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Review

The "new" chemosystematics: Phylogeny and phytochemistry

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Abstract

For almost a decade it has been acknowledged that the flowering plant dichotomy of monocotyledons and dicotyledons does not reflect the evolution of angiosperms. Despite this, conclusions in the field of chemosystematics are still drawn from, and rely on, non-phylogenetic botanical classifications such as those of Cronquist, Dahlgren and Takhtajan. In this paper the two alkaloids colchicine and camptothecin are used as examples of how phylogenetic systematics may be applied to alkaloid chemosystematics.

Keywords: Alkaloid; Camptothecin; Colchicine; Chemosystematics; Phylogeny

Contents

1. Introduction

Discussions of occurrence of specific chemical characters within the plant kingdom, chemosystematics, have always been dependent on botanical classification. Among recent systems commonly used we find those of Dahlgren (1980); Cronquist (1981) and Takhtajan (1980, 1997) – splitting the flowering plants into groups such as Dicotyledoneae and Monocotyledoneae, and commonly used subdivisions such as Asteridae, Rosidae, Dilleniidae, Hamamelididae and Caryophyllidae. From the first phylogenetic analyses using the nucleotide sequence of the chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*), and sampling a large number of flowering plant groups, it was evident that the dicotyledon-monocotyledon division was artificial, and that only monocotyledons were monophyletic (Chase et al., 1993). Results indicated that especially the subclasses Dilleniidae and Hamamelididae were grossly polyphyletic. Thus previous thoughts about angiosperm evolution were in need of re-evaluation and the angiosperm systematics recircumscribed. The angiosperm phylogeny group (APG) thus presented a synthesis of cladistic analyses of angiosperm phylogenies emphasizing monophyletic families and orders in the end of the 20th century (APG, 1998). A summary tree of this classification can be seen in Fig. 1. Further analyses using other genes than *rbcL* (e.g. the *matK* by Hilu et al., 2003) or combinations of genetic sequences (e.g. 18S rDNA, *rbcL* and *atpB* by Soltis et al., 2000) have largely confirmed the outline of the APG-system.

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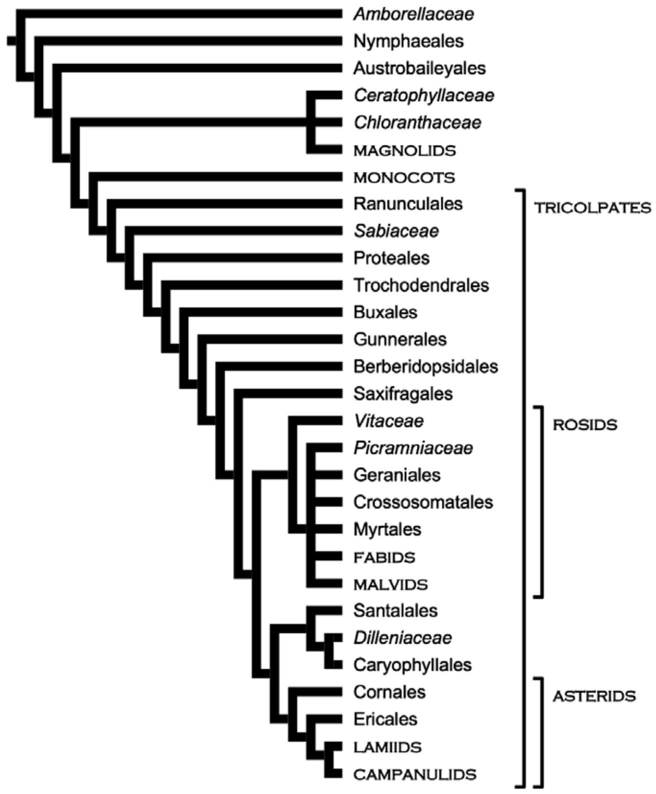


Fig. 1. Summary tree of the Angiosperm Phylogeny Group II classification (compiled from Haston et al., 2007; with names of informal groups from Judd and Olmstead, 2004).

Table 1
Genera outside the family Colchicaceae reported to contain colchicine (Klein and Pollauf, 1929; Šantavý, 1956), with their current family and order assigned (APG, 1998, 2003; Haston et al., 2007)

Genus	Family	Order
Tofieldia	Tofieldiaceae	Alismatales
Anthericum	Agavaceae	Asparagales
Asphodelus	Asphodelaceae	Asparagales
Chlorogalum	Asphodelaceae	Asparagales
Hemerocallis	Hemerocallidaceae	Asparagales
Muscari	Hyacinthaceae	Asparagales
Ornithogalum	Hyacinthaceae	Asparagales
Narthecium	Nartheciaceae	Dioscoreales
Fritillaria	Liliaceae s.s.	Liliales
Lloydia	Liliaceae s.s.	Liliales
Tulipa	Liliaceae s.s.	Liliales
Chamaelirium	Melanthiaceae	Liliales
Veratrum	Melanthiaceae	Liliales
Xerophyllum	Melanthiaceae	Liliales
Zigadenus	Melanthiaceae	Liliales

2. Discussion

One of the families commonly encountered in alkaloid chemosystematics is Liliaceae *sensu latissimo*, known for an abundance of different alkaloid types as represented by e.g. galanthamine **1**, jervine **2** and colchicine **3**. The latter has a widespread use in medicine and molecular biology, and is one of the earliest isolated (Pelletier and Caventou, 1820; Geiger, 1833). It has been reported from a large number of genera from the polyphyletic family Liliaceae *sensu latissimo* (see Table 1; Klein and Pollauf, 1929; Šantavý, 1956), information that is available through, and repeated in, common sources such as *The Plant-Book* (Mabberley, 1997). Dahlgren et al. (1985) split the Liliaceae *sensu latissimo* into several smaller families including Uvulariaceae. However, the genera of this family were shown to be polyphyletic belonging to both Colchicaceae and Liliaceae *sensu stricto* (Vinnersten and Bremer, 2001). One former Uvulariaceae genus, *Tripladenia*, was proposed to contain colchicine (investigated under the name *Kreysigia*; Šantavý, 1967) and this led us to reinvestigate the distribution of the alkaloid. In our investigation using mass spectrometry detection we have found colchicine throughout Colchicaceae as circumscribed by Vinnersten and co-workers (Vinnersten and Reeves, 2003; Vinnersten and Manning, 2007), but been unable to detect it in any of the previously reported genera outside the family (Vinnersten and Larsson, *in prep.*; Vinnersten, 2003; Larsson, 2004). This suggests that the biosynthetic pathway leading to colchicine-type alkaloids evolved in the ancestor of this family. A parallel case may be the alkaloid group in which galanthamine is a member, as this seem to be restricted to the family Amaryllidaceae (Zhong, 2005; Unver, 2007), while the steroidal alkaloids have a much wider distribution e.g. also involving dicotyledonous families such as Solanaceae and Apocynaceae (Atta-ur-Rahman and Choudhary, 1998).

The alkaloid camptothecin **4** also illustrates how up-to-date knowledge influence the conclusions that may be drawn from chemosystematics. In the article by Lorence and Nessler (2004), the distribution of the monoterpene-indole alkaloid camptothecin includes the orders (and

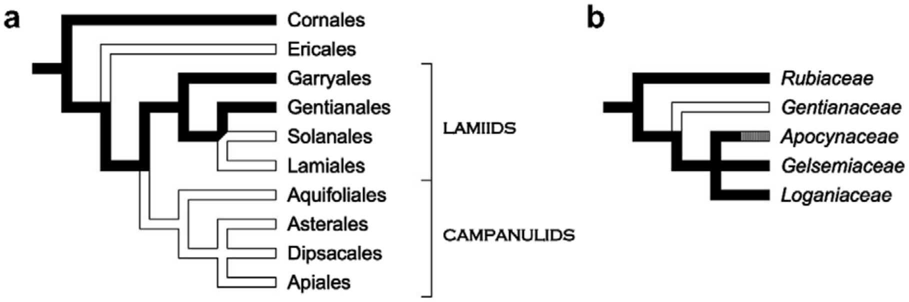
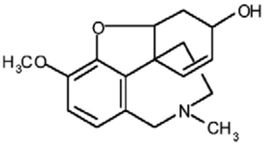


Fig. 2. Mapping of camptothecin on the asterid orders (a), and the occurrence of monoterpene-indole alkaloids within the order Gentianales (b).

families): Celastrales (Icacinaceae), Cornales (Nyssaceae) and Gentianales (Apocynaceae, Gelsemiaceae and Rubiaceae). This indicates that camptothecin may have evolved twice as Celastrales is a rosid order in the fabid clade, while Cornales and Gentianales are asterid orders, the latter in the lamiid clade (cf. Fig. 1). Or one may argue that an ancestral evolution of a biosynthetic pathway can be silent (''switched off'') in large parts of the extant plant lineages and only active (''switched on'') in specific groups (e.g. discussed by Wink, 2003). The choice between these two lines of argument relies upon a correct phylogenetic interpretation, and in Lorence and Nessler (2004) the phylogeny of the discussed genera is wrong. The family Icacinaceae as defined pre-APG is polyphyletic, and neither of its segregates is of rosid affinity. Kårehed (2001) showed that Icacinaceae did not belong in the order Celastrales, instead the family contained several lineages associated with either Garryales in the lamiid clade or Aquifoliales in the campanulid clade (cf. Fig. 1). The genera *Nothapodytes*, *Merriliodendron* and *Pyrenacantha* are proposed to be part of Icacinaceae *sensu stricto* of the order Garryales (Kårehed, 2001), which limits the distribution of camptothecin to asterid taxa. So instead of having two independent evolutions of the biosynthetic pathway in rosids and asterids, or multiple losses in rosid and asterid lineages, the phylogeny proposes that camptothecin biosynthesis is acquired only in taxa also being able to produce other monoterpene-indole alkaloids, namely within early diverging asterids (Cornales) and lamiids (Garryales and Gentianales) as shown in Fig. 2a. The question about silencing or loss of biosynthetic genes thus relates to the orders Ericales, Solanales and Lamiales, as well as to the campanulid clade. When mapping the ability to synthesize monoterpene-indole alkaloids on the families of Gentianales, see Fig. 2b, a similar pattern is seen as such alkaloids are absent in the family Gentianaceae and in the apocynaceous genera previously treated as the family Asclepiadaceae. Investigation for presence of genes necessary for the production of monoterpene-indole alkaloids, such as strictosidine synthase, would shed light upon the question whether this pathway is silenced or lost within these lineages.

Only investigations of biosynthetic pathways can lead to answers regarding why a certain chemical is present or absent in particular plants. This is necessary to discuss aspects such as monophyly of biosynthetic potential or parallel/convergent evolution of such pathways (e.g. discussed in Waterman, 1998; Firn and Jones, 2003; Wink, 2003). The benzylisoquinoline alkaloids are biosynthesized from (*S*)-norcoclaurine **5** and investigations of its synthase have revealed that this group have a monophyletic origin (Liscombe et al., 2005). In accordance it is reasonable to assume that the monoterpene-indole alkaloids have a monophyletic origin as they are biosynthesized from 3α(*S*)-strictosidine **6**. Examples of non-monophyletic alkaloids would include the pyrrolizidine alkaloids, as the most common precursor homospermidine can be produced by deoxyhypusine synthase (DHS, EC 2.5.1.46) present in all eukaryotes and normally involved in activation of eukaryotic initiation factor 5A (Ober et al., 2003). Plants rich in pyrrolizidine alkaloids have a specific homospermidine synthase (HSS, EC 2.5.1.45) that in each plant lineage is evolutionary derived from the corresponding DHS (Ober and Hartmann, 1999, 2000). There are also examples of pyrrolizidine alkaloids not synthesized from homospermidine, such as the endophyte-produced lolines of Poaceae (Schardl et al., 2007).



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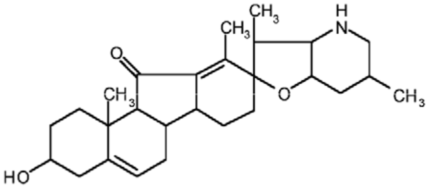

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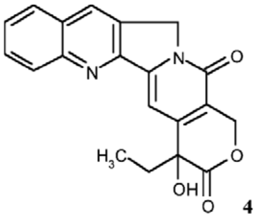

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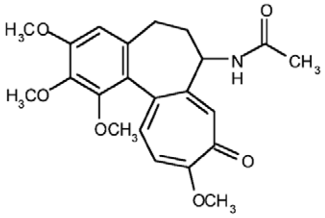

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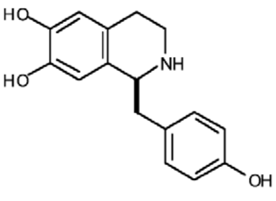

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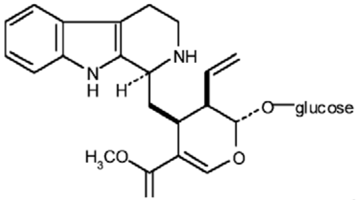

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3. Concluding remarks

Correct interpretation of alkaloid distribution in a systematic sense relies upon good phylogenies, and such interpretations may be used as hypotheses for investigations of biosynthetic pathways. As molecular phylogenies have redrawn the classification of plants, any chemosystematic worker needs to check that their study object have not been subject to family or ordinal recircumscription. Families known to have undergone major revisions within the ordinal classification of the Angiosperm Phylogeny Group (APG, 1998, 2003; Haston et al., 2007), include e.g. Flacourtiaceae, Scrophulariaceae/Plantaginaceae and Euphorbiaceae.

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