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Review

Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Secondary metabolites, at least the major ones present in a plant, apparently function as defence (against herbivores, microbes, viruses or competing plants) and signal compounds (to attract pollinating or seed dispersing animals). They are thus important for the plant's survival and reproductive fitness. Secondary metabolites therefore represent adaptive characters that have been subjected to natural selection during evolution. Molecular phylogenies of the Fabaceae, Solanaceae and Lamiaceae were reconstructed and employed as a framework to map and to interpret the distribution of some major defence compounds that are typical for the respective plant families; quinolizidine alkaloids and non-protein amino acids for legumes; tropane and steroidal alkaloids for Solanaceae, and iridoids and essential oils for labiates. The distribution of the respective compounds appears to be almost mutually exclusive in the families studied, implying a strong phylogenetic and ecological component. However, on a closer look, remarkable exceptions can be observed, in that certain metabolites are absent (or present) in a given taxon, although all the neighbouring and ancestral taxa express (or do not express, respectively) the particular trait. It is argued that these patterns might reflect differential expression of the corresponding genes that have evolved earlier in plant evolution. The inconsistent secondary metabolite profiles mean that the systematic value of chemical characters becomes a matter of interpretation in the same way as traditional morphological markers. Thus, the distribution of secondary metabolites has some value for taxonomy but their occurrence apparently reflects adaptations and particular life strategies embedded in a given phylogenetic framework.

Keywords: Secondary metabolites; Ecological function; Defence; Attraction; Molecular phylogeny; Fabaceae; Lamiaceae; Solanaceae; Chemotaxonomy

Contents

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1. The production of secondary metabolites in plants: an ecological and evolutionary perspective

1.1. Defence and signal molecules

Secondary metabolites (SM) are present in all higher plants, usually in a high structural diversity (Table 1). As a rule, a single group of SM dominates within a given taxon. A few major compounds are often accompanied by several derivatives and minor components. Altogether, the pattern of SM in a given plant is complex; it changes in a tissue- and organ specific way; regularly, differences can be seen between different developmental stages (e.g., organs important for survival and reproduction have the highest and most potent SM), between individuals and populations. SM can be present in the plant in an active state or as a "prodrug" that becomes activated upon wounding, infection or in the body of a herbivore (Table 2). The biosynthesis of some SM is induced upon wounding or infection and SM are made de novo ("phytoalexins").

The high structural diversity of plant secondary metabolites (Table 1) has puzzled botanist and natural product chemists for some time. More than 100 years ago Ernst Stahl (Jena, Germany) had shown experimentally (Stahl, 1888) that secondary metabolites serve as defence compounds against snails and other herbivores. The corresponding defence hypothesis was not accepted by most botanists at that time because most of them were not convinced of evolution and adaptive explanations. Botanists preferred the simpler interpretation that secondary metabolites were waste products of primary metabolism and that structural diversity would only reflect a play of nature. Today, adaptive explanations are more favoured again to explain the existence and diversity of secondary metabolites (overviews in Levin, 1976; Swain, 1977; Waterman and Mole, 1989; Rosenthal and Berenbaum, 1991, 1992; Harborne, 1993; Bernays and Chapman, 1994; Roberts and Wink, 1998; Wink, 1988, 1993a,b, 1999a,b).

Being sessile organisms plants cannot run away when they are attacked by snails, insects or vertebrate herbivores, nor can they rely on an immune system when challenged by bacteria, fungi or viruses. Since herbivores and microbes were already present when the evolution of angiosperms started about 140 million years ago, plants had to evolve survival strategies very

Table 1
Structural diversity of plant secondary metabolites

Number of natural products	
With nitrogen	
•Alkaloids (1)	12,000
•Non-protein amino acids (2)	700
•Amines (3)	100
•Cyanogenic glycosides (4)	60
•Glucosinolates (5)	100
•Alkamides	150
Without nitrogen	
•Monoterpenes (incl. Iridoids) (6)	2,500
•Sesquiterpenes (7)	5,000
•Diterpenes (8)	2,500
•Triterpenes, Saponins, Steroids (9)	5,000
•Tetraterpenes	500
•Phenylpropanoids, coumarins, lignans	2,000
•Flavonoids (10)	4,000
•Polyacetylenes, fatty acids, waxes (11)	1,000
•Polyketides (12)	750
•Carbohydrates	>200

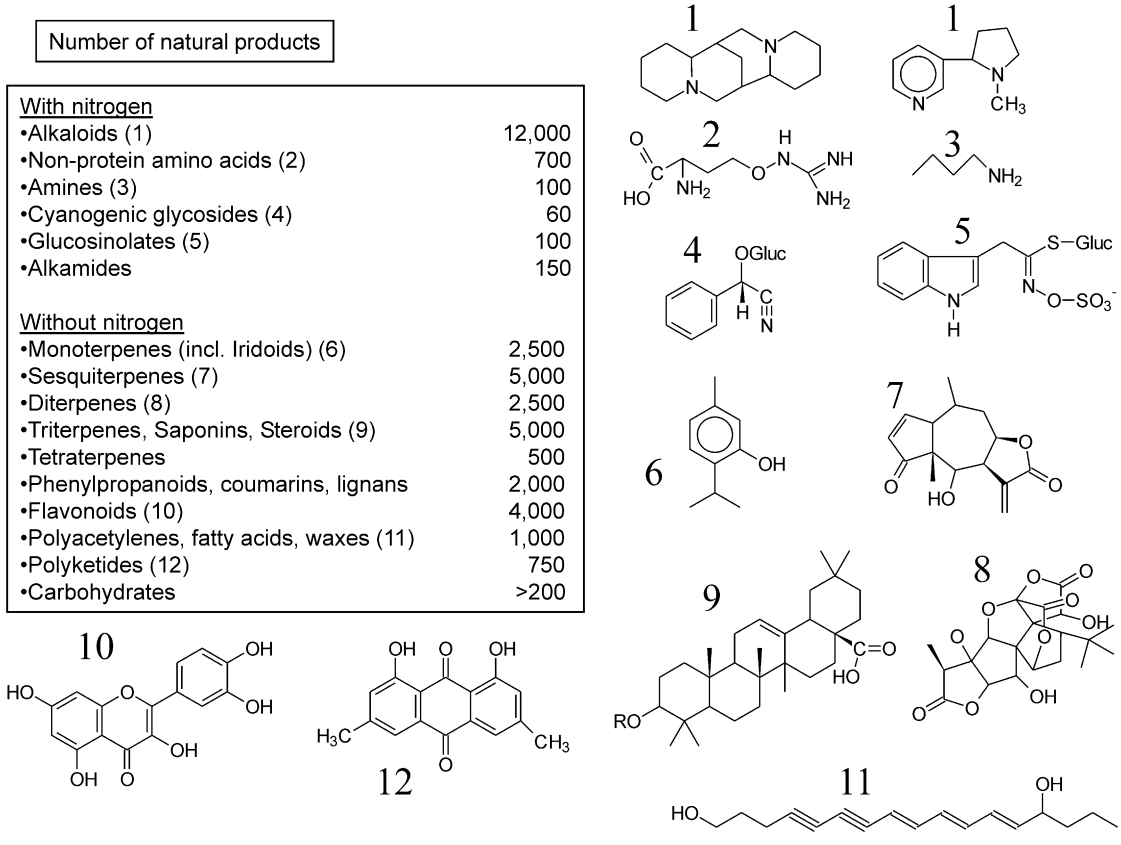

Table 2
Typical "prodrugs" present in plants that are activated after wounding, infection or metabolically in a herbivore

SM of undamaged tissue	Active allelochemical
Cyanogenic glycoside	HCN
Glucosinolate	Isothiocyanate
Alliin	Allicin
Coumaroylglycoside	Coumarin
Arbutin	Naphthoquinone
Bi-desmosidic saponines	Mono-desmosidic saponins
Cycasin	Methylazoxymethanol (MAM)[a]
Aristolochic acid	Nitrenium ion of aristolochic acid[a]
Pyrrolizidine alkaloids	Mono- and bifunctional pyrrolic intermediates[a]
Salicin	Salicylic acid[a]
Methylsalicylates	Salicylic acid[a]
Ranunculin	Protoanemonin

[a] Activation in animal liver.

early in their history. In common with other sessile or slow moving organisms (amphibians, marine sponges, corals, nudibranchs, and others) plants have evolved defence chemicals to ward off, inhibit or kill their enemies. Some insects, often aposematically coloured, either produce toxins themselves or sequester them from their host plants. Zoologists have never doubted that these compounds serve for chemical defence against predators. A large body of experimental evidence supports the view that many alkaloids, cyanogenic glycosides, glucosinolates, terpenes, saponins, tannins, anthraquinones, and polyacetylenes are allelochemicals. They represent adaptive traits that have diversified during evolution by natural selection in order to protect against viruses, bacteria, fungi, competing plants and most importantly against herbivores.

However, plants often need animals for pollination or seed dispersal. In this case SM can serve to attract animals (fragrant monoterpenes; coloured anthocyanins or carotenoids in flowers). To reward these animals, the plant provides nectar or nutritious fruit pulp. Often immature fruits are rich in toxic SM, whereas ripe fruits are digestible. However, plants need to control bacteria and fungi, that could easily live on nectar and pulp; this would explain the presence of SM in nectar and pulp (often phenolic compounds, tannins, essential oils, saponins), which prevents that plant material start to rot inappropriately. In several instances attractant and defensive activities are exhibited by the same compounds: anthocyanins or monoterpenes can be insect attractants in flowers, but are insecticidal and antimicrobial at the same time.

In addition, some secondary metabolites concomitantly carry out physiological functions, for example alkaloids, NPAAs, and peptides (lectins, protease inhibitors) can serve as mobile and toxic nitrogen transport and storage compounds or phenolics, such as flavonoids, can function as UV-protectants (Harborne, 1993; Wink, 1988, 1999a,b).

The observed multiple functions are typical and do not contradict the main role of many secondary metabolites as chemical defence and signal compounds. If a costly trait can serve multiple functions (and the maintenance of the biochemical machinery to produce and store SM is energetically costly; Wink 1999a), it is more likely that it is maintained by natural selection.

1.2. Allelochemical properties of secondary metabolites

Allelochemicals can only function as chemical defence compounds if they are able to influence molecular targets in herbivores or microbes in a negative way. Molecular targets range from proteins, nucleic acids, biomembranes to metabolites that are widely present in herbivores and microbes (for review see Wink and Schimmer, 1999; Wink, 2000). Despite the structural diversity of SM, a few structure-function relationships are apparent. A distinction can be made between specific and unspecific interactions.

Several specific interactions of SM with proteins (enzymes, receptors, ion-channels, structural proteins) and other cellular components have been discovered (review see Harborne, 1993; Wink and Schimmer, 1999; Wink, 1993b, 2000; Wink et al., 1998). Structures of these allelochemicals appear to have been shaped during evolution in such a way that they can mimic the structures of endogenous substrates, hormones, neurotransmitters or other ligands. This process can be termed "evolutionary molecular modelling" in analogy to the molecular modelling of modern science. For example, several alkaloids form a quaternary nitrogen configuration under physiological conditions, thus showing a structural motif present in most neurotransmitters. Not surprisingly, many alkaloids are agonists or antagonists of neurotransmitters and neuroreceptors (review in Wink and Schimmer, 1999; Wink, 1993b, 2000). These allelochemicals are useful for plants against most vertebrates, since the elements of

neuronal signalling pathways are quite similar throughout the animal kingdom. These inhibitors, usually do not help against microbes or competing plants. They have the advantage, that corresponding molecular targets are not present in the plant producing these compounds; by this strategy, an autotoxicity is avoided. Other examples for specific interactions are cardiac glycosides (inhibiting Na^+, K^+-ATPase), cyanogenic glycosides (HCN blocking cytochrome oxidase in respiratory chain), or salicylates (inhibiting cyclooxygenase and consequently prostaglandin formation).

Planar and lipophilic SM (often tri- or tetra-cyclic systems) can intercalate DNA (e.g. alkaloids, such as emetine, sanguinarine, berberine or quinine; and several furanocoumarins); other SM with reactive functional groups can alkylate DNA (e.g. pyrrolizidine alkaloids, cycasin, aristolochic acid) resulting in disturbance of replication and transcription and finally in frameshift and other mutations.

Mustard oil, quinones, allicin, protoanemonine, furanocoumarins, thiophenes, polyacetylenes and several sesquiterpene lactones have chemically reactive substituents that can form covalent bonds with proteins under physiological conditions and consequently alter their bioactivity.

Widely distributed are SM (such as phenolics, terpenoids, saponins) that affect molecular targets in animals and microbes in a more unspecific way. Tannins and other phenolics have a large number of phenolic hydroxyl groups that can form multiple hydrogen and ionic bonds with all sorts of proteins. Proteins (enzymes, transporters, ion-channels, receptors, cytoskeletal and structural proteins) change their conformation when a tannin–protein complex is formed and therefore lose their activity and function. Lipophilic terpenes (volatile mono- and sesqui-terpenes, etc.) are soluble in biomembranes. At higher concentration, they influence the environment of membrane proteins (ion channels, transporters, receptors) and thus change their conformation and bioactivity.

Saponins (especially the mono-desmosidic form that is generated upon tissue breakdown) are amphiphilic compounds that strongly interact with biomembranes. They can form pores in membranes and thus make cells leaky; a broad cytotoxic or antimicrobial effect is usually the consequence. A disturbance of membrane permeability can also be caused by lipophilic mono-, sesqui- and di-terpenes, if membranes are exposed to them at higher concentrations.

SM often contain more than one functional group; they therefore often exhibit multiple functionalities and bioactivities. Furthermore, since SM are present in the complex mixtures, consisting of several structural types, these strategies guarantee an interference with more than one molecular target in herbivores and microbes and can thus protect against a wide variety of enemies. Even if the individual interaction of a particular SM might be unspecific and weak, the sum of all interactions leads to a substantial effect.

In conclusion, experimental and circumstantial evidence support the assumption that many of the major SM present in a given plant are important for the fitness of the plant producing them (e.g. as defence or as signal compounds). SM must therefore be regarded as adaptive traits that have been shaped and modified by natural selection during evolution. This finding should have implications for using the distribution of SM as a taxonomic marker. This topic will be analysed and discussed in the second part of this contribution.

2. Occurrence of secondary metabolites: a molecular phylogenetic perspective

2.1. Distribution patterns of secondary metabolites and chemotaxonomy

The systematic and phylogenetic analysis of plants was traditionally based on macroscopic and microscopic morphological characters. Since secondary metabolites are often similar within members of a clade, their occurrence or absence might be taken as an indication of common descent and thus relatedness. While the potential value of plant secondary metabolites to taxonomy has been recognised for nearly 200 years (Candolle, 1804; Abbott, 1896) their practical application has been restricted to the 20th century, and predominantly to the last 40 years (reviews in Hegnauer, 1966, 1973, 1989, 1990; Harborne and Turner, 1984; Waterman and Gray, 1988; Hegnauer and Hegnauer, 1994, 1996, 2001; Wink and Waterman, 1999). Chemotaxonomy had a considerable impact on plant systematics and new systems of classification were being developed that took account of the distribution of these metabolites (Thorne, 1968, 1976; Dahlgren, 1980). Dahlgren's framework allowed chemotaxonomists to plot out known distribution patterns against a phylogenetic system of classification for the Angiospermae. The results of such analyses were very revealing and more than a little disconcerting for many chemotaxonomists since a strict attribution of a given SM to a clade is quite rare. Quite often, even allelochemicals of high structural specificity occur simultaneously in unrelated families of the plant kingdom.

For example, the anti-tumour alkaloid camptothecin (affecting DNA-topoisomerase) has been found in the following unrelated orders and families: Order Celastrales: *Nothapodytes foetida*, *Pyrenacantha klaineana* (Icacinaceae); order Cornales: *Camptotheca acuminata* (Nyssaceae); order Rubiales: *Ophiorrhiza mungos, O. pumila, O. filistipula* (Rubiaceae); *Ervatamia heyneana* (Apocynaceae) and *Mostuea brunonis* (Loganiaceae).

Cardiac glycosides (CGs) inhibit the Na^+,K^+-ATPase and are therefore strong poisons that provide potent chemical defence against herbivores. Cardiac glycosides are produced in a limited number of genera in many unrelated plant families, such as the Scrophulariaceae, Apocynaceae, Asclepiadaceae, Ranunculaceae, Brassicaceae, Hyacinthaceae, Liliaceae, Celastraceae and a few others. Even a few animals, such as toads and beetles can produce their own CGs.

The inevitable conclusion drawn from these observations is that the expression of secondary metabolites of a given structural type has almost invariably arisen in a number of occasions in different parts of the plant kingdom. Consequently the co-occurrence of a structural class in two taxa could, but not necessarily, be an indication of a monophyletic relationship. This discrepancy could be due, either to convergent evolution or differential gene expression: It is likely that in some cases the genes that encode the enzymes for the production of a given structure or structural skeleton have evolved early during evolution. These genes are not lost during phylogeny but might be "switched off" . On the other hand such genes might be "switched on" again at some later point (Wink and Witte, 1983).

2.2. Distribution of secondary metabolites mapped onto molecular phylogenies

The analysis of DNA sequences, among them chloroplast or nuclear DNA (Soltis et al., 1992; Chase et al., 1993; Doyle, 1993), has been increasingly employed to reconstruct the phylogeny of higher and lower plants. This powerful approach, which provides the best phylogenetic resolution so far, is facilitated by rapid DNA amplification techniques, such as polymerase chain reaction (PCR), by rapid DNA sequencing methods (manual and increasingly automated sequencing systems) and by powerful computation with adequate software programs (such as PAUP, MEGA) (Kumar et al., 1993; Swofford, 2001) all of which have been developed during the last 10 or 20 years. Using the molecular trees as a phylogenetic framework, there is an opportunity to examine and discuss similarities and dissimilarities of secondary metabolite profiles (e.g. Käss and Wink, 1995; Wink and Waterman, 1999; Gemeinholzer and Wink, 2001; Wink and Mohamed, 2003).

For this contribution, the molecular phylogeny of three large plant families (Fabaceae, Solanaceae and Lamiaceae) has been studied and used as a framework to evaluate the occurrence of some of their typical secondary metabolites.

2.2.1. Fabaceae

The Fabaceae (= Leguminosae) is a very large plant family with 750 genera and more than 18,000 species (ILDIS, 2001). Several types of alkaloids, non-protein amino acids, amines, flavonoids, isoflavonoids, coumarins, phenylpropanoids, anthraquinones, di-, sesqui- and triterpenes, cyanogenic glycosides, protease inhibitors and lectins have been described in this family (see reviews and compilations in Harborne et al., 1971; Polhill et al., 1981a,b; Kinghorn and Balandrin, 1984; Stirton, 1987; Hegnauer and Hegnauer, 1994, 1996, 2001; Bisby et al., 1994; Southon, 1994; Wink, 1993a, Wink et al., 1995; Sprent and McKey, 1994; DNP, 1996).

For the following analysis, a large *rbc*L data set, established in my laboratory (Wink and Mohamed, 2003) was reduced to 95 taxa, by selecting representative taxa covering a broad range of tribes. A strict Maximum Parsimony cladogram (Figs. 1 and 2) was chosen as a phylogenetic framework which is mostly concordant with other molecular phylogenetic studies of legumes. Papilionoideae and Mimosoideae form monophyletic subfamilies, whereas the Caesalpinioideae appear to be paraphyletic. Furthermore, the Mimosoideae do not form the base of the legume tree, but clearly derive from the ancestral Caesalpinoideae. (for a discussion of the systematic implications see Doyle, 1994; Doyle et al., 1997, 2000; Käss and Wink, 1995, 1996, 1997a,b; Bruneau et al., 2000, 2001; Crisp et al., 2000; Luckow et al., 2000; Pennington et al., 2001; Kajita et al., 2001; Wink and Mohamed, 2003).

We have mapped the occurrences of two major groups of legume defence compounds on the trees in order to understand whether a character occurs consistently within a monophyletic taxon or whether a given structural type has almost invariably arisen in a number of occasions.

2.2.1.1. Nitrogen-containing secondary metabolites. Legumes are able to fix atmospheric nitrogen via symbiotic *Rhizobia* in root nodules. Thus nitrogen is easily available for secondary metabolism and it is probably not surprising that nitrogen-containing secondary metabolites (alkaloids, non-protein amino acids, cyanogens, protease inhibitors, lectins) are a common theme in legumes.

Quinolizidine alkaloids (QA) are typical secondary metabolites and defence chemicals in some phylogenetically related tribes of the Fabaceae (Wink 1992; 1993a), but they have also been found in a few unrelated genera of Chenopodiaceae, Berberidaceae, Ranunculaceae, Scrophulariaceae and Solanaceae (Kinghorn and Balandrin, 1984; Wink and Waterman, 1999). Since traces of QAs could be detected in plants and cell cultures of other unrelated taxa, we have postulated (Wink and Witte, 1983) that the genes which encode the basic pathway leading to lupanine, must have evolved early during evolution, but that these genes are turned off in most instances, but turned on in plants which use the alkaloids as chemical defence substances (Wink, 1992, 1993a, 2000).

Quinolizidine alkaloids are especially abundant in members of the "genistoid alliance s.l." of the subfamily Papilionoideae including the tribes Genisteae, Crotalarieae, Podalyrieae/Liparieae, Thermopsideae, Euchresteae, Brongniartieae, and Sophoreae (Kinghorn and Balandrin, 1984; Wink, 1993a). Also dipiperidine alkaloids of the ammodendrine type, which also derive from lysine as a precursor, exhibit a comparable distribution pattern. As can be seen from Fig. 1, nearly all taxa in "genistoid alliance s.l." accumulate quinolizidine alkaloids. An obvious exception are members of the large tribe Crotalarieae: *Crotalaria* species sequester pyrroli-

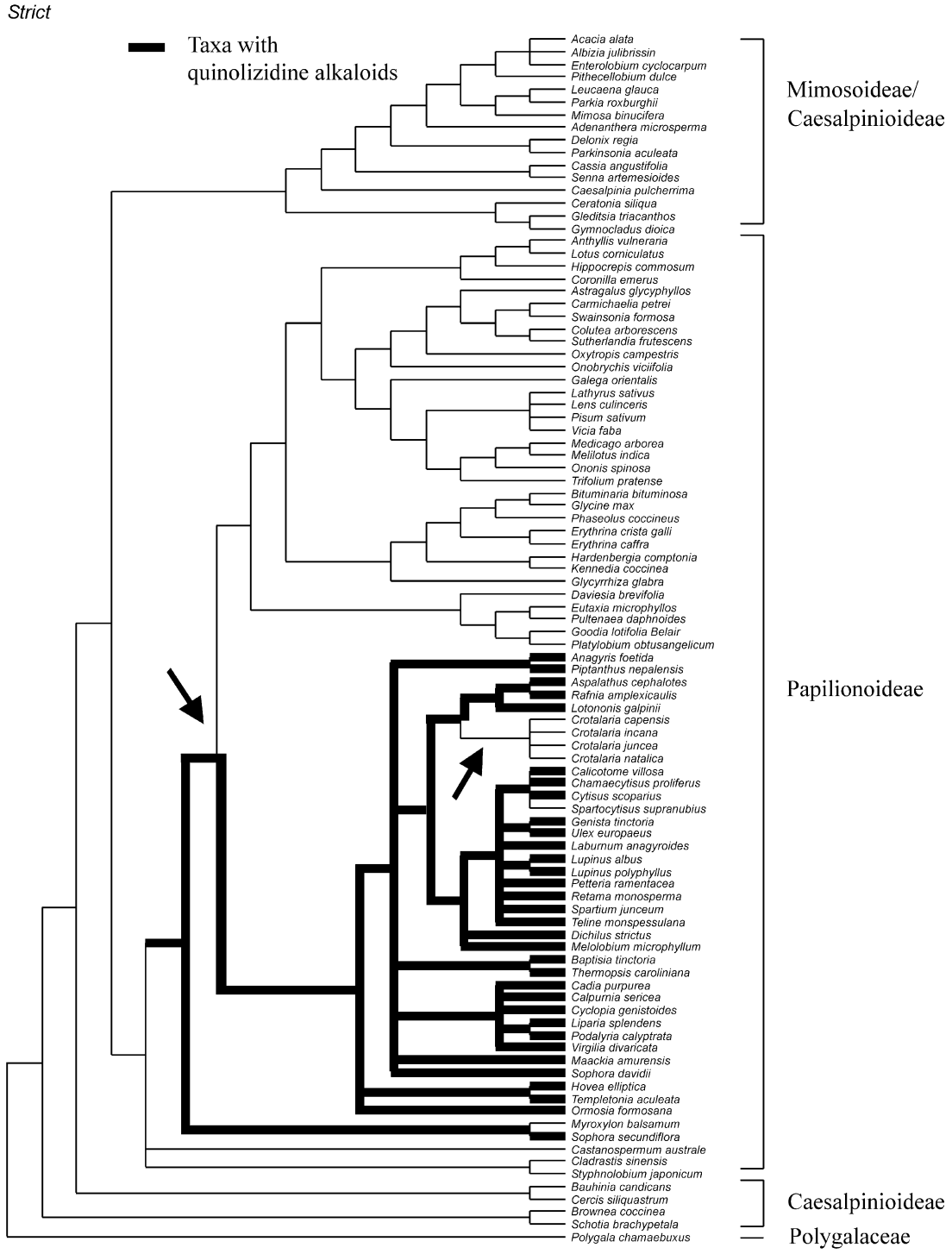

Fig. 1. Distribution of quinolizidine alkaloids in Fabaceae. MP-tree; strict consensus. Because the phytochemical analysis is still incomplete for some compounds or taxa, and since a few published findings may actually be wrong, inconsistencies should be interpreted with caution. The arrows point to critical bifurcations, splitting clades producing QA from those that do not produce QA. In case of *Crotalaria*, pyrrolizidine alkaloids replace QA as defence compounds.

zidine alkaloids and/or non-protein amino acids but not QA. In the genus *Lotononis* some taxa produce quinolizidine alkaloids, others pyrrolizidine alkaloids. Since *Crotalaria* and *Lotononis* derive from ancestors, which definitely produced quinolizidine alkaloids but not pyrrolizidine alkaloids, the genes encoding biosynthetic enzymes of quinolizidine alkaloid formation must still be present. It is unlikely that corresponding genes have been lost. More likely the quinolizidine alkaloid genes have been turned off in *Crotalaria* and partially in *Lotononis*. The formation of pyrrolizidine alkaloids (which are typical SM of the Boraginaceae and some Asteraceae) instead appears to be a new acquisition for chemical defence, which probably evolved independently. The occurrence of simple pyrrolizidine alkaloids in *Laburnum* and *Adenocarpus* (Wink and Mohamed, in press) might be interpreted accordingly.

Within the genistoid alliance all taxa (except the few examples mentioned before) produce alkaloids of the sparteine/lupanine type, at least as minor alkaloids. α-Pyridone alkaloids, such as anagyrine and cytisine, show a more patchy distribution. They occur already in the more ancestral tribes of the Papilionoideae, but also in the more advanced *Cytisus*/*Genista* complex of the Genisteae, suggesting that the biosynthetic capacity to produce these alkaloids is ancestral. These QA are especially potent allelochemicals: Cytisine and *N*-methylcytisine are strong agonists at nicotinic acetylcholine receptors (Wink, 2000; Wink et al., 1998); anagyrine is known to induce mutations and malformations in vertebrate embryos. Lupins form a monophyletic clade in the tribe Genisteae. Whereas Old World lupins sequester QA of the lupanine and multiflorine type, a number of New World lupins produce anagyrine and other α-pyridone alkaloids in addition. The occurrence of α-pyridones in North American lupins is erratic and apparently not helpful as a reliable taxonomic marker.

In a few other taxonomic groups that cluster within quinolizidine alkaloid accumulating genera, quinolizidine alkaloids are hardly detectable or levels are very low, such as in *Ulex, Calicotome* or *Spartocytisus*. These taxa have extensive spines in common that apparently have replaced chemical defence; in this case the presence or absence of quinolizidine alkaloids is clearly a trait reflecting rather different ecological strategies than taxonomic relationships.

Besides quinolizidine alkaloids, legumes accumulate a wide range of other alkaloids, deriving from different precursors. Most of them show occurrences which are restricted to a few, often non-related taxa. For example, *Erythrina* alkaloids, which derive from tyrosine as a precursor, are typical for members of the large genus *Erythrina* and have not been found elsewhere in the plant kingdom. This would be an excellent example for a close correlation of SM contribution and phylogeny. Indolizidine alkaloids, that inhibit hydrolytic enzymes, have been reported in *Swainsonia, Astragalus* (tribe Galegeae) and *Castanospermum* (Sophoreae). β-Carboline alkaloids have been detected in a few mimosoid taxa of the tribes Mimoseae and Acacieae. Also a number of simple phenylethylamine or simple indole alkaloids have been reported, usually in taxa which do not accumulate quinolizidine alkaloids. Interestingly, the occurrence of quinolizidines and other alkaloids is usually mutually exclusive, indicating the parsimonious utilisation of chemical defence resources.

It is likely that the genetic capacity to synthesise quinolizidine alkaloids must be present in the very early members of the Papilionoideae (Fig. 1); they are absent however in many other tribes that cluster as a sister group to the genistoid alliance (see arrow in Fig. 1). Similar to the situation in *Crotalaria*, which no longer accumulates quinolizidine alkaloids we suggest that all the other tribes of the Papilionoideae that diverge from quinolizidine alkaloid producing ancestors, have secondarily lost this trait or have just turned off the corresponding genes. These tribes accumulate other defence compounds, especially non-protein amino acids instead (Fig. 2).

The Fabaceae are a major source of "non-protein amino acids" such as albizziine, canavanine, mimosine and lathyrine. Non-protein amino acids have been considered to be useful taxonomic markers throughout the family, albizziine being characteristic of the Mimosoideae and lathyrine for the genus *Lathyrus*. The distribution of canavanine in the Papilionoideae was examined very extensively and has been used in the compilation of phylogenies for that subfamily (Bell et al., 1978; Polhill et al., 1981a,b). The pattern of non-protein amino acid accumulation (Fig. 2) again is almost complementary to the distribution of alkaloids (Fig. 1), if all non-protein amino acids with different structures and activities are grouped together. Similar to the functions of quinolizidine alkaloids and other nitrogen containing defence compounds, non-protein amino acids also serve at least two purposes, both as chemical defence compounds and as mobile nitrogen storage compounds of seeds that are used as a nitrogen source for the seedling. Considering different types of non-protein amino acids, a more differentiated picture becomes apparent. At least three groups of non-protein amino acids are common in legumes, such a canavanine, pipecolic acid and derivatives, and the sulfur-containing djenkolic acids. Canavanine is common in the tribes Galegeae, Loteae, Tephrosieae, Robinieae, and some Phaseoleae. It could be assumed that the trait of canavanine accumulation was acquired by an ancestor, from which all the other tribes derived, but that the canavanine genes are turned off in Vicieae, Trifolieae, Cicereae, and Abreae which produce pipecolic acids instead. Whether pipecolic acid biosynthesis was independently invented in Caesalpinioideae/Mimosoideae and in the papilionoid tribes Vicieae and Trifolieae, or whether the

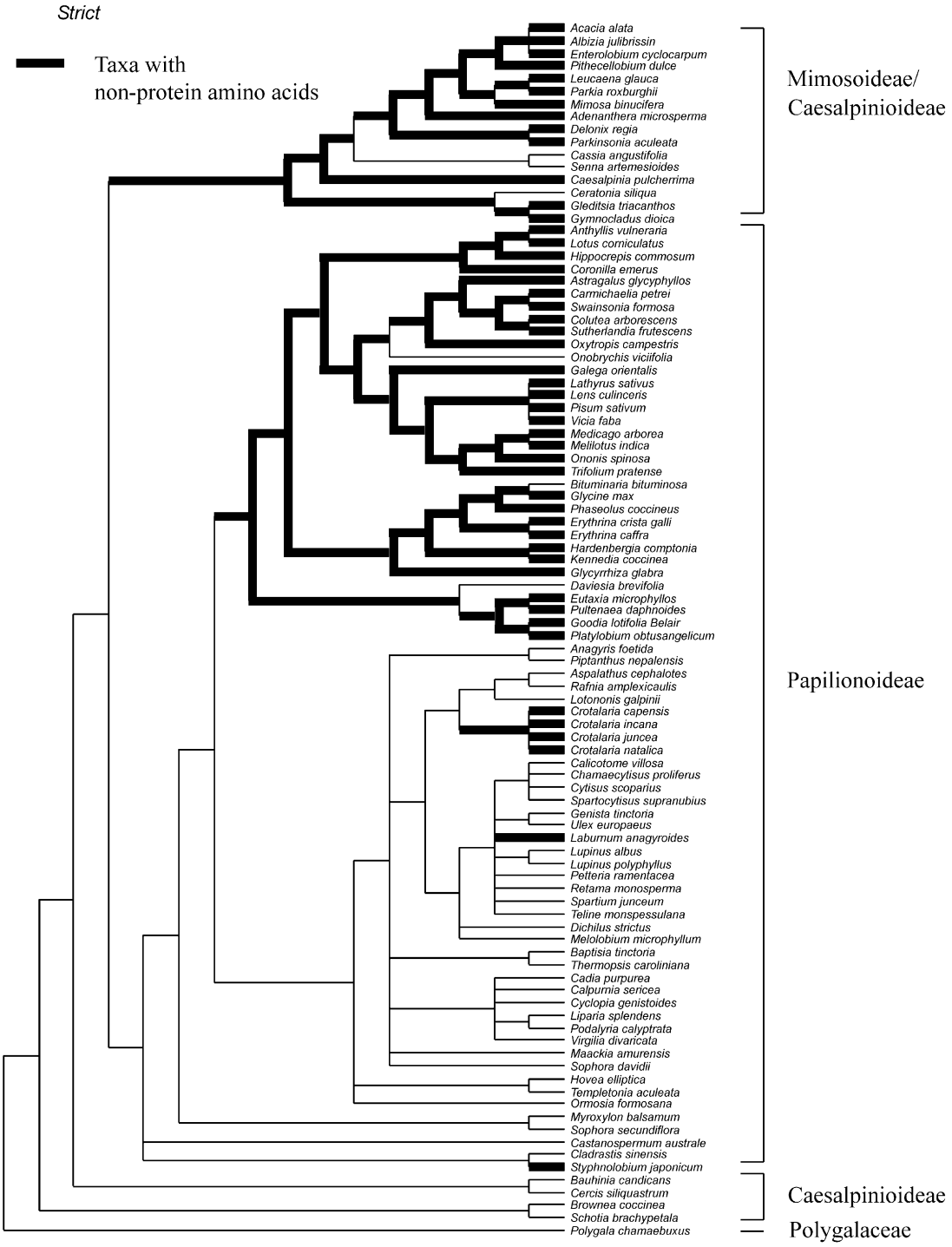

Fig. 2. Distribution of non-protein amino acids in Fabaceae.

canavanine genes were only inactivated in Vicieae and Trifolieae is open to debate. Several other non-protein amino acids have been described from legumes (Harborne et al., 1971; Polhill et al., 1981a,b; Stirton, 1987; Hegnauer and Hegnauer, 1994, 1996, 2001; Southon, 1994; Sprent and McKey, 1994). Most of them have a more restricted occurrence, and presence or absence in phylogenetically related taxa is a common theme. As strict taxonomic markers, non-protein amino acids are therefore of limited value, since they would create wrong monophyletic groups in several instances.

Cyanogenic glycosides appear to be more common in the more ancestral than the more advanced legume tribes (Wink and Waterman, 1999; Wink and Mohamed, 2003). Whether the isolated occurrences of

cyanogenic glycosides are based on common genes, which are turned off in most instances and turned on in a few places, cannot be answered yet; a convergent and independent evolution might also be possible. The distribution of protease inhibitors (i.e. trypsin and chymotrypsin inhibitors) exhibit an almost complementary pattern to quinolizidine alkaloids (Wink and Waterman, 1999). The members of the Caesalpinoideae and many Mimosoideae accumulate protease inhibitors in their seeds, where they serve concomitantly as chemical defence and nitrogen storage compounds. Within the Papilionoideae, protease inhibitors are prominent in the tribes Vicieae, Trifolieae, Cicereae, Abreae, Galegeae, Loteae, Phaseoleae, and Tephrosieae, but have not been described in the Mirbelieae. Since some of the genes for protease inhibitors are known, it would be interesting to analyse whether protease inhibitor genes are present or absent in taxa not producing protease inhibitors.

Concluding, the numerous nitrogen containing metabolites seem to function both as chemical defence and nitrogen storage compounds in legumes and are thus open to natural selection. Although they appear as plausible taxonomic markers on several occasions, they fail to do so in other parts of the legume tree.

2.2.1.2. Nitrogen-free secondary metabolites. Whereas flavonoids are found in all three subfamilies, and are thus of limited systematic value at the family/tribal level, isoflavonoids are obviously restricted to the subfamily Papilionoideae. Except for a few tribes and genera, among which are several Australian taxa, all taxa accumulate isoflavonoids and derivatives, including several phytoalexins of the pterocarpan type.

Catechins and proanthocyanins, or galloylcatechins occur in all three subfamilies; their occurrence rather reflects life style, i.e. growth as trees, than taxonomic relatedness. In Caesalpinioideae and Mimosoideae, both traits are almost congruent, since woody life style dominates in both subfamilies.

Coumarins and furanocoumarins, which serve as potent defence compounds in Apiaceae, occur in a few, but mostly unrelated legume species. Only in the genus *Psoralea*/*Bituminaria* and *Melilotus* do they have a wider distribution. Interestingly, *Psoralea* does not sequester canavanine, as do most of the taxa of related tribes. Anthraquinones, which are potent Na^+, K^+ATPase inhibitors and strong purgatives, ubiquitously occur in the genus *Cassia*, but occasionally only in *Andira* and *Abrus*.

Among terpenoids, all classes of terpenes have been found in legumes, such as triterpenes, triterpene and steroidal saponins (including cardiac glycosides in *Securigera* and *Coronilla*, both Loteae). Triterpenes and saponins, which are powerful defence compounds against microbes and herbivores, are more common in the ancestral Caesalpinioideae/Mimosoideae and in the basal tribes of the Papilionoideae, but also in the more advanced Vicieae, Trifolieae, Cicereae, and Phaseoleae. Whether they have appeared independently, or whether the genes have evolved at the beginning of legume evolution, but switched on or off according to ecological needs, cannot be answered with certainty. The wide distribution of triterpenes and triterpene saponins in the plant kingdom and their common basic structures, favours the latter possibility.

2.2.2. Solanaceae

The Solanaceae are of special economical, agricultural, and pharmaceutical importance; they comprise about 96 genera and 3000 species. The Solanaceae show a cosmopolitan distribution with the main centre of taxonomic diversity and endemism in South America. Solanaceous plants produce a wide variety of secondary metabolites such as tropane, pyridine, and steroid alkaloids, withanolides, ecdysteroids, sesquiterpenes, diterpenes and even anthraquinones (Hegnauer, 1973, 1990; Harborne and Baxter, 1993; Griffin and Lin, 2000). Within the Solanaceae, certain tribes and genera are well characterised by the presence or absence of these natural products. Therefore, the distribution of SM has been used to build a classification of the Solanaceae (Tetenyi, 1987).

Because the molecular studies of the Solanaceae apparently describe the most likely evolutionary framework (Olmstead et al., 1992, 1999; Olmstead and Sweere, 1994; Olmstead and Palmer, 1992), we have therefore tried in our study to use a molecular framework, based on nucleotide sequences of a combined data set of *rbc*L and *matk* genes to re-analyse the occurrence of chemotaxonomic characters within the Solanaceae. Our molecular phylogeny is mostly congruent with other phylogenetic reconstructions (e.g. Olmstead et al., 1999) and it widely agrees with morphological and karyotype data (Gemeinholzer and Wink, 2001).

Tropane alkaloids, such as hyoscyamine, scopolamine and other esters of tropine, constitute one of the most distinctive groups of secondary metabolites of the Solanaceae (Griffin and Lin, 2000). Many plants containing them have long been utilised for their medicinal, hallucinogenic, and poisonous properties (e.g. Wink, 1998). Tropane alkaloids have also been discovered outside the Solanaceae in unrelated families such as the Erythroxylaceae, Proteaceae, Euphorbiaceae, Rhizophoraceae, Convolvulaceae and Brassicaceae (Griffin and Lin, 2000).

Within the Solanaceae, tropane alkaloids were found in the subfamilies Schizanthoideae, Solanoideae and Nicotianoideae. They are especially abundant in certain tribes, e.g. Datureae, Hyoscyameae, and Mandragoreae and especially the genera *Datura, Brugmansia, Hyoscyamus, Atropa, Scopolia, Anisodus, Przewalskia, Atro-*

panthe, Physochlaina, Mandragora, Anthotroche, Cyphantera, and *Duboisia* are well known for the presence of these compounds (Fig. 3). In Physaleae, Solaneae, Solandreae only a limited number of taxa produce these alkaloids, which are completely absent in other tribes and subfamilies (e.g., Petunioideae, Cestroideae).

As can be seen from Fig. 3, tropane alkaloid producing taxa do not cluster in a single monophyletic group; they are apparently unrelated. Within the tribes Datureae, Hyoscyameae, and Mandragoreae, all members produce these alkaloids. In other tribes tropane alkaloids are only present in a few members, thus forming an autapomorphic trait. Other members of the tribe produce other defence compounds. According to cladistic rules, the occurrence of tropane alkaloids does not represent a consistent trait and if plants would be classified according to this trait alone, wrong taxonomic groupings would be obtained within the Solanaceae.

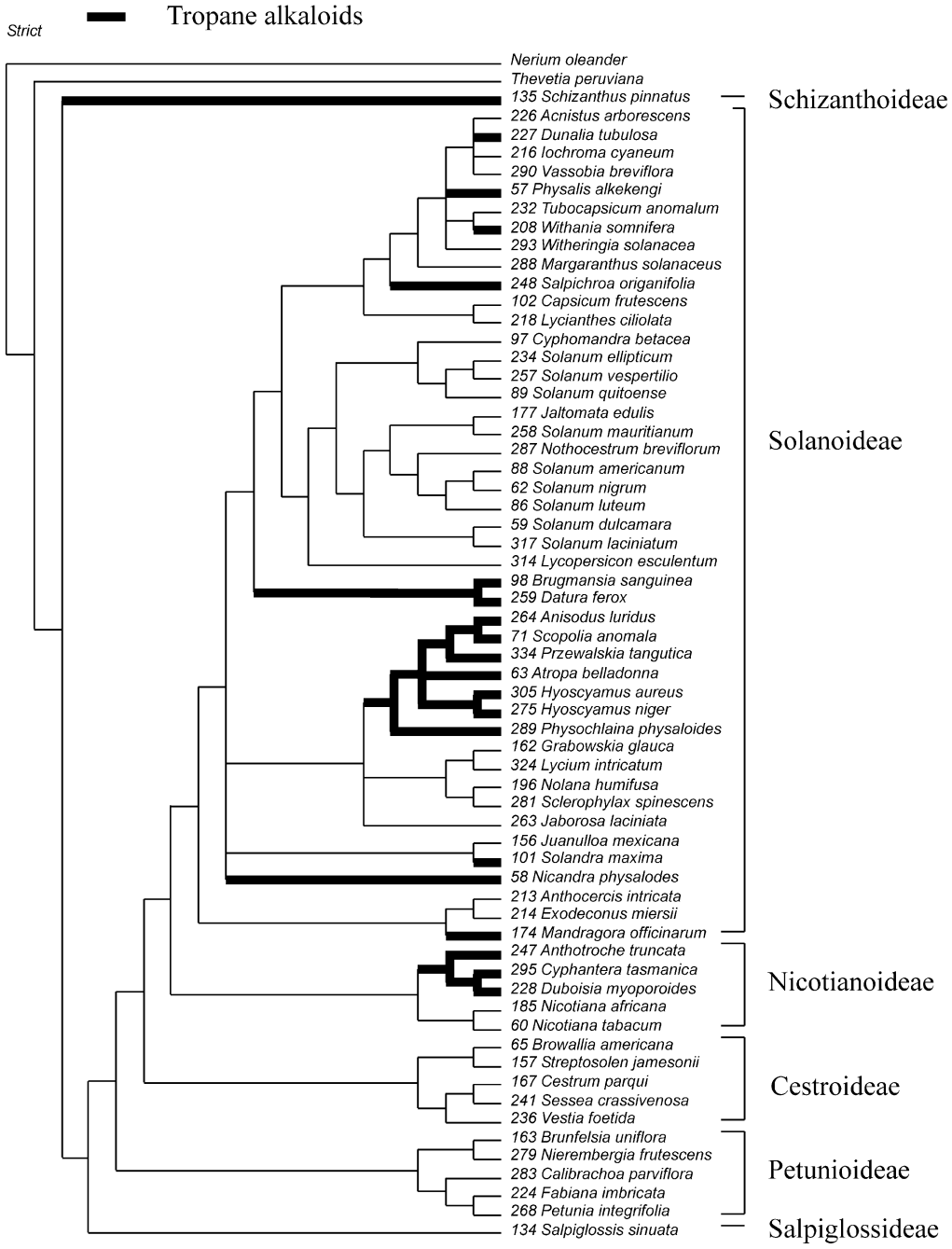

Fig. 3. Distribution of tropane alkaloids in Solanaceae. MP tree; strict consensus. Tree lengths 2082 steps; CI = 0.622; RI = 0.609; Note, that members of the genus *Solanum* which comprises a large number of species, cluster as a polyphyletic group. Molecular data according to Gemeinholzer and Wink (2001).

Steroid alkaloids are typical secondary metabolites of the Buxaceae, Liliaceae, Apocynaceae and Solanaceae (Harborne and Baxter, 1993). Steroid alkaloids are dominant characters in the genera *Solanum* and *Lycopersicon* (Fig. 4). A few isolated occurrences have been reported from the genera *Lycianthes, Cyphomandra* and *Cestrum parqui*.

The pyridine alkaloid nicotine is a typical and major allelochemical of the genus *Nicotiana*. As it is present in all its members, it forms a derived and consistent character for this group. It has also been found in a few other genera of the Nicotianoideae, such as *Cyphantera* and *Duboisia*. Isolated occurrences of nicotine, usually as a minor component, have been reported for a few genera of the Solanoideae, which are not closely related to the Nicotianoideae. According to Hegnauer (1973) nicotine has been discovered as a minor alkaloid in many other genera of

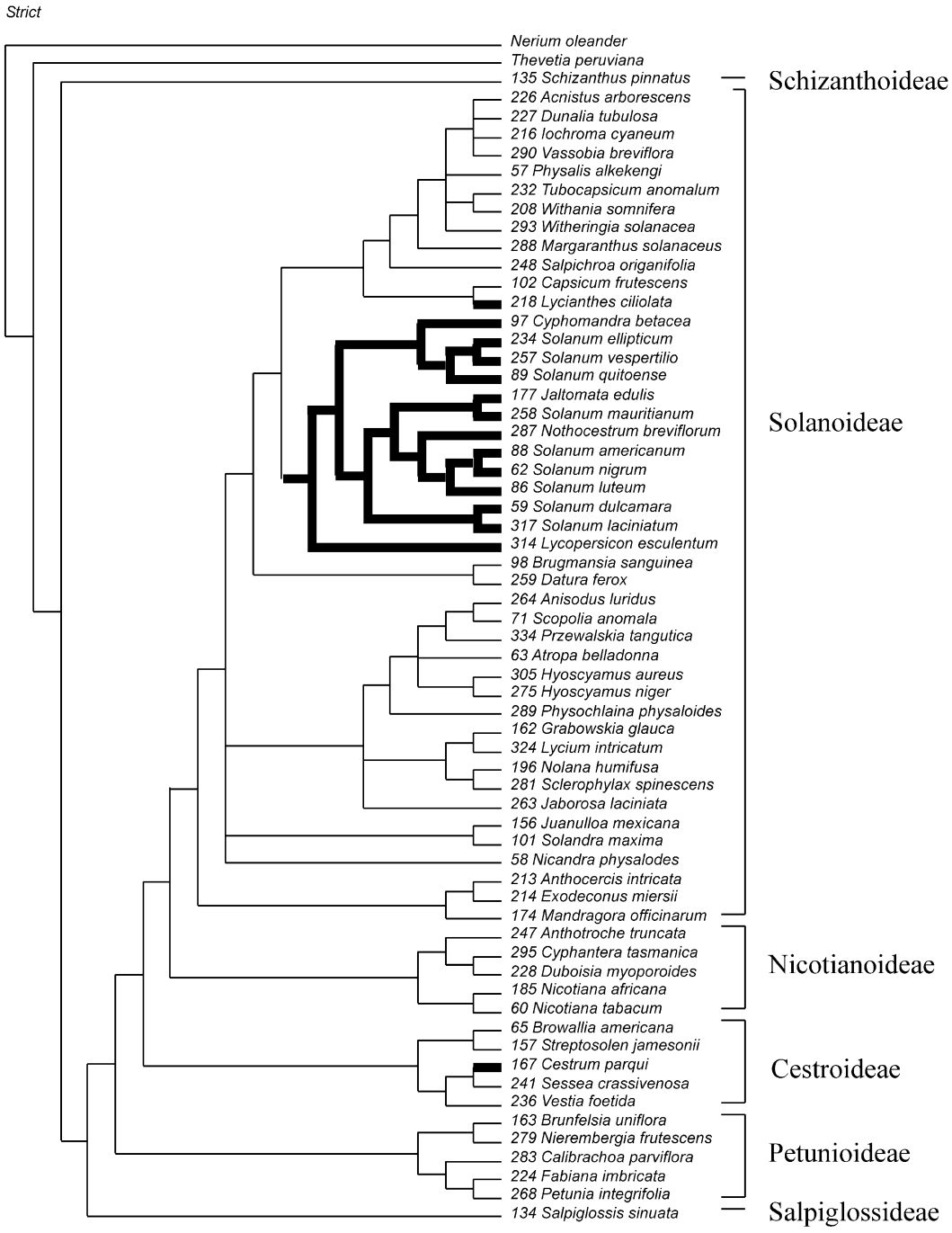

Fig. 4. Distribution of steroid alkaloids in Solanaceae.

the Solanaceae and other plant families. Apparently, nicotine distribution alone does not represent a good taxonomic marker as it would group unrelated taxa together.

Withanolides represent a group of steroidal lactones with strong insecticidal properties which appear to be restricted to the Solanaceae (Hegnauer, 1973, 1990; Harborne and Baxter, 1993). Withanolide producing genera are typical for the tribe Physaleae, but isolated occurrences have been reported for *Brugmansia* (Datureae), *Hyoscyamus* (Hyoscyameae), *Lycium* (Lycieae), *Jaborosa* (Jaboroseae), *Nicandra* (Nicandreae) and *Browallia* (Browallieae).

If we consider the occurrence of the major secondary metabolites of the Solanaceae, a general pattern becomes visible. The distribution of tropane alkaloids, steroid alkaloids, nicotine and withanolides is often mutually exclusive (Figs. 3 and 4) in that steroid alkaloids are typical for members of the Solaneae, withanolides for the Physaleae, nicotine for *Nicotiana* and tropane alkaloids for Datureae, Hyoscyameae, and Mandragoreae. Since these natural products are active against animals (herbivoral insects and vertebrates) (Wink and Schimmer, 1999; Wink, 2000) we can assume that they serve as chemical defence compounds in the plants producing them. Therefore, these compounds constitute important fitness traits and represent adaptive characters with some, but usually limited value as a taxonomic marker.

2.2.3. Lamiaceae

According to their flower structure the Lamiaceae constitute a highly developed plant family. The structural variability of its members has made it difficult to establish an unequivocally accepted system for their classification. Whereas the attribution of the approximately 4000 species to 220 genera is not a matter of much debate (Hedge, 1992), their grouping in subtribes, tribes and subfamilies has been a challenge since Bentham published his classification system in 1876. Follow-up classifications were produced by Junell (1934), Erdtmann (1945), Wunderlich (1967), Sanders and Cantino (1984), Cantino and Sanders (1986), Cantino et al. (1992), Cantino (1992). On account of pollen morphology, Erdtmann (1945) subdivided the Labiatae into the two subfamilies Lamioideae and Nepetoideae. Whereas the Lamioideae are characterized by tricolpate, binucleate pollen, albuminous seeds, spatulate embryos, the Nepetoideae have hexacolpate, trinucleate pollen, exalbuminous seeds, and investing embryos (Erdtman, 1945; Wunderlich, 1967; Harley and Reynolds, 1992). It has now been accepted that the Lamiaceae are subdivided into two major groupings: the Lamioideae and Nepetoideae and are closely related to the Verbenaceae (Chase et al., 1993; Bremer et al., 1998). A subdivision of the Lamiaceae into these two subfamilies is also supported by nucleotide sequences of the *rbc*L gene (Kaufmann and Wink, 1994; Wink and Kaufmann, 1996) which is also taken as a marker in this contribution.

Typical SM of Lamiaceae include various terpenoids, especially mono-, sesqui- di- and tri-terpenes. Also various phenolic compounds, especially phenolic acids, such as rosmarinic acid and flavonoids are abundant. Nitrogen containing SM play a minor role, such as stachydrine and other simple alkaloids (Hegnauer, 1966, 1989).

Iridoid glycosides, which derive from monoterpenes have been regarded as a good taxonomic marker in labiates (Hegnauer, 1989; Kooimann, 1972). Iridoid glycosides, such as aucubin, catalpol and harpagoside are also common outside the Lamiaceae, such as in Verbenaceae, Scrophulariaceae, Loganiaceae, Rubiaceae, Apocynaceae, Gentianaceae, Menyanthaceae, Oleaceae, Caprifoliaceae, Plantaginaceae, Pedaliaceae, Valerianaceae and others (Frohne and Jensen, 1973). Within the Lamiaceae, iridoid glycosides are common in members of the subfamily Lamioideae (Fig. 5). Iridoids have however also been recorded in a few members of the Nepetoideae, such as *Nepeta cataria* and *Satureja vulgaris* (syn. *Clinopodium vulgaris*), which are not more closer related to the Lamioideae than other members of the Nepetoideae. Since iridoids are also common in the sister family of the Lamiaceae, the Verbenaceae, a likely evolutionary scenario could be that the genes encoding the pathway to iridoids have evolved in an ancestor of both Verbenaceae and Lamiaceae. The absence of iridoids in most members of the Nepetoideae could be due to an inactivation of the corresponding genes. Iridoid glycosides are pharmacologically active compounds; among other targets they inhibit the formation of prostaglandins and leucotrienes that are important mediators in animals. Since iridoids glycosides are sequestered by a few adapted insects, there is good experimental evidence that they can serve as potent defence compounds (Bowers and Stamp, 1997).

The apparent absence of iridoid glycosides in most members of the Nepetoideae raises the question of the major chemical defence compounds in the Nepetoideae. A typical and most characteristic feature of most Nepetoideae is the production and accumulation of comparably large amounts of volatile monoterpenes, which are usually sequestered in specialised glands and trichomes. A few genera also produce sesquiterpenes. As can be seen from Fig. 6, the distribution of these compounds is almost mutually exclusive to that of iridoids, suggesting that they should contribute significantly to chemical defence in the Nepetoideae. In addition to the essential oils, the Nepetoideae (but not the Lamioideae) produce a special "tannin", mainly represented by the phenolic

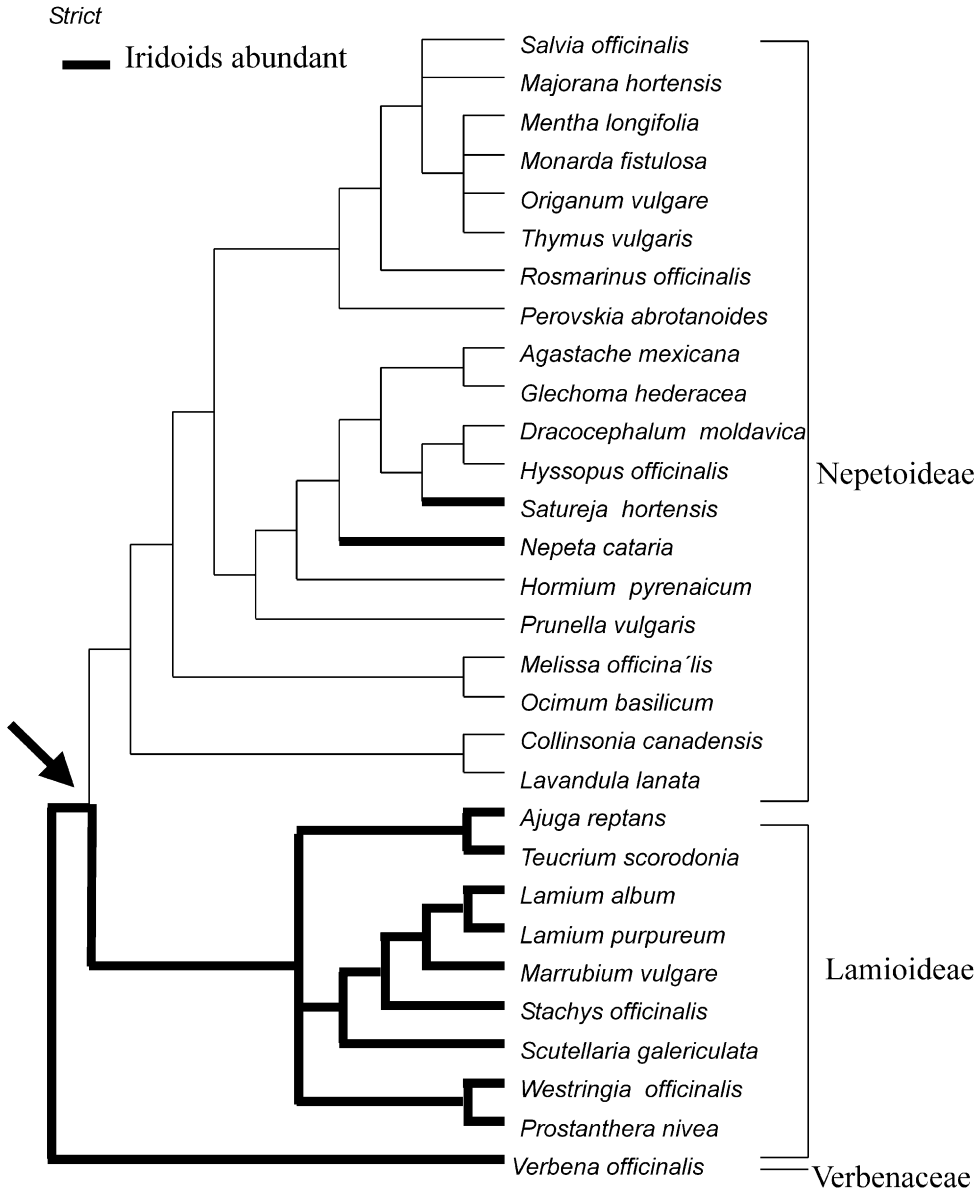

Fig. 5. Distribution of iridoids in Lamiaceae. A strict MP tree (out of 6 most parsimonious trees) was reconstructed from complete *rbc*L sequences; a single sequence per genus was selected (lengths 447 steps; CI = 0.622, RI = 0.686) (molecular data according to Kaufmann and Wink, 1994; Wink and Kaufmann 1996). Branches leading to taxa accumulating iridoid glycosides as major SM are printed in bold.

compound, rosmarinic acid. Furthermore, biologically active diterpenes have been found in some members of the Nepetoideae. Since the biosynthetic pathways leading to mono- and sesquiterpenes are also present in the Verbenaceae, it is likely that the Lamioideae possess the corresponding genes, but do not express them. It is less likely that the biosynthetic pathways leading to iridoids and various other terpenoids have evolved convergently in the Lamiaceae. Since several genes of the terpenoid pathways have been cloned already, their absence or presence in Lamioideae or Nepetoideae, could be studied experimentally.

3. Conclusions

When analysing the profiles of typical secondary metabolites in Fabaceae, Solanaceae, Lamiaceae and other plant families we observe in some instances that almost all members of a monophyletic clade share a chemical characteristic; this would favour its use as a taxonomic marker. In other instances a particular SM may occur in several unrelated clades and/or plant families (Wink and Waterman, 1999; Gemeinholzer and Wink, 2001; Wink and Mohamed, 2003). The erratic SM distribution can be due to simple convergence, in

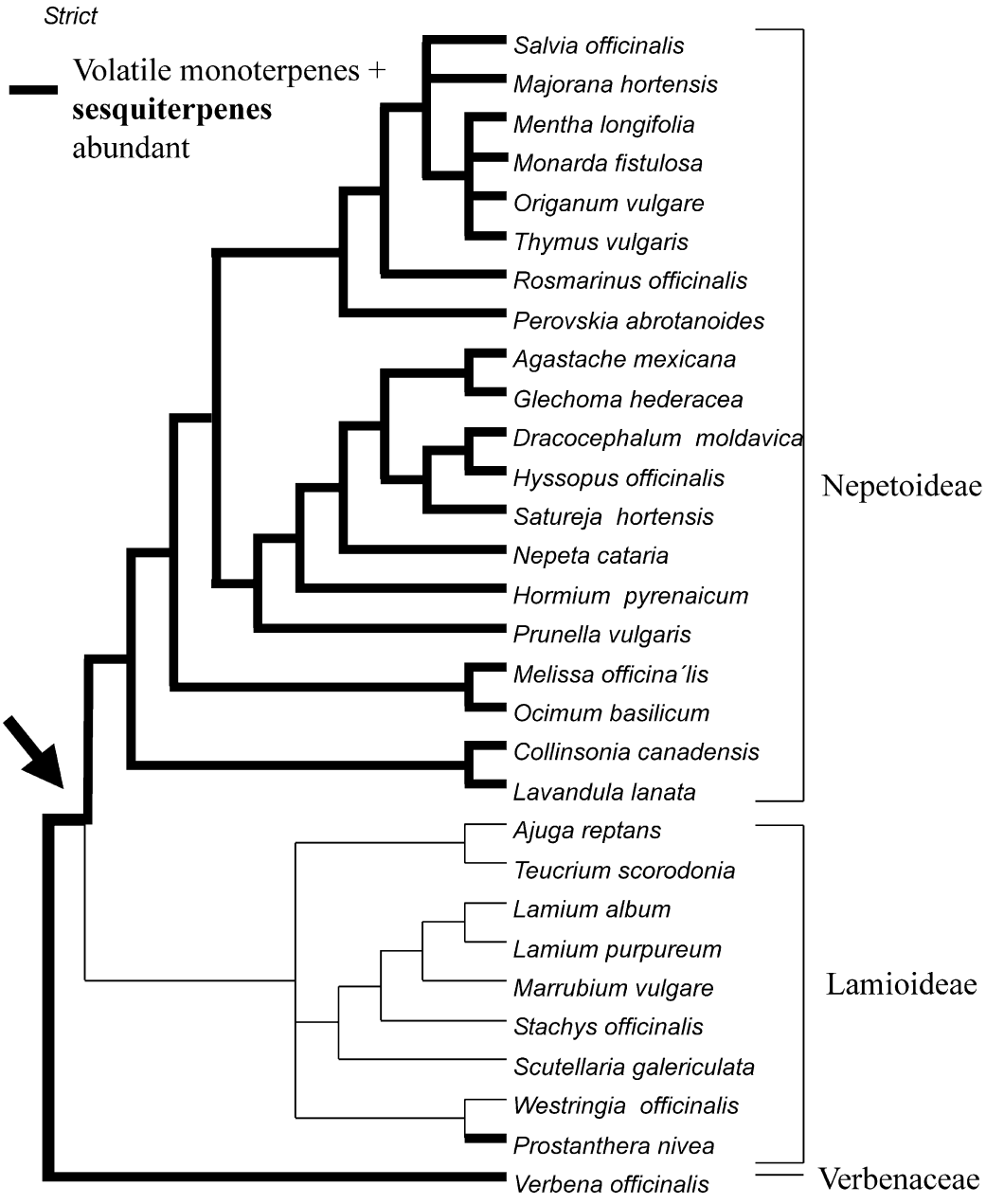

Fig. 6. Distribution of volatile mono- and sesqui-terpenes in Lamiaceae. Branches leading to taxa accumulating volatile oil (mainly monoterpenes) as major SM are printed in bold. If sesqui-terpenes are accumulated, then the taxon name is also printed in bold.

that the genes that encode a particular biosynthetic pathway evolved independently in several parts of a phylogeny. There is evidence however for an alternative explanation: In several cases it is apparent that ancestral members of a group evolved the biosynthetic capacity to produce a certain SM. The absence of such a trait in phylogenetically derived groups is probably due to differential gene expression, in that the corresponding genes are not lost but switched off. Since secondary metabolites play a vital role as defence and signal compounds, their occurrence apparently reflects adaptations and particular life strategies embedded in a particular phylogenetic framework.

The inconsistent secondary metabolite profile mean that the systematic value of chemical characters becomes a matter of interpretation in the same way as traditional morphological markers despite the fact that they can be defined unambiguously in terms of both origin and structure. The distribution of secondary metabolites apparently has some value for taxonomy

but it has to be analysed carefully and critically, as any other adaptive trait.

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