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The value of chemosystematics in clarifying relationships in the genistoid tribes of papilionoid legumes

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Abstract

Taxonomic patterns in the distribution of alkaloids, phenolic compounds and cyanogenic glycosides in the genistoid group, which includes the tribes Brongniartieae, Genisteae, Thermopsideae, Crotalarieae, Podalyrieae, Euchresteae and part of the Sophoreae, are reviewed. Discontinuities in the distribution of secondary metabolites agree well with recent modifications to generic and tribal delimitations, and also with new phylogenetic hypotheses based on DNA sequence data. Examples of potential synapomorphies include the presence of quinolizidine alkaloids (genistoid group), *Ormosia*-type alkaloids (*Ormosia* group of Sophoreae, the Brongniartieae and Thermopsideae), absence of α-pyridone alkaloids (Podalyrieae, Crotalarieae and some Sophoreae), carboxylic acid esters of alkaloids (*Cadia purpurea*, *Sophora inhambanensis* and the Podalyrieae), quinolizidines of the matrine type (Euchresteae and *Sophora* species), 5-*O*-methylgenistein (Genisteae), vicenin-2 (Crotalarieae, Podalyrieae and some Sophoreae) and esters of cyanidin (purple-flowered Podalyrieae, excluding *Hypocalyptus*). Despite incomplete data and extensive homoplasy, secondary metabolites provide phylogenetic clues at all taxonomic levels.

Keywords: Genistoid legumes; Podalyrieae; Crotalarieae; Genisteae; Sophoreae; Quinolizidine alkaloids; Flavonoids; Anthocyanins; Chemotaxonomy

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1. Introduction

Secondary metabolites often show erratic or counterintuitive distribution patterns so that their usefulness as taxonomic characters is sometimes questioned (e.g. Käss and Wink, 1995; Wink and Waterman, 1999). The emphasis has shifted to DNA sequencing as the main source of data for phylogenetic studies. An advantage is that subjective interpretations of morphological or chemical patterns are no longer necessary. On the other hand, it has also become clear that all classes of taxonomic evidence (morphological, anatomical, ontogenetic, cytological, palynological, etc.) are potentially valuable in our quest for a classification system that reflects evolutionary relationships. Examples of various classes of compounds, selected mainly from own studies of African genistoid legumes, are used in this paper to show that rigorous comparative studies of secondary metabolites can indeed contribute to a more profound understanding of relationships at all taxonomic levels.

The concept of a genistoid alliance was first proposed by Polhill (1981) but it has been slightly modified as a result of molecular studies (Doyle et al., 2000; Crisp et al., 2000; Pennington et al., 2000, 2001). As currently circumscribed, the true genistoids comprise a part of the tribe Sophoreae and the whole of the tribes Euchrestiae, Podalyrieae, Crotalarieae, Thermopsideae, Genisteae and Brongniartieae (including the Australian *Templetonia* group). Much progress has been made in the taxonomy of the temperate tribes, but the relationships within and between the mainly tropical Sophoreae genera are still poorly known. DNA sequencing studies have shown that the Sophoreae are in need of taxonomic realignment (Käss and Wink, 1995; Doyle et al., 2000; Crisp et al., 2000; Pennington et al., 2000, 2001). In contrast, most of the recent changes in the genistoid tribes (summarised in Van Wyk and Schutte, 1995 and Schutte and Van Wyk, 1998a, 1998b) have been influenced mostly by chemotaxonomic studies.

2. Limitations of chemosystematics

Much effort has gone into chemosystematic studies of the Fabaceae over the last few decades—Southon (1994) and Hegnauer and Hegnauer (1992–2001) give elegant and comprehensive summaries of the available data. Despite the massive amount of information that has accumulated, much remains to be done to obtain good quality comparative data that will allow for sensible chemosystematic interpretations at higher taxonomic levels. Many genera and species that are rare or difficult to sample have remained unstudied. This is particularly true for woody, tropical (and presumably basal) genistoids. Some genera hitherto misplaced within the system have never been critically compared to their true relatives (e.g. *Hypocalyptus*, see below). The plant parts extracted are often not mentioned, or different plant parts are studied, so that the results are not comparable. In many cases, there are large quantitative and even qualitative differences between plants parts (such as roots, stems, leaves and seeds). False negative reports are common—partly because minor compounds may be ignored, or partly because it may not be worthwhile to spend time identifying or reporting on the occurrence of well known compounds (a disproportionate effort is thus spent on new or unusual compounds, even if they occur in minute quantities). False positive reports are quite common (especially in the older literature), perhaps resulting from wrongly identified material, contamination in the laboratory or the uncritical use of paper- and thin layer chromatography for the separation and identification of complex mixtures. It is therefore not only the possibility of homoplasy, but also a lack of good quality data and inadequate sampling that limit the use of chemical characters in determining relationships.

3. Chemotaxonomic patterns in the genistoid alliance

3.1. Alkaloids

Quinolizidine alkaloids, once thought to be widely distributed in legumes, now appear to be a useful synapomorphy for the genistoid tribes. As stated by Wink and Witte (1983); Käss and Wink (1995) and Greinwald et al. (1996), it seems likely that most of the major pathways were already present in the basal groups, and that there have been repeated losses in some tribes and genera of the genistoid group, while other tribes and genera developed various modifications to the basic pathways. It is particularly noteworthy that genera of the Sophoreae that accumulate quinolizidines all appear to be related to other genistoids (Käss and Wink, 1995, 1997; Doyle et al., 2000; Pennington et al., 2000, 2001). Puzzling exceptions are *Sophora secundiflora*, *Styphnolobium japonicum* and *Ormosia formosana* (Käss and Wink, 1997; Wink and Mohamed, 2001) and an earlier report of *Dalbergia monetaria* (Kinghorn et al., 1982). The presence of quinolizidine alkaloids and the taxonomic placement of these taxa require confirmation. The Sophoreae genera shown by DNA sequence data to belong to the genistoid alliance are *Acosmium*, *Bolusanthus*, *Bowdichia*, *Cadia*, *Clathrotropis*, *Dicraeopetalum*, *Euchresta*, *Maackia*, *Ormosia*, *Platycelyphium*, *Salweenia* and *Sophora* (some species) and will here be referred to as basal woody genistoids. It is also interesting that the results of Pennington et al. (2000, 2001), based on the chloroplast *trnL* intron, showed the quinolizidine-bearing Brongniartieae included in a monophyletic genistoid clade. Probably the best support for the genistoid clade comes from *matK* data (Hu et al., 2000). Alkaloid data for *Acosmium* (Veitch et al., 1997), *Bowdichia* (Torrenegra et al., 1989), *Lamprolobium* (Greinwald et al., 1993), *Poecilanthe* (Greinwald et al., 1995) and *Dicraeopetalum*, *Platycelyphium* and *Sakoanala* (Van Wyk et al., 1993) give added confidence that the chemical patterns are in harmony with the emerging DNA phylogeny of the genistoid legumes.

At least six major types of quinolizidine alkaloids, presumably representing six major biochemical pathways, are present in the genistoid tribes. A summary of this pattern is presented in Table 1.

Bicyclic alkaloids (e.g. lupinine, lusitanine and lamprolobine) are widely distributed in all except the Crotalarieae and perhaps Thermopsideae and Euchrestieae (the two last-mentioned tribes need closer scrutiny, as some genera are likely to produce

Table 1
Distribution of some secondary metabolites in the genistoid group as defined by Pennington et al. (2000, 2001), with the monotypic Hypocalyptieae added for comparison. Data from Southon (1994), Hegnauer and Hegnauer (1992–2001) and various other publications as cited in the reference list. The arrangement of genera follows Polhill (1994) except for some modifications to reflect the emerging DNA phylogeny of the genistoid group[a]

Tribes, groups and genera	CHEMICAL CHARACTERS (see notes below)													
	Q1	Q2	Q3	Q4	Q5	Q6	P1	P2	F1	F2	F3	F4	A1	C1
Tribe Sophoreae p.p. (“basal woody genistoids”) :	+	+	+	+	+	−	−	−	+	−	−	+	?	−
Tribe Euchresteae:	?	−	+	+	−	−	−	−	?	?	?	?	?	−
Tribe Brongniartieae s.l. (incl. the *Templetonia* group):	+	+	−	+	−	+	−	−	?	?	?	?	?	−
Tribe Podalyrieae s. l.	+	−	−	−	+	+	−	−	+	+	+	+	+	−
Cadia purpurea:	+	−	−	−	+	+	−	−	+	−	+	−	?	−
Sophora inhambanensis:	+	−	−	−	+	+	−	−	+	−	+	−	−	−
Tribe Hypocalyptieae: (non-genistoid, for comparison only)	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Tribe Crotalarieae:	−	−	−	−	+	−	+	+	+	+	−	+	+	+
Tribe Thermopsideae:	?	+	−	+	+	−	−	−	?	?	?	?	?	−
Tribe Genisteae														
African genera:	+	−	−	+	−	−	+	−	−	−	−	−	−	−
South American genera:	+	−	−	+	−	−	−	−	?	?	?	?	?	−
North Temperate genera:	+	−	−	+	+	−	+	−	?	?	?	?	?	−

[a] Chemical characters: Q1—bicyclic quinolizidine alkaloids (lupinine, lusitanine and lamprolobine) Q2—tetracyclic *Ormosia*-type quinolizidine alkaloids Q3—tetracyclic matrine-type quinolizidine alkaloids Q4— α-pyridone alkaloids [(+)-sparteine pathway] Q5— hydroxylated lupanines and their esters [(–)-sparteine pathway] Q6—carboxylic acid esters of quinolizidine alkaloids P1—simple pyrrolizidine bases P2—macrocyclic pyrrolizidine alkaloids F1—vicenin-2 (major flavone in seeds) F2—butin (major flavanone in seeds) F3—3’-hydroxydaidzein (major isoflavone in seed) F4—orobol (major isoflavone in seeds) A1—hydroxylated anthocyanins and their esters C1—cyanogenic glycosides (prunasin and prunasin 6’-malonate)

bicyclic alkaloids). Tetracyclic *Ormosia*-type alkaloids, once thought to be restricted to the Sophoreae (present in *Ormosia*, *Acosmium*, and *Bowdichia*), are now known to occur in Brongniartieae, where they are present in the South American *Brongniartia* (Greinwald et al., 1996) as well as the Australian *Templetonia* and *Hovea*. The only other report of *Ormosia*-type alkaloids is in the Asian genus *Ammopiptanthus* (Thermopsideae), suggesting that a comparison between the Thermopsideae, Brongniartieae and the above-mentioned basal woody genistoids would be most worthwhile. This is particularly interesting in light of a suggestion by Doyle et al. (2000) that the Thermopsideae may not be monophyletic. Several species of *Sophora* (including the type species, *S. tomentosa*—Murakoshi et al., 1981—) has tetracyclic matrine alkaloids in combination with the usual and widespread α-pyridone alkaloids (summary in Southon, 1994). Matrine alkaloids are also present in the monotypic Euchrestieae, showing that the two groups may indeed be very closely related, as suggested by Polhill (1981). The possible presence of matrine alkaloids in *Ammodendron* (Polhill, 1981) needs confirmation.

The Podalyrieae (including Liparieae) and Crotalarieae differ from all other genistoid tribes in the absence of α-pyridone alkaloids and from most other tribes in the accumulation of hydroxylated lupanines and their esters, particularly in seeds. These two types of alkaloids seem to represent two independent, non-homologous biochemical pathways—they do not co-occur in any species even though they are very rarely present in the same genus, e.g. *Brongniartia* (Greinwald et al., 1996) and *Lupinus* (Wink et al., 1995). Van Wyk and Verdoorn (1995) have shown that the two pathways are enantiomer-specific and that the optical rotation of sparteine and lupanine can be used to test the homology of the alkaloid patterns in two or more taxa. The distribution of (+)-lupanine and (–)-lupanine in *Podalyria* species provided circumstantial evidence that hydroxylation and esterification of lupanine is only possible when (-)-sparteine and (+)-lupanine are the precursors. All literature data (where optical rotation has actually been measured) agrees with the assumption that (+)-spartiene and (-)-lupanine lead to α-pyridone alkaloids. When sparteine or lupanine is present in the absence of hydroxylated compounds or α-pyridones, the optical rotation becomes important for meaningful chemotaxonomic interpretations. The presence of both pathways in the woody basal genistoids, e.g. ester alkaloids in *Ormosia*, α-pyridones in *Pericopsis* (Kinghorn et al., 1988), shows that the chemical divergence must have evolved early in the phylogenetic history of the genistoid group. According to literature data (Southon, 1994) and own experience, the two pathways have never been found in a single species. Despite important differences in morphology, the genus *Cadia* seems close to the Podalyrieae because it has the same unusual carboxylic acid esters of alkaloids as is found in *Virgilia* (Greinwald et al., 1989), *Calpurnia*, *Stirtonanthus* (Van Wyk et al., 1992) and some species of *Liparia* (Van Wyk, 1991). Interestingly, Greinwald et al. (1996) found a combination of α-pyridones and ormosanine-type alkaloids in three species of *Brongniartia*, while two other species showed carboxylic acid esters of alkaloids in the absence of α-pyridones. This report is significant because it shows that esterification with carboxylic acid is not peculiar to Africa—all other genera accumulating these compounds are associated with the Podalyrieae and are from Africa or Madagascar. The chemical distinction between the Podalyrieae and other groups is blurred even more by the discovery of carboxylic acid esters of alkaloids in the southern African *Sophora inhambanensis* (Van Wyk, unpublished data). The link between *S. inhambanensis*, *Cadia* and the Podalyrieae is further supported by flavonoid data (see below). In contrast, the genus *Hypocalyptus* was clearly shown to be misplaced in the Podalyrieae-Liparieae because none of its chemical features agreed with other members of the two tribes (Schutte and Van Wyk, 1998a, 1998b). Unlike all other Podalyrieae, it accumulates the non-protein amino acid canavanine in seed (Bell et al., 1978) and is totally devoid of alkaloids.

The tribes Genisteae and Crotalarieae share the sporadic occurrence of pyrrolizidine alkaloids but the homology of the characters is in doubt. Some species of *Adenocarpus* and *Laburnum* (Genisteae) have simple pyrrolizidine bases of the norloline type, but none of the macrocyclic compounds as are present in rich abundance and diversity in *Lotononis* and *Crotalaria* (Crotalarieae—for review see Van Wyk and Verdoorn, 1990). To this can be added a new record for the Crotalarieae, namely the presence of a partially identified pyrrolizidine base in *Lebeckia wrightii* (Van Wyk, unpublished data). Quinolizidine alkaloids are absent from *Crotalaria*, but are present in several species of *Lotononis* (Van Wyk and Verdoorn, 1990). Pyrrolizidine alkaloids do not co-occur with quinolizidine alkaloids in the same species of *Lotononis* and provided convincing support for the inclusion of the genus *Buchenroedera* into *Lotononis*. Pyrrolizidine alkaloids show a definite link between *Crotalaria*, *Lotononis* and *Lebeckia* and possibly also between the tribes Crotalariae and Genisteae (the only two tribes with pyrrolizidine-bearing genera).

3.2. Flavonoids

Despite the large data set on flavonoids of the Fabaceae (Southon, 1994; Hegnauer and Hegnauer, 1992–2001), very few taxonomic conclusions can be reached. In general, the unique occurrence of isoflavonoids in the subfamily Papilionoideae and perhaps the frequent absence of a hydroxyl group in position five are interesting features. The patterns seem to be erratic and complex, but there are very few rigorous comparisons of genera or species. An exception is the study of Harborne (1969), which showed that the combination of four isoflavones (daidzein, formononetin, genistein and 5-*O*-methylgenistein) in the leaves of several species of the tribe Genisteae is highly characteristic. The rare 5-*O*-methylgenistein is a potential synapomorphy for a large part of the tribe (Genisteae sensu stricto) that excludes *Lupinus* and *Argyrolobium*. De Nysschen et al. (1998) showed that flavonoids behave rather poorly as cladistic characters but observed the same interesting absence of isoflavonoids in seeds of *Argyrolobium* and other African Genisteae (*Melolobium*, *Dichilus* and *Polhillia*). Discontinuities of taxonomic interest were found in the seed flavonoids of some genera and tribes (Table 1). The Podalyrieae and Crotalarieae were shown to have the same four major seed flavonoids (two isoflavones, one flavone and one flavanone), while none of these compounds occurred in African Genisteae. Of special interest was the presence of the isoflavone 3’-hydroxydaidzein as major seed flavonoid in the Podalyrieae and in both *Cadia purpurea* and *Sophora inhambanensis*, but not in the Crotalarieae or Genisteae. *Hypocalyptus* was again shown to be misplaced in the Liparieae, as it had only trace amounts of flavonoids in the seeds, and none of them occurred in any of the 25 genera and 87 species of genistoids examined. Seed flavonoids tend to be much more conservative than leaf flavonoids (Hegnauer and Grayer-Barkmeijer, 1993; De Nysschen et al., 1998) and their obvious potential as taxonomic characters has yet to be explored in most groups.

3.3. Anthocyanins

Pink or purple flower pigments are only sporadically present in the genistoid group (most genera and species have yellow flowers), so that anthocyanins are rarely studied. Interesting discontinuities were found in the Podalyrieae, Liparieae and Crotalarieae (Van Wyk and Winter, 1994; Van Wyk et al., 1995). Esterification appears to be a unique apomorphy for the tribe Podalyrieae (including Liparieae). Anthocyanins, if present, are esterified with coumaric or acetic acid, while species from other tribes (as far as is known) have glycosides only. In the Podalyrieae and in the related Crotalarieae, only hydroxylated anthocyanins (derivatives of cyanidin and peonidin) were found, while *Hypocalyptus* again showed itself to be misplaced by the unique presence of a methoxylated compound (malvidin-3-glucoside) as the only pigment in the flowers of all three species. It would be interesting to compare *Hypocalyptus* with purple-flowered members of the Milletieae, Indigofereae and other potential relatives. Similarly, it would be worthwhile to check if *Cadia* have esterified anthocyanins as is found in *Virgilia* and other Podalyrieae.

3.4. Cyanogenic glycosides

Cyanogenesis is a rare character in genistoid legumes (Seigler et al., 1989) and has so far been reported only from 57 species of *Lotononis* and four species of the closely related *Buchenroedera* (Van Wyk, 1989). Prunasin and prunasin 6’-malonate have been identified as main compounds (Lechtenberg et al., 1999). A report of cyanogenesis in two species of *Lupinus* needs confirmation (Seigler et al., 1989). Cyanogenesis agreed with several other characters that *Buchenroedera* is better placed as a section of *Lotononis*, close to the section *Krebsia* and other predominantly cyanogenic groups (Van Wyk, 1991). The sectional classification system for *Lotononis* was much improved when cyanogenic and acyanogenic groups of species were realigned. Cyanogenesis also contributed to the exclusion of the cyanogenic *Lebeckia microphylla* from *Lebeckia* (no other species are cyanogenic) and its inclusion in *Lotononis*.

4. Conclusions

The distribution of chemical characters in the genistoid tribes is clearly not random, but gives important clues about relationships. *Cadia* and *Hypocalyptus* are interesting examples of how morphological characters alone were insufficient to make sound taxonomic judgements. Indeed, evidence from both DNA sequence data and secondary chemistry are in agreement in the placement of these genera, *Cadia* with the genistoids close to genera of Podalyrieae, and Hypocalyptus elsewhere (probably close to Millettieae).

Chemical diversity within genera and species is quite common and can perhaps best be explained by the activation or deactivation of existing genes for the biosynthesis of particular compounds. As a result, multiple losses or gains may result in incomprehensible patterns, but it would be wrong to dismiss the characters outright simply because they are not always reliable. There are many cases where anomalies turned out to be reliable synapomorphies once the taxon and its relations became better known. The extensive rearrangements at tribal and generic levels within the Podalyrieae, Crotalarieae and Genisteae over the last decade (Van Wyk and Schutte, 1995; Schutte and Van Wyk, 1998a) were based on morphological, cytological and chemical evidence, and predate molecular phylogenetic studies based on DNA sequences.

Homology of chemical characters in two or more taxa (as opposed to similarity) is rarely investigated and remains poorly known, because the biochemical pathways and biogenetic relationships of the compounds present are rarely studied. An exception is the study of *Podalyria* alkaloids. The presence of two optical isomers of lupanine in this genus showed that chemical homology (or non-homology in this case) may help to explain confusing patterns. Even more important is the need for rigorous comparisons (such as the use of seeds rather than above-ground parts, because they are likely to be more uniform and conservative). Many genera remain chemically unknown or poorly known, so that much work remains to be done. It is an exciting challenge to explore the congruence between chemical characters and the emerging molecular phylogeny for the tribes and genera of genistoid legumes.

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