounds (phenylpropanoids, lignin, cyanogenic glucosides) present in different taxonomic groups are shown in color. Occurrence of aromatic and aliphatic cyanogenic glucosides are shown using blue and red letters, respectively. Ages of taxonomic groups are according to (Labandeira and Sepkoski, 1993; Regier et al., 2005; Willis and McElwain, 2002). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

enzymes, to the exterior (Duffey, 1981). The cyanogenic glands of *H. haydeniana* were shown to contain both β-glucosidase and α-hydroxynitrile lyase activity and *H. haydeniana* also contains β-cyanoalanine synthase and rhodanese for detoxification of the CNglc degradation products (Blum, 1981). *H. haydeniana* and *Oxidus gracilis* have been shown to possess biochemical pathways for CNglc biosynthesis which involve similar or identical intermediates to those known from higher plants (N-hydroxy amino acids, oximes, nitriles) (Duffey, 1981). *O. gracilis* produce HCN and benzaldehyde (Towers et al., 1972), probably representing biosynthesis from phenylalanine with mandelonitrile as the final stored product. *Apheloria corrugate* contains mandelonitrile which is stored separate from the degrading enzymes. The enzyme and substrate are only mixed after stimulation of the millipede which results in the production of benzaldehyde and HCN (Blum, 1981). *Polydesmus vicinus* stores *p*-isopropylmandelonitrile glucoside, *Polydesmus collaris* stores mandelonitrile benzoate and *Pachydesmus crassicutis* stores a mandelonitrile glucoside (Towers et al., 1972). HCN has been detected in the glandular secretions of the following millipedes: *Euryurus maculates*, *Cherokia georgiana ducilla*, *C. g. georgiana*, *C. g. latassa*, *Motyxia tularea*, *Sigmoria nantahalae* and *Pseudopolydesmus erasus* (Duffey et al., 1977). Benzoyl cyanide, mandelonitrile and benzaldehyde have been identified in the glandular exudates of the millipedes *Pseudopolydesmus serratus*, *A. corrugate* and *Apheloria trimaculata* (Conner et al., 1977). *P. serratus* additionally contain mandelonitrile benzoate, benzoic acid, isovaleric acid, myristic acid and stearic acid. Defense secretions from 17 polydesmoid milipeds were analyzed with GLC-MS (Duffey et al., 1977) and benzoyl cyanide and mandelonitrile benzoate together comprise up to 35% by weight of the secretion of the various millipedes (Conner et al., 1977; Duffey, 1981). Some of the compounds present in the defensive fluid also have antibiotic properties and could protect the millipede from attack by several types of fungi and bacteria (Duffey et al., 1977).

4.2. Chilopoda

Centipedes of the order Geophilomorpha produce a defensive secretion which in the case of *Geophillus vittatus* contains mandelonitrile, benzoyl cyanide, benzaldehyde and benzoic acid (Jones et al., 1976). The secretion is also used by females to guard their eggs. The centipede *Asanada* sp. has a defensive secretion containing HCN, an unknown carbonyl compound, and protein (Maschwitz et al., 1979).

4.3. Coleoptera

Larvae of the beetle *Paropsis atomaria* contain HCN in their defense secretions as well as benzaldehyde and glucose, which were probably at some stage derived from degradation of a CNglc. Since their food plants (*Eucalyptus blakelyi*, *Eucalyptus fastigata* and *Eucalyptus polyanthemos*) were shown to be acyanogenic in this study, these insects are probably able to *de novo* biosynthesize the compounds (Moore, 1967). *Megacephala virginica* contains mandelonitrile, HCN and benzaldehyde (Davis and Nahrstedt, 1985), with the latter two obviously being degradation products of the former.

It has been assumed that the ultimate cyanogen in arthropods (found in Chilopoda, Diplopoda and Coleoptera) is mandelonitrile (Fig. 2) (Witthohn and Naumann, 1987). Some researchers have also found glucosides in the defense secretions and have proposed mandelonitrile glucoside as the *in vivo* HCN storage form but other researchers found no trace of glucosides. The free mandelonitrile may represent a degradation product of a stabilized acylated form present in the insect which is degraded by esterases during the extraction procedure. The precise storage forms of HCN in Chilopoda, Diplopoda and Coleoptera remain an open question.

4.4. Heteroptera

The scentless plant bugs *Jadera haematoloma* and *Jadera sanguinolenta* emit HCN when reared on food plants containing cyanolipids but not when reared on acyanogenic plants (Aldrich et al., 1990). This demonstrates that this true bug is able to sequester the cyanogenic principle. *Leptocoris isolata* contains the CNglc cardiospermin which it most likely synthesizes from a cyanolipid sequestered from its food plant (Braekman et al., 1982; Davis and Nahrstedt, 1985).

4.5. Lepidoptera

Many aposematic Lepidoptera are strongly associated with poisonous plants and sequester toxic compounds from their food plant instead of or in addition to manufacturing their own (Nishida, 1994, 2002). Many non-toxic Lepidoptera mimic the toxic

species, to gain protection from predators. The thyridid caterpillar *Calindoea trifascialis* emits a defensive secretion which among its constituents contains mandelonitrile, benzaldehyde and benzoic acid and serves as defense against ants (Darling et al., 2001). *Malacosoma americanum* (Ditrysia) regurgitate a droplet of enteric fluid containing food plant derived HCN and benzaldehyde when attacked by predatory ants. Ants were shown to be repelled by the odor of benzaldehyde but not by HCN (Peterson et al., 1987). *M. americanum* feed on the cyanogenic *Prunus serotina*, which contains prunasin in its leaves, and preferentially feeds on young leaves, which contain more prunasin. Thus, *M. americanum* is envisioned to sequester the defense compounds from the food plant. *Seirarctia echo* (Arctiidae) sequesters the pseudo-CNglc cycasin from its Cycad food plants (Duffey, 1981). The glucoside is hydrolyzed in the gut and the aglycone diffuses into tissues where it is re-converted into cycasin which remains dissolved in the insect body fluids. Imagines of butterflies from the Heliconiinae, Acraeinae, Nymphalinae and Polyommatinae (Papilionoidea) groups (Fig. 4) accumulate linamarin and often lotaustralin at all life-stages (Brown and Francini, 1990; Nahrstedt, 1988; Nahrstedt and Davis, 1981, 1983). The accumulation of the compounds in these butterflies is not achieved by sequestering because linamarin and lotaustralin are absent from the food plants of the larvae (Passifloraceae) (Engler et al., 2000; Nahrstedt and Davis, 1983). Accordingly, the butterflies must *de novo* biosynthesize linamarin and lotaustralin from valine and isoleucine, respectively. The amount of linamarin is higher than that of lotaustralin in imagines as was also observed in imagines of Zygaenidae (Zagrobelny et al., 2007a), and the CNglcs have the same body distribution as observed in *Z. trifolii* (Davis and Nahrstedt, 1982; Franzl et al., 1986). The examined butterflies also contain monoglycosidic cyclopentenyl derived cyanogens, probably sequestered from their food plants at the larval stage (Engler et al., 2000). *Eutoptia hegesia* (Heliconiinae, Papilionoidea) has as the only insect species outside of the Zygaenoidea clade been suggested to *de novo* biosynthesize as well as sequester cyclopentenyl glycine derived CNglcs from its primary food plant *Turnera ulmifolia* (Schappert and Shore, 1999).

Detoxification of CNglcs involves the incorporation of HCN into β-cyanoalanine as catalyzed by β-cyanoalanine synthase. β-Cyanoalanine and in some cases β-cyanoalanine synthase have been found in the following species of Lepidoptera: Papilionidae, Pieridae, Lycaenidae, Hesperidae, Lymantriidae, Arctiidae, Notodontidae, Megalopygidae, Limacodidae, Cymatophoridae, Noctuidae, Geometridae, Yponomeutidae, Nymphalidae, Zygaenidae and Heterogynidae (Witthohn and Naumann, 1987) (Fig. 4). Taxonomic, geographical and seasonal variation in the amount of β-cyanoalanine has been found in a number of cases, as would be expected because the CNglc content typically varies with the same parameters. Some species from the abovementioned species groups do not contain CNglcs, and their defensive biology is based on other compounds. These species probably only produce β-cyanoalanine to detoxify ingested CNglcs.

The ability to utilize aromatic CNglcs for defense has evolved prior to the use of aliphatic CNglcs in both plants and insects (Fig. 3). Lepidoptera may have evolved the ability to use aliphatic CNglcs for defense purposes before plants, and species within Zygaenoidea which both sequester and biosynthesize aliphatic CNglcs must therefore have developed sequestering as a secondary ability to minimize the amount of energy allocated to biosynthesis of defense compounds.

5. Presence and evolution of cyanogenic glucosides in zygaenidae

At least 45 species of Zygaenidae (burnet moths, Fig. 4) contain the CNglcs linamarin and lotaustralin (Davis and Nahrstedt, 1982, 1985; Zagrobelny et al., 2004) which they are able to *de novo* biosynthesize from the parent amino acids valine and isoleucine (Nahrstedt, 1988) as well as to sequester from food plants (Fabaceae) (Zagrobelny et al., in press). The ability to both *de novo* bio-

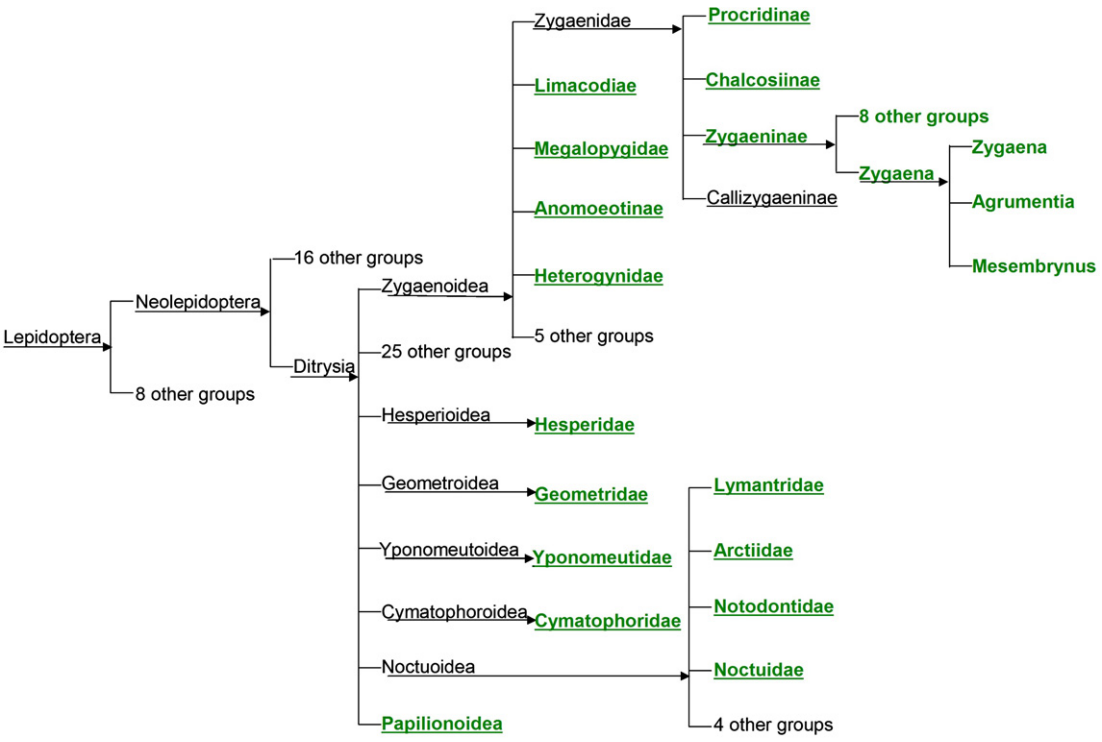


Fig. 4. Evolution of cyanogenesis within Lepidoptera. Groups containing cyanogenic glucosides are shown in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

synthesize and sequester the same CNglcs appear to be restricted to Lepidopteran species. The CNglcs linamarin and lotaustralin are the most abundant CNglcs in both plants and insects. They usually co-occur (Lechtenberg and Nahrstedt, 1999), which can be explained by the ability of the enzyme system involved to use both of the structurally related amino acids valine and isoleucine as substrates. Administration of the radiolabelled amino acids valine and isoleucine to *Zygaena* and *Heliconius* species, demonstrated preferential incorporation of linamarin compared to lotaustralin. This indicates that the biosynthetic enzymes for CNglc biosynthesis in *Zygaena* have a higher affinity for valine and intermediates derived from it than for isoleucine (Davis and Nahrstedt, 1987). This may explain why linamarin is the major CNglc in most tissues of *Zygaena filipendulae* larvae and imagines (Zagrobelny et al., 2007a). The 1:1 ratio in larval integument could reflect that the HCN resting potential of *Z. filipendulae* larvae is derived mainly from linamarin degradation. The higher amount of linamarin present in the most vulnerable stages (imagines, eggs and newly hatched larvae) could indicate that linamarin serve as a better deterrent of predators than lotaustralin. This could be achieved by a higher turn-over rate for lotaustralin in comparison to linamarin (Nahrstedt and Davis, 1983) during pupation, perhaps by recycling the nitrogen for the production of chitin. This is possible, *e.g.* if the β-glucosidases functioning in CNglc catabolism in *Z. filipendulae* have different affinity for the two substrates as observed, *e.g.* for the cyanogenic β-glucosidases in barley (Nielsen et al., 2002) and *L. japonicus* (Morant et al., 2008). Another explanation could be preferential biosynthesis of linamarin in the imagines. *De novo* biosynthesis was demonstrated in imagines of *H. melpomone* (butterfly) (Nahrstedt and Davis, 1983), but there is yet no evidence of this in imagines of *Zygaena*.

The Zygaenidae are brightly colored, diurnal moths with extensive individual and geographical variation. Zygaenid moths are capable of colonizing a great variety of natural and occasionally secondary habitats, from coastal dunes and cliffs and dry Mediterranean maqui to various arboreal habitats and even high alpine and extreme boreal regions. The larvae of zygaenid moths are usually oligophagous, *i.e.* they are restricted to a very small number of food plant species which usually belong to the same family, generic group, genus or even species group. Larvae of the primitive (nonpalaearctic) genera of Zyganinae (Fig. 4) feed on Celestraceae. The principal plant families associated with the genus *Zygaena* is the Fabaceae, Apiaceae, Asteraceae and Lamiaceae (Naumann et al., 1999). For zygaenids, orientation and localization of a food plant from a distance probably involves visual and olfactory senses, whereas testing the suitability of a plant at close range is mainly based on gustation and mechanoreception (Bernays et al., 1977). Adult females are able to distinguish between the larval food plant, *e.g. Lotus corniculatus* for *Z. filipendulae*, and other plants on the level of olfactory perception, while larvae may be using contact chemoreception to identify their proper food plant (Bernays et al., 1977). Specific odor components arise from the breakdown of secondary plant substances, so CNglcs or by-products thereof might be involved in food plant recognition. It is well established that ovipositing females rely on chemotactile stimuli evoked by plant secondary metabolites as cues to assess the suitability of potential food plants they land on (Nishida, 2005). *Z. filipendulae* larvae prefer to feed on highly cyanogenic *L. corniculatus* over low cyanogenic or acyanogenic plants probably to optimize the amount of CNglcs available for sequestering. *L. japonicus* and cyanogenic *L. corniculatus* contain about the same amount of CNglcs, but larvae develop faster and to a larger size on the latter food plant (Zagrobelny et al., 2007a). In spite of this, the *Z. filipendulae* larvae prefer *L. japonicus* over *L. corniculatus* in laboratory free choice feeding trials but the cue was diluted to insignificant levels in field trials (Zagrobelny et al., in press). The nature of the feeding cue detected in the laboratory experiments is not known but it is different from linamarin, lotaustralin or rhodiocyanosides. The latter are present only in *L. japonicus*.

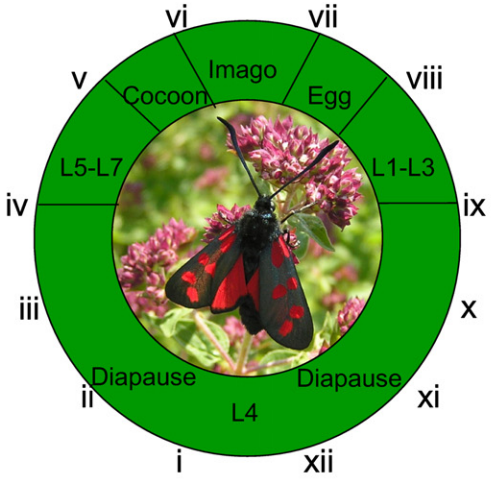
All instars of Zygaenidae release HCN by enzymatic breakdown of CNglcs and are extremely resistant to cyanide. As a defensive reaction against predators (shrews, hedgehogs, starlings, frogs and carabid beetles), larvae of Zygaeninae species release highly viscous, colorless fluid droplets from cuticular cavities placed on their dorsal side. Droplets appear on the cuticular surface upon contraction of irritated segments (Franzl and Naumann, 1985). Defense droplets may be reabsorbed a few seconds after irritation has stopped. In contrast to most diplopods and chilopods that have specialized cyanogenic glands (Duffey, 1981), there are no gland cells or cuticular ducts leading through the cuticle into the cavities in *Zygaena* larva, and no special morphological adaptation for secretion has been developed in the epidermis (Franzl and Naumann, 1985). Defense droplets contain CNglcs, proteins and water (Witthohn and Naumann, 1984) with a ratio of linamarin:lotaustralin from 1:1 to 1:2 (Zagrobelny et al., 2007a). Droplets do not contain β-glucosidase for breakdown of the CNglcs, but CNglcs are in themselves bad-tasting and could deter predators. If the presence of defense droplets does not deter the predator and the larva is damaged or eaten, haemolymph containing β-glucosidase and α-hydronynitrile lyases will be released resulting in CNglc degradation and HCN release. CNglcs are transported from the cuticular cavities into the body before the molt and transported back into newly formed cuticular cavities after molting (Franzl et al., 1988; Zagrobelny et al., 2007a).

The *Zygaena* imago does not contain cuticular cavities but retain CNglcs from their larval stage. Histamine, acetylcholine and 2-methoxy-3-alkylpyrazines have also been detected in adult *Zygaena lonicerae* and *Z. filipendulae* (Rothschild et al., 1984; Tremewan, 2006). When an adult *Zygaena* is attacked by a predator it secretes a fluid smelling of pyrazines from around the mouthparts and haemolymph smelling of HCN from the legs. Pyrazines appear to function as a general warning signal in many insect species (Nishida, 2002).

Z. trifolii is able to biosynthesize and degrade the two CNglcs linamarin and lotaustralin using intermediates similar to those identified in plants (Davis and Nahrstedt, 1987; Holzkamp and Nahrstedt, 1994; Wray et al., 1983). Accordingly, the insect enzyme systems involved in these processes may have similar characteristics as those from plants. Activity towards 2-methylbutanal-oxime (derived from isoleucine) was detected from microsomes from the integument of *Z. filipendulae* L7 larvae (Zagrobelny et al., 2007b). The activity was NADPH-dependent and is likely derived from a membrane-bound P450 enzyme catalyzing the second step in CNglc biosynthesis (Fig. 1). This indicates that CNglcs are produced in the larval integument and transported to other tissues. It agrees well with the fact that most CNglcs (75%) are present in the integument of L7 larvae (Zagrobelny et al., 2007a). In contrast to the situation in plants, the enzymes involved in the degradation of CNglcs in *Zygaena* as well as the CNglcs are localized in the same compartment, the haemolymph. The degradative enzymes have very low activity at the neutral pH of the haemolymph. It has been hypothesized that this points to a situation where upon ingestion, the stomach acid from a predator will activate the enzymes leading to a rapid and strong release of HCN. Zygaenid moths contain β-cyanoalanine synthase which catalyzes formation of β-cyanoalanine from cystein and HCN (Witthohn and Naumann, 1987). β-Cyanoalanine may possibly be recycled into protein metabolism by transformation into L-asparagine or L-aspartate.

In the *Z. filipendulae* life cycle, content and ratio of CNglcs are tightly regulated with a 1:1 ratio of linamarin:lotaustralin in L4–L7 larval stages and at least a 2:1 ratio in subsequent stages, rather

A



B

	Egg	L1	L4	L7	Female Imago	Male Imago	Empty eggshell	Exuviae	Empty Cocoon	Haemolymph	Defensive fluid
Linamarin	7±5	4.6±2.5	73±25	2200 ±800	1500 ±660	870 ±320	0.12±0.12	2.1±1.1	38±18	560±280	80±50
Lotaustralin	2.1±1.6	1.8±0.3	70±25	2800 ±1400	620 ±270	200 ±120	0.02±0.02	3.2±2.4	58±37	200±160	170±100
Total CNglcs	9±3	6.4±2.4	150±40	5100 ±2000	2100 ±880	1100 ±340	0.14±0.14	5.5±3.8	96±48	780±400	290±140

Fig. 5. CNglcs in the *Z. filipendulae* life cycle. (A) Overview of the *Z. filipendulae* life cycle. (B) Content of CNglcs in *Z. filipendulae* life stages and tissues in μg pr. individual.

independently of the CNglc-composition of the food plant (Zagrobelny et al., 2007a) (Fig. 5). Larvae are in part able to compensate for the level and composition of CNglcs in their food plants by *de novo* biosynthesis of the lacking components. However, larval sequestration of CNglcs from the food plant does play the dominant role in the overall acquisition of CNglcs and *de novo* biosynthesis does not allow the larvae to fully adjust. When *Zygaena* larvae are feeding on acyanogenic plants and therefore forced to acquire all of their CNglcs by *de novo* biosynthesis, the energy spent on CNglc synthesis reduces the amount of energy left for growth and development (Zagrobelny et al., 2007a). *Z. filipendulae* change the linamarin:lotaustralin ratio from roughly 1:1 in the last larval instar to 2:1 in the imago (Fig. 5). A ratio of linamarin and lotaustralin of 2:1 was also observed in imagines of *Zygaena transalpina*, *Zygaena purpuralis* and *Z. lonicerae* (Zagrobelny, unpublished results), indicating that this is the generally observed ratio at least in *Zygaena*, implying that a lot of CNglcs have been broken down during pupation. The transition from larvae to imago in *Zygaena* proceeds concomitant with biosynthesis of chitin which is a nitrogen containing polymer. The nitrogen for chitin biosynthesis may be acquired by rapid turn-over of the CNglcs stored in the larvae permitting remobilization of reduced nitrogen and explaining the 55% loss of CNglcs during this transition. The change in the ratio of CNglcs during the *Zygaena* life cycle could reflect lotaustralin being more easily degraded than linamarin or linamarin acting as a better defense compound than lotaustralin in the more vulnerable stages (imagines and eggs).

The large differences in CNglc content between virgin and mated *Z. filipendulae* male and female imagines indicate that a nuptial gift of CNglcs is being transferred from the male to the female during mating (Zagrobelny et al., 2007b) (Fig. 5). The female probably use this gift for her own defense and to protect her eggs. Transfer of nuptial gifts of pyrrolizidine alkaloids in *Utetheisa ornatrix* has been shown to serve these purposes (Rossini et al., 2001). In laboratory experiments, females refused to mate with males with a low content of CNglcs (Zagrobelny et al., 2007b), indicating that the females are able somehow to assess how much CNglc the male possesses and would be able to contribute to her. A resting potential of HCN, 19-fold higher in female imagines than in male imagines, indicates that HCN may serve an additional role in male attraction in *Z. filipendulae* (Zagrobelny et al., 2007b). Defense purposes are certainly only one of several functions CNglcs have acquired in the *Zygaena* life cycle and this may also hold true for other arthropods. In many arthropods, defense compounds serve an additional function as pheromones, possibly to conserve energy (Blum, 1981; Eisner et al., 2002), so the notion of HCN serving as a pheromone in *Zygaena* is likely. If the female use HCN, acetone and/or 2-butanone to attract males, then she will have even more use for the nuptial gift of CNglcs offered her during mating, especially if she mates more than once, which *Z. filipendulae* imagines have occasionally been observed to do. In conclusion, the ratio and content of CNglcs could be equally important for defense, nitrogen metabolism, mate attraction, and as a nuptial gift, which of course render a tight regulation of ratio and content important.

The Zygaenidae form part of the lepidopterous superfamily Zygaenoidea which includes 10 families (Naumann et al., 1999) (Fig. 4). The ability to biosynthesize linamarin and lotaustralin has been used as an evolutionary character of the Zygaenidae (Niehuis et al., 2006b), although the Callizygaeninae has not been tested for presence or absence of CNglcs. Furthermore, this characteristic is present in other groups within the Zygaenoidea (Fig. 4). Cuticular cavities are present in both the Zygaeninae and Pryeria, both groups within the Zygaenidae (Naumann and Feist, 1987). According to the current idea of the phylogeny of Zygaenidae, cuticular cavities are a basic trait of the Zygaenidae which subsequently has been lost in some species of Procridinae and Chalcosiinae (Niehuis et al., 2006b). Cuticular cavities could even be part of the ground plan of the superfamily Zygaenoidea, since defense droplets have been found in larvae of Dalceridae, Lacturidae and Limacodidae (Niehuis et al., 2006b). Dalceridae and Lacturidae

are groups within Zygaenoidea where CNglcs have not been found. This implies that the defense system based on the emerging of droplets charged with toxic or bad-tasting compounds was present even before Zygaeninae began to biosynthesize CNglcs for use in defense. *Zygaena* is split into three subgenera: *Zygaena* (26 species), *Mesembrynus* (35 species) and *Agrumentia* (37 species) (Fig. 4). *Mesembrynus* larvae feed on acyanogenic plants while *Zygaena* and *Agrumentia* both feed on cyanogenic plants. Since *Zygaena* and *Agrumentia* are not a monophyletic group with respect to *Mesembrynus*, the ancestor of all three groups probably fed on cyanogenic plants while an ancestor of species from the *Mesembrynus* group later switched back to acyanogenic plants (Niehuis et al., 2006a). The ability of Zygaenidae species to detoxify HCN (Witthohn and Naumann, 1987) and to carry out *de novo* biosynthesis of CNglcs (Davis and Nahrstedt, 1987) is probably a basic characteristic of Zygaenidae. The ability to detoxify HCN would have enabled many species to initiate feeding on cyanogenic plants, while the ability to *de novo* biosynthesize CNglcs would have rendered Zygaenidae unpalatable to many predators. This would have enabled ancestors of Zygaenidae to colonize a new niche of food plants largely free of competition and to escape most of their predators. The ability to sequester CNglcs from the food plant may also turn out to be an essential and common old trait, which enabled *Zygaena* species to optimize their supply of CNglcs. In Chrysomelid beetles, *de novo* biosynthesis of chemicals for defense is a primitive state while sequestration of chemicals from the food plant appears to be a subsequently acquired character (Pasteels et al., 1990). Since the Lepidopteran radiation happened much earlier than the radiation of their current food plants, the angiosperms (Fig. 3) (Labandeira et al., 1994), most instances of biosynthesis of defense compounds probably evolved before occurrences of plant product sequestration.

6. Discussion

In many instances arthropods sequester their defense compounds or precursors for their defense compounds from food plants (Blum, 1981; Roth and Eisner, 1962) thereby minimizing the energy spent for defense. But many arthropods probably evolved a strategy for detoxifying the toxic food plant compounds first, thereby avoiding competition from other herbivores and providing a separate niche in the environment. When the arthropod had adapted to feeding on the toxic plant, the foundation would be laid for the invention of a pathway for sequestration of the toxic compounds from the food plant for utilization in their own defense. Arthropods who biosynthesize their defense compounds spend more energy on defense than arthropods who sequester their defense compounds from food plants, but they also have better control of the amount of toxic compounds they contain in their bodies. The pathway for biosynthesis of CNglcs in Arthropods has most likely been assembled by recruitment of enzymes which through evolutionary changes most easily could be adapted to acquire the required catalytic properties for CNglc biosynthesis. The presence of CNglcs in a wide range of arthropods indicates that these compounds are fairly "manageable" compounds which in many instances provide an effective defense.

The reason why CNglcs are so widespread among plants may reflect that their biosynthesis involves only three enzymes, one or two enzymes for degradation and one enzyme for detoxification. The enzymes working in CNglc metabolism in arthropods are unknown except for β-glucosidases and α-hydroxynitrile lyases from *Zygaena* and a few millipedes. In both groups it has been suggested that the entire pathway for CNglc biosynthesis is largely similar to that known from plants. Since cyanogenesis is present in species of arthropods widely separated by evolution, it is likely that the enzymes involved in CNglc metabolism have been invented independently more than once. Another scenario could be horizontal gene transfer between food plants and arthropods, but since many cyanogenic arthropods do not feed on cyanogenic plants this scenario is unlikely. The most likely explanation is that the biosynthesis of CNglcs follows one particular route and that enzymes are then recruited to perform according to this pathway (convergent evolution). If, as suggested, Zygaenidae and millipedes have recruited the same types of enzymes as plants use in their CNglc metabolism, then it is feasible that other groups of arthropods did the same. Since two P450s and a glycosyl transferase have been shown to operate in the biosynthesis of CNglcs in plants, similar enzymes could also be recruited for biosynthesis in arthropods. P450s and glycosyltransferases constitute very large and versatile gene families and it is easy to envision organisms recruiting genes from these families for developing new defense compounds. One P450 has already been linked to the biosynthesis of CNglcs in *Zygaena* (Zagrobelny et al., 2007b) making this theory even more plausible. It would be very interesting to identify and characterize the enzymes responsible for CNglc metabolism in different arthropods to test this hypothesis.

Multienzyme complexes, *i.e.* metabolons improve catalytic efficiency by bringing co-operating active sites into close proximity. Metabolon formation may also prevent undesired metabolic crosstalk (Jørgensen et al., 2005b; Nielsen et al., 2008). Metabolons additionally provide possibilities for swift redirection of metabolism by the formations of new metabolons that have altered enzyme composition and product output as might be demanded by environmental challenges. Because of these advantages it could be hypothesized that arthropods may also favor metabolon assembly of enzymes functioning in, *e.g.* CNglc biosynthesis.

7. Conclusion and perspectives

CNglcs are important defense compounds in many plants and arthropods. As more plant and arthropod species are being examined, it may be discovered that CNglcs are even more widespread than formerly thought. Defense is only one of many roles of the compounds. It is reasonable to think that when a plant or an insect has developed a mechanism to produce and store/sequester defense compounds, the organism would try to take advantage of the compounds in as many ways as possible to conserve energy and not having to produce additional compounds. In arthropods this could for example be as pheromones if the compounds or degradation products thereof happened to be volatiles. It could also be as nuptial gifts to ensure better protection of the offspring. Furthermore CNglcs, which are nitrogen containing, have the unique ability to take part in the nitrogen metabolism of both plants and insects. Another aspect is the ongoing arms race between insects and predators and/or parasitoids. Defense compounds often become antiquated after a while as many predators and parasitoids learn to overcome them. If the defense compounds have acquired new roles in the life cycle of the plant as well as of the insect, elaborate systems of biosynthesis, sequestration and detoxification of the defense compounds will not become obsolete. Compounds that initially served defense purposes may now acquire new functions in host-insect recognition or be recruited as storage compounds that are mobilized when needed to counteract imbalances in primary metabolism. Thus as we learn more about the intricate functions of secondary metabolites in plants and arthropods, the distinction between primary and secondary metabolites is vanishing and becoming meaningless.

Since the ability of *de novo* biosynthesis of CNglcs was developed independently in arthropods and plants and even multiple times in arthropods at very different time points, it is peculiar that the pathways for synthesis and catabolism seem to be so similar at the global level. This indicates that the universal route by which

CNglcs appear to be produced enables easy recruitment of the required enzyme activities. The enzymes involved in CNglc biosynthesis and degradation in arthropods are largely unknown, and a numerous questions concerning the roles of CNglcs could be answered if more knowledge was gained within this area.

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