

Alkaloids as Taxonomic Characters in the Tribe Crotalariaeae (Fabaceae)

BEN-ERIK VAN WYK* and GERHARD H. VERDOORNT

*Department of Botany and †Department of Chemistry and Biochemistry, Rand Afrikaans University, P.O. Box 524, Johannesburg 2000, South Africa

Key Word Index—Crotalariaeae; Leguminosae; piperidyl alkaloids; pyrrolizidine alkaloids; quinolizidine alkaloids; chemotaxonomy; generic relationships.

Abstract—Recent studies of alkaloids have led to a better understanding of the intricate taxonomic relationships in the tribe Crotalariaeae. Data from alkaloidal metabolites are largely congruent with morphological data, but allow refinement of the understanding of the phylogeny. A well-supported and almost fully resolved cladogram is presented, which shows that the genera can be grouped into four main clades: (1) an unspecialized group without α -pyridone alkaloids and without esters of alkaloids (*Aspalathus*, *Lebeckia*, *Rafnia*, *Wiborgia* and perhaps *Spartidium*); (2) a group specializing in lupanine-type esters (*Pearsonia* and *Rothia*); (3) a group with macrocyclic pyrrolizidine alkaloids (*Crotalaria* and *Lotononis*); and (4) a specialized group with α -pyridone alkaloids (*Argyrolobium*, *Dichilus*, *Melolobium* and *Polhillia*).

Introduction

Detailed morphological studies have shown the widespread occurrence of convergence and conflicting character information in the tribe Crotalariaeae [1]. Only a few of the genera are monothetic taxa and very few characters are available for cladistic analyses. This is not so much due to a lack of data but rather to ambiguity as to how morphological characters should be interpreted. Our studies of alkaloids as independent criteria have now reached a stage where meaningful comparisons can be made within the tribe as a whole. With the exception of *Crotalaria* L., virtually nothing, until recently, was known about the alkaloids of the tribe [2, 3]. In the tribe Genisteae, alkaloid patterns show a high degree of conformity with evolutionary patterns based on morphological evidence [1] and it is accepted that alkaloids have value as generic characters in the Leguminosae [3]. In this paper, we present the results of cladistic analyses of relationships in the tribe Crotalariaeae using alkaloidal and morphological evidence.

The intricate relationships amongst the genera of the Crotalariaeae are not yet resolved [1, 4–8]. In the latest treatment at the tribal level [9], 16 genera are included: *Lebeckia* Thunb. (ca 35 spp.); *Wiborgia* Thunb. (10 spp.); *Rafnia* Thunb. (ca 23 spp.); *Aspalathus* L. (278 spp.);

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Spartidium Pomel (one sp.); *Crotalaria* (ca 600 spp.); *Bolusia* Benth. (five spp.); *Lotononis* (DC.) Eckl. & Zeyh. (150 spp. as presently circumscribed); *Buchenroedera* Eckl. & Zeyh. (11 spp., now treated as a section of *Lotononis*); *Pearsonia* Dummer (12 spp.); *Rothia* Pers. (two spp.); *Robynsiophyton* Wilczek (one spp.); *Melolobium* Eckl. & Zeyh. (ca 20 spp.), *Dichilus* DC. (five spp.); and two other genera that were only tentatively included, namely *Anarthrophyllum* Benth. (15 spp.) and *Sellocharis* Taub. (one sp.). It was recently suggested [8] that *Argyrolobium* Eckl. & Zeyh. (ca 70 spp.) and the newly described *Polhillia* Stirton (seven spp.) are closely related and that both should be included in the Crotalariaeae near *Melolobium*. The correct taxonomic position of the two highly modified South American genera *Anarthrophyllum* and *Sellocharis* is very doubtful [1, 9] and is not considered here. Cytological evidence [10] indeed suggests a position in the tribe Genisteae near *Lupinus* L. The two small genera *Bolusia* and *Robynsiophyton* could also not be included in this study (their alkaloids have not yet been investigated), but they are assumed to be local derivatives of, respectively, *Crotalaria* and *Pearsonia* [1].

Conclusions about generic relationships, as reflected in the sequence of genera in Polhill [9], were based on morphological similarities and

considerable experience in legume taxonomy. It is possible to translate the sequence into the phylogenetic tree given in Fig. 1. Polhill never gave explicit ideas about the sequence of branching and the figure is used here only to show what is probably a fairly accurate summary of presumed taxonomic relationships based on morphological evidence. *Argyrolobium* and *Polhillia* are included in the group of genera with bilabiate calyces, as suggested previously [8]. *Polhillia* is not shown separately in Fig. 1. The "Lotononis group" [9] includes all the genera with zygomorphic calyces, i.e. those which have the upper and lateral lobes on either side fused higher up in pairs. This group is shown as the polychotomy B in Fig. 1

A preliminary study of alkaloid patterns in

some genera [11] indicated that chemical evidence may have considerable value as a means of testing the predictivity of the presumed affinities as given in Fig. 1. A systematic survey of alkaloids in all the major genera confirmed the presence of definitive qualitative and quantitative discontinuities. Several quinolizidine (1–29), imidazole (30, 31), piperidyl (32–43) and pyrrolizidine (44–113) alkaloids were identified in extracts from species of *Argyrolobium* [12, 13], *Aspalathus* [14], *Dichilus* [15], *Lebeckia* [16, 17], *Lotononis* [18, 19], *Melolobium* [20], *Pearsonia* [21], *Polhillia* [22], *Rafnia* [14], *Rothia* [23], *Spartidium* [24] and *Wiborgia* [14]. The known distribution of all alkaloids reported from 13 genera of the tribe is summarized in Table 1.

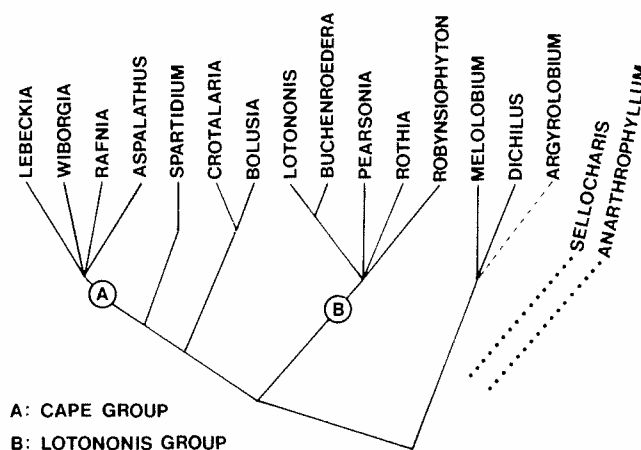
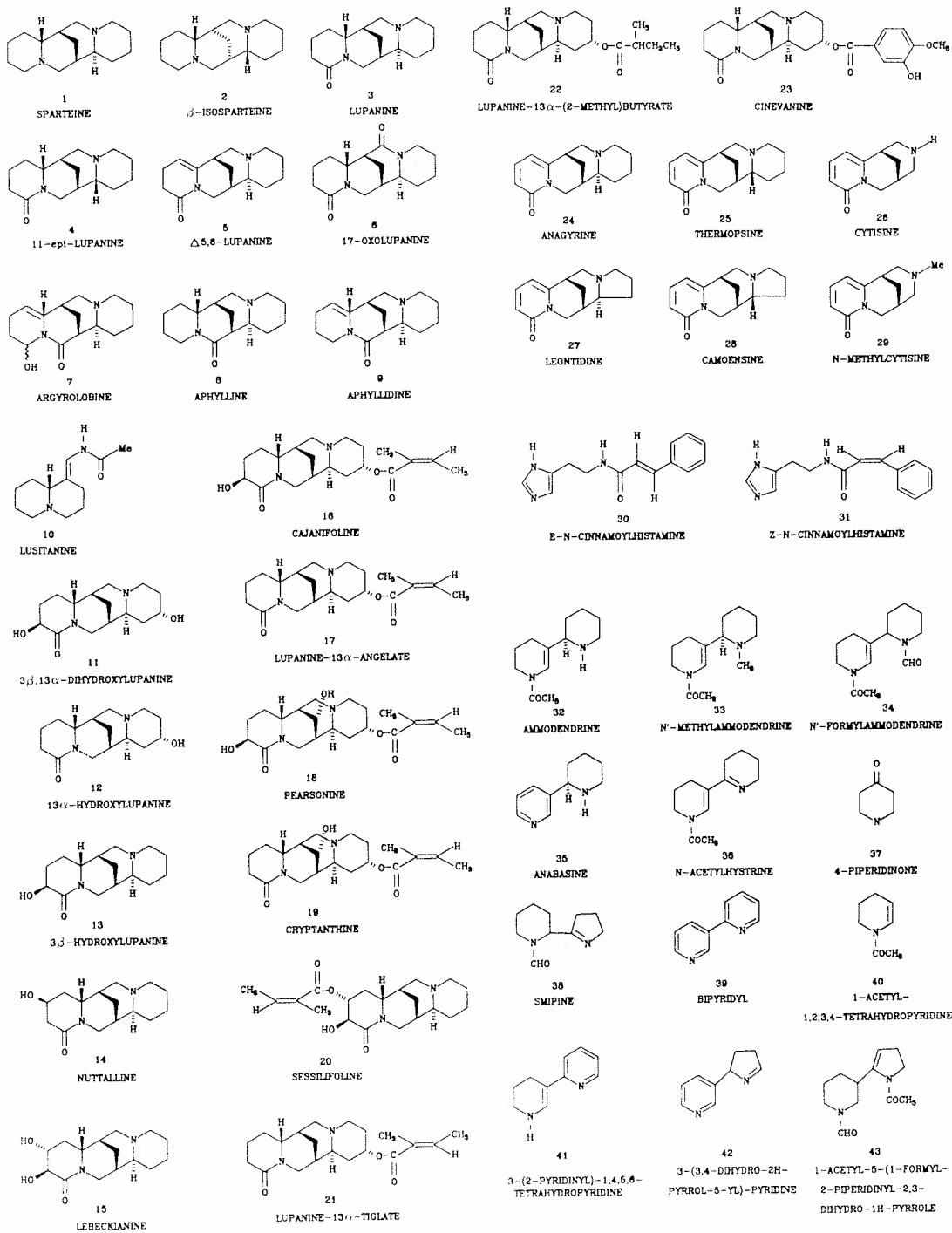
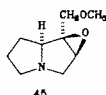
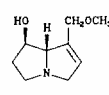
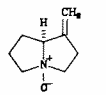
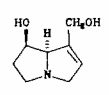
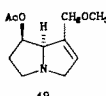
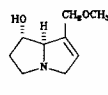
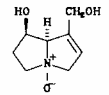
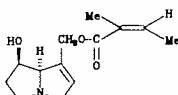
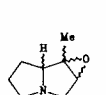
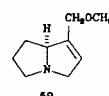
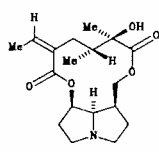
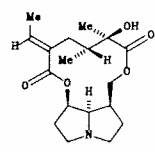
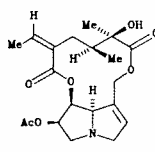
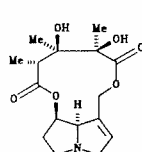
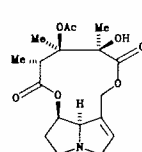
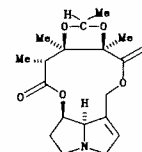
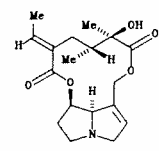
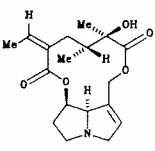
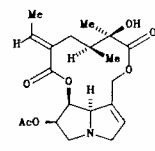
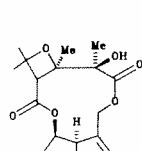
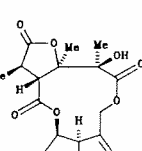
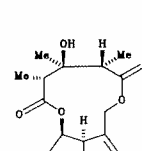
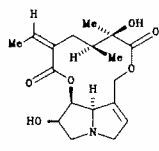
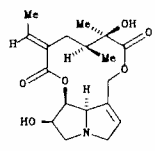
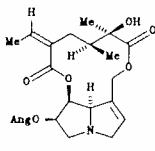
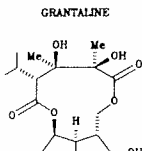
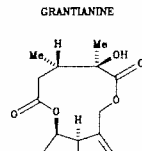
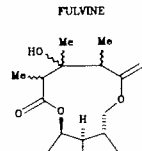


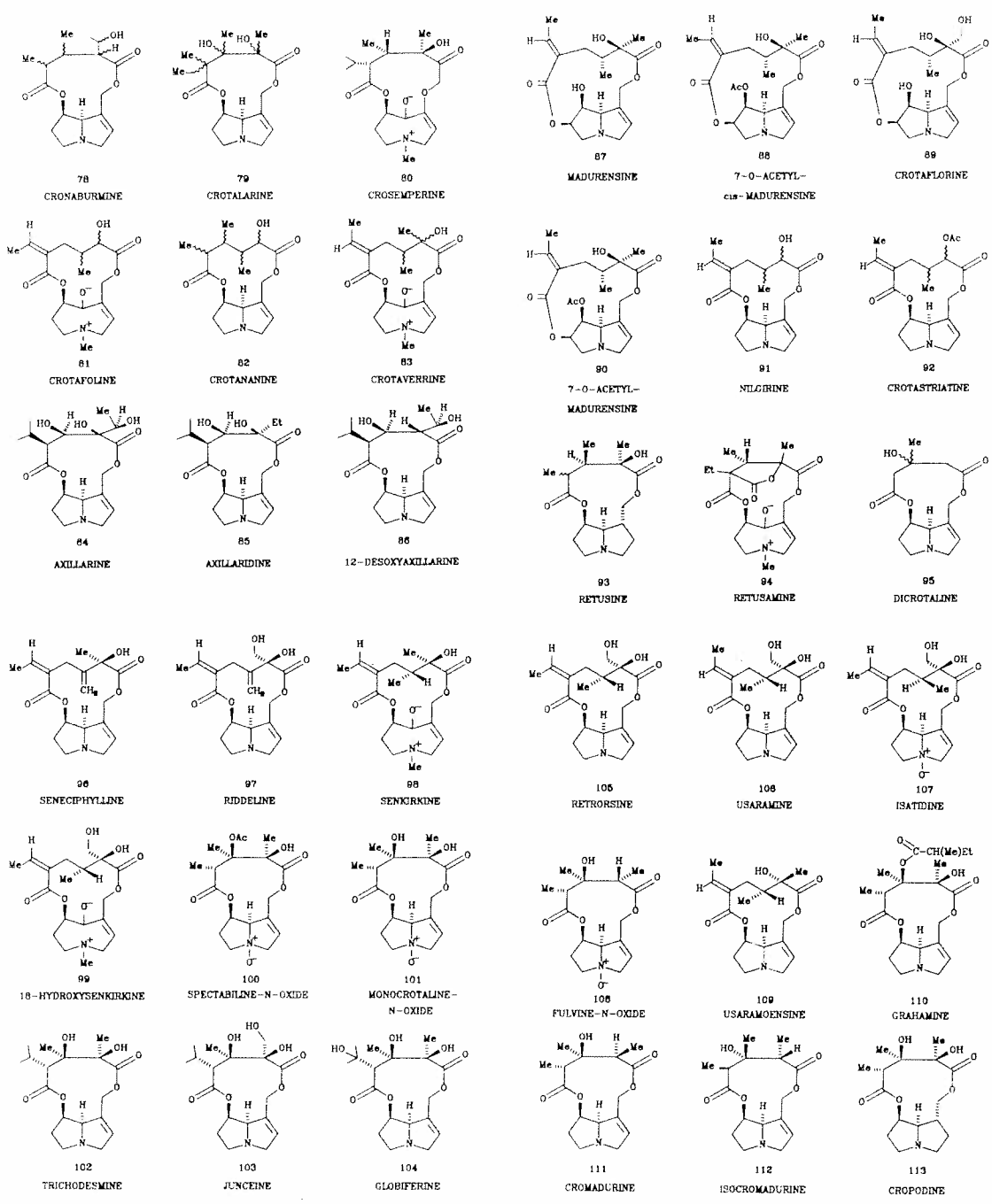
FIG. 1. PHYLOGENETIC TREE SHOWING RELATIONSHIPS BETWEEN GENERA OF THE TRIBE CROTALARIEAE. The topology (based on morphological evidence) as suggested by the sequence of genera in the latest tribal revision [1, 9].

TABLE 1. SUMMARY OF ALKALOIDS REPORTED FROM 13 GENERA OF THE TRIBE CROTALARIEAE

Genus	(No. of spp. studied /total no. of spp.)	Compounds reported	Refs
<i>Argyrolobium</i>	(15/70)	1, 3, 5, 7, 8, 10, 24, 26, 29, 32	[12, 13, 22]
<i>Aspalathus</i>	(11/278)	1, 3, 4, 13, 32	[14]
<i>Crotalaria</i>	(49/600)	44–113, except 60, 61, 63	[25–27]
<i>Dichilus</i>	(5/5)	3, 24, 25, 29, 32–43	[15]
<i>Lebeckia</i>	(14/35)	1, 3, 4, 13, 15, 32	[16, 17]
<i>Lotononis</i>	(52/150)	1, 3, 4, 13, 32, 60, 61, 63, 64	[18, 19]
<i>Melolobium</i>	(11/20)	1, 3–6, 13, 24–29, 32	[20]
<i>Pearsonia</i>	(4/11)	1, 3, 4, 13, 16–20, 32	[21, 28]
<i>Polhillia</i>	(5/7)	1, 3, 10, 24, 26, 29, 32	[22]
<i>Rafnia</i>	(6/23)	1, 3, 13, 32	[14]
<i>Rothia</i>	(2/2)	2, 3, 12, 14, (13?), 17, 21–23	[23]
<i>Spartidium</i>	(1/1)	1, 30–32, 34	[24]
<i>Wiborgia</i>	(3/10)	1, 3, 4, 13, 32	[14]



44
1,β,2β-EPOXY-1α-HYDROXY-
METHYL-8α-PYRROLIZIDINE45
1α-METHOXYMETHYL-1,β,2β-EPOXY-
8α-PYRROLIZIDINE52
7β-HYDROXY-1-METHYLENE-
8α-PYRROLIZIDINE53
7β-HYDROXY-1-METHOXYMETHYL-1,2-
DEHYDRO-8β-PYRROLIZIDINE46
1-METHYLENE-8α-
PYRROLIZIDINE47
1-METHYLENE-8α-
PYRROLIZIDINE-N-OXIDE54
1-HYDROXYMETHYL-1,2-DEHYDRO-
8β-PYRROLIZIDINE55
RETRONECINE48
7β-HYDROXY-1-METHOXYMETHYL-
1,2-DEHYDRO-8α-PYRROLIZIDINE49
7β-ACETOXY-1-METHOXYMETHYL-
1,2-DEHYDRO-8α-PYRROLIZIDINE56
9-O-METHYL-
HELIOTRIDINE57
ISATIDINE60
RIVULARINE51
BASE C58
7β-HYDROXY-1-METHYLENE-
8β-PYRROLIZIDINE59
1-METHOXYMETHYL-1,2-DEHYDRO-
8α-PYRROLIZIDINE60
PLATYPHYLLINE61
NEOPLATYPHYLLINE62
ACETYLANACROTINE69
MONOCROTALINE70
SPECTABILINE71
MONOCROTALININE63
INTEGERRIMINE64
SENECIOTIONINE65
ACETYL-trans-ANACROTINE72
GRANTALINE73
GRANTIANINE74
FULVINE66
ANACROTINE67
ISOANACROTINE68
8-ANGELOYLANACROTINE75
CROALBIDINE76
CROBARBATINE77
CROCANDINE



Of particular significance was the discovery of macrocyclic pyrrolizidine esters in *Lotononis* and *Buchenroedera* [18, 19], which confirmed the idea of a close relationship between the two genera and also indicated a possible relationship with the genus *Crotalaria*. The isolation of several lupanine-type esters from species of the genus *Pearsonia* [21, 28] was also of special interest, because these angelate and tiglate esters appear to be restricted to *Rothia* and *Pearsonia*. Some new compounds with unusual substitution patterns were found in *Pearsonia* and this discovery led to the structural elucidation of 3 β -hydroxylupanine (**13**), a compound previously known as 4 β -hydroxylupanine (nuttalline) (**14**) [28].

The biochemical pathways along which most of the alkaloids are formed have been studied and reviewed by several workers [25, 26, 29–31] and are now rather well-known. Detailed summaries of the pathways for quinolizidine alkaloids are available [32–34]. This information is very useful because it allows meaningful comparisons of the biosynthetic routes in which the different genera seem to specialize.

Results and Discussion

All available data on the distribution of alkaloids in the tribe were summarized in the form of "alkaloid profiles" for each of the genera as shown in Fig. 2. This figure also gives a diagrammatic summary of the most likely biosynthetic pathways of quinolizidine and pyrrolizidine alkaloids, the various steps of which are explained in the caption. The alkaloid patterns suggest four major groups. (1) An unspecialized group without α -pyridone alkaloids and without esters of alkaloids (*Aspalathus*, *Lebeckia*, *Rafnia*, *Wiborgia* and *Spartidium*). (2) A group specializing in lupanine-type esters (*Pearsonia* and *Rothia*). (3) A group with macrocyclic pyrrolizidine alkaloids (*Crotalaria* and *Lotononis sensu lato*, i.e. *Buchenroedera* included). (4) A specialized group with α -pyridone alkaloids (*Argyrolobium*, *Dichilus*, *Melolobium* and *Polhillia*).

Furthermore, it was possible to polarize the character states for a cladistic analysis based on alkaloid data alone. These characters and the way in which they were polarized are shown in Table 2 (the first 12 characters are from alkaloid data, characters 13–25 were added for later

analyses). Cladistic analyses were performed to evaluate the significance of the alkaloid data in determining the most likely phylogenetic relationships between the genera. The computer program HENNIG 86 (Farris 1988, Hennig 86 reference, Version 1.5) was used in the following three analyses. The "mhennig*" and "bb*" commands were used to make sure that all possible topologies were found. The three analyses were as follows. (A) Alkaloid data only (characters 1–12, polarized as in Table 2). (B) Morphological data and chromosome base number (characters 13–25, polarized as in Table 2). (C) All characters combined (complete data set as in Table 2).

Analysis A (with autapomorphies excluded) resulted in a single tree with a length of 12 character state changes and a consistency index of 75. This cladogram is shown in Fig. 3a. As would be expected for such a small number of characters, the cladogram is only partly resolved and includes several polychotomies. Nevertheless, the high consistency index shows that alkaloids are useful as cladistic characters and that the grouping based on similarities ("alkaloid profiles" in Fig. 2) is valid.

The similarity between the alkaloid cladogram (Fig. 3a) and the phylogenetic tree based on morphological evidence (Fig. 1) is obvious. Both show an early divergence of the group of genera with bilabiate calyces, followed by the two genera with "gullet-type" flowers (*Pearsonia* and *Rothia*). The cladogram differs from the phylogenetic tree mainly in the relationships between Polhill's [9] "Cape genera" and the *Lotononis* group. The Cape genera still forms a polychotomy but it now also includes *Crotalaria* and *Lotononis*, the latter shown to be misplaced in the *Lotononis* group. The distribution of major alkaloids strongly suggests that *Crotalaria* and *Lotononis* are sister groups (defined by the presence of pyrrolizidine alkaloids) and that *Pearsonia* and *Rothia* represent a separate specialization (presence of lupanine-type esters). This conflict between morphological and alkaloidal evidence was explored in more detail in a second analysis, using only morphological data and chromosome base number (analysis B).

The virtual absence of monothetic characters in the tribe Crotalariaeae is well-known, and makes it difficult to find reliable characters for

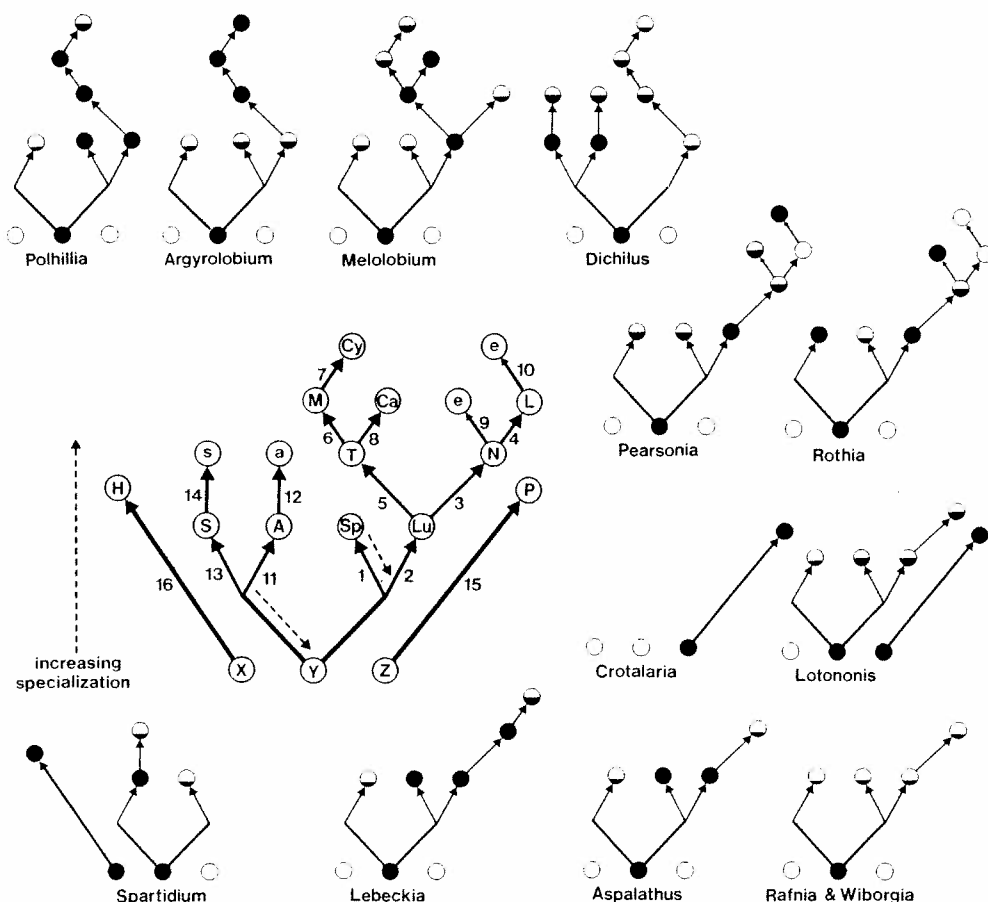


FIG. 2. DIAGRAMMATIC SUMMARY OF MAJOR BIOGENETIC PATHWAYS OF THE ALKALOIDS REPORTED FROM THE TRIBE CROTALARIEAE AND A COMPARISON OF ALKALOID PROFILES OF 13 GENERA FOR WHICH ALKALOID DATA ARE AVAILABLE. Directions of arrows suggest increasing specialization. Major pathways: X=imidazole alkaloids; Y=quinolizidine alkaloids (lysine pathway); Z=pyrrolizidine alkaloids (ornithine pathway). Basic type(s) of major compounds (indicated by letters): H=histamine derivatives; S=smipine; s=N'-derivatives of smipine; A=ammodendrine; a=N'-derivatives and aromatic derivatives of ammodendrine; Sp=sparteine; Lu=lupanine and lupanine stereoisomers; T=lupanine-type structures with α -pyridone ring (thermopsine and anagyrine); M=N-methylcytisine; Cy=cytisine; Ca=camoensine and leontidine; N=monohydroxylated lupanines (nuttalline and 13 α -hydroxylupanine); L=polyhydroxylated lupanines (lebeckianine the only example reported from the Crotalarieae thus far); e=mono-esters of hydroxylated compounds. Steps in pathways (indicated by numbers): 1=cadaverine to sparteine (loss of ability to accumulate sparteine is taken to be a derived character state as indicated by broken arrow); 2=transformation of α -ring to carbonyl; 3=hydroxylation of lupanine; 4=increased hydroxylation; 5=olefination of α -ring; 6=fissure of δ -ring and removal of a propyl moiety; 7=demethylation of N-methylcytisine; 8=fissure of δ -ring, demethylation of N-butyl moiety and ring fusion; 9=esterification with angelic-, tiglic- and Z and E cinnamic acids on C-13 position; 10=mono-esterification of C-4 or C-13 positions with angelic acid; 11=formation of piperidine ring; 12=N'-derivatization of ammodendrine and aromatization of piperidine rings; 13=formation of pyrrole ring; 14=N'-derivatization; 15=formation of macrocyclic ester from pyrrolizidine base and necic acid; 16=amidation of histamine by Z and E cinnamic acids.

cladistic analyses. Many morphological characters are distributed in such a way that they can only be used if the present generic limits are ignored and totally artificial groups are created. Some of these characters would lead to an unsatisfactorily high incidence of homoplasy as a result of convergence. It has been argued that variable characters should be left out of analyses or should be coded as unknown [38], but this

seems very restrictive and, except for some generic autapomorphies, would exclude virtually all of the few morphological characters that are available. Table 2 shows 13 characters (mainly morphological) that seemed potentially useful and which could be added to the alkaloid data. Polarity decisions (except for obvious ones) are given in the footnotes of Table 2. This data set of 13 characters produced 38 equally parsimonious

TABLE 2. CHARACTERS AND POLARIZATION OF CHARACTER STATES IN 13 GENERA OF THE TRIBE CROTALARIEAE*

Taxa	Characters and character states																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Hypothetical ancestor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Argyrobolium</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aspalathus</i>	0	0	0	1	0	0	0	0	1	0	0	1	0	0	2	0	1	0	1	0	0	1	0	0	0
<i>Crotalaria</i>	0	1	0	1	0	1	0	0	0	0	1	1	0	1	0	1	0	0	0	1	0	0	1	0	0
<i>Dichilus</i>	0	0	1	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
<i>Lebeckia</i>	0	0	0	1	0	0	0	0	0	2	0	0	1	0	1	0	1	0	1	0	1	0	1	0	2
<i>Lotononis</i>	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1	0	1	1	0	1	0	1	0	0
<i>Melolobium</i>	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	2
<i>Pearsonia</i>	0	0	0	0	0	0	0	0	0	1	2	0	0	0	1	0	1	1	0	1	0	1	0	0	0
<i>Polhillia</i>	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1
<i>Rafnia</i>	0	0	0	1	0	0	0	0	0	1	0	0	1	0	1	0	1	0	1	0	1	0	0	0	1
<i>Rothia</i>	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	1	0	1	0	1	0	0	0
<i>Spartidium</i>	1	0	1	0	0	0	0	0	0	0	0	0	1	0	2	0	1	0	1	0	0	0	0	0	?
<i>Wilborgia</i>	0	0	0	1	0	0	0	0	0	1	0	0	0	0	2	0	1	0	1	0	1	0	1	0	2

Characters and polarization of character states:

Alkaloidal evidence (comments in parentheses refer to Fig. 2):

1. Histamine derivatives (pathway X): absent⁰, present¹.
2. Quinolizidine and piperidyl alkaloids (lysine pathway, Y): present⁰, absent¹.
3. Smpine and derivatives (steps 13 and 14): absent⁰, present¹.
4. Ammodendrine (step 11): present as a major compound in at least some species⁰, at best in trace quantities¹.
5. Ammodendrine derivatives (step 12): absent⁰, present¹.
6. Sparteine (step 1): present⁰, absent¹.
7. α -Pyridone alkaloids (step 5): absent⁰, present¹.
8. Thermopsine, rather than anagryrine (T): absent⁰, present¹.
9. C₁₄ α -pyridones (step 8): absent⁰, present¹.
10. Hydroxylated lupanines (steps 3 and 4): † absent⁰, monohydroxy type; (step 3): present¹, polyhydroxy type; (step 4): present².
11. Lupanine-type esters (steps 9 and 10): ‡ absent⁰, monohydroxy type; (step 9): present¹, polyhydroxy type; (step 10): present².
12. Pyrrolizidine alkaloids (pathway Z): absent⁰, present¹.

Morphological evidence

13. Leaf type: digitate⁰, often unifoliate or simple¹.
14. Petiole anatomy: fibres usually not present on the adaxial side of the main vascular bundle⁰; fibres invariably present¹. (This character state has been found in some species of *Crotalaria* and *Lotononis*, but so far not in any of the other genera—see ref. [8].)
15. Stipules: invariably present⁰, often dimorphic and/or absent¹, invariably absent².
16. Stipule attachment: narrow⁰, clasping the stem¹.
17. Calyx structure (Genisteae or Podalyriaceae/Lipariaceae as outgroup): with a trifid lower lip⁰, without a trifid lower lip¹.
18. Calyx (lateral sinuses), as deep or deeper than the upper and lower ones⁰, shallower¹.
19. Carinal anther (Genisteae or Podalyriaceae/Lipariaceae as outgroup), similar to basifixed ones (5+5 arrangement)⁰, intermediate in size (6+4 arrangement)¹.
20. Anther dimorphism (lack of dimorphism is considered to be a derived condition in *Pearsonia* and *Rothia* [35]): dimorphic⁰, all similar in size and shape¹.
21. Gynoecium base: sessile and subsessile⁰, often stipitate¹.
22. Style curvature: curved upwards⁰, straight or down-curved¹.
23. Fruit appendages: pod not winged⁰, pod with at least the upper suture winged (in some species)¹.
24. Seed surface: smooth⁰, often rugose or tuberculate¹.

Cytological evidence

25. Chromosome base number (see refs. [10, 36, 37] and discussion in the text): 7⁰, 8¹, 9².

*Autapomorphies in alkaloid characters were not removed because they show the way in which these characters were polarized.

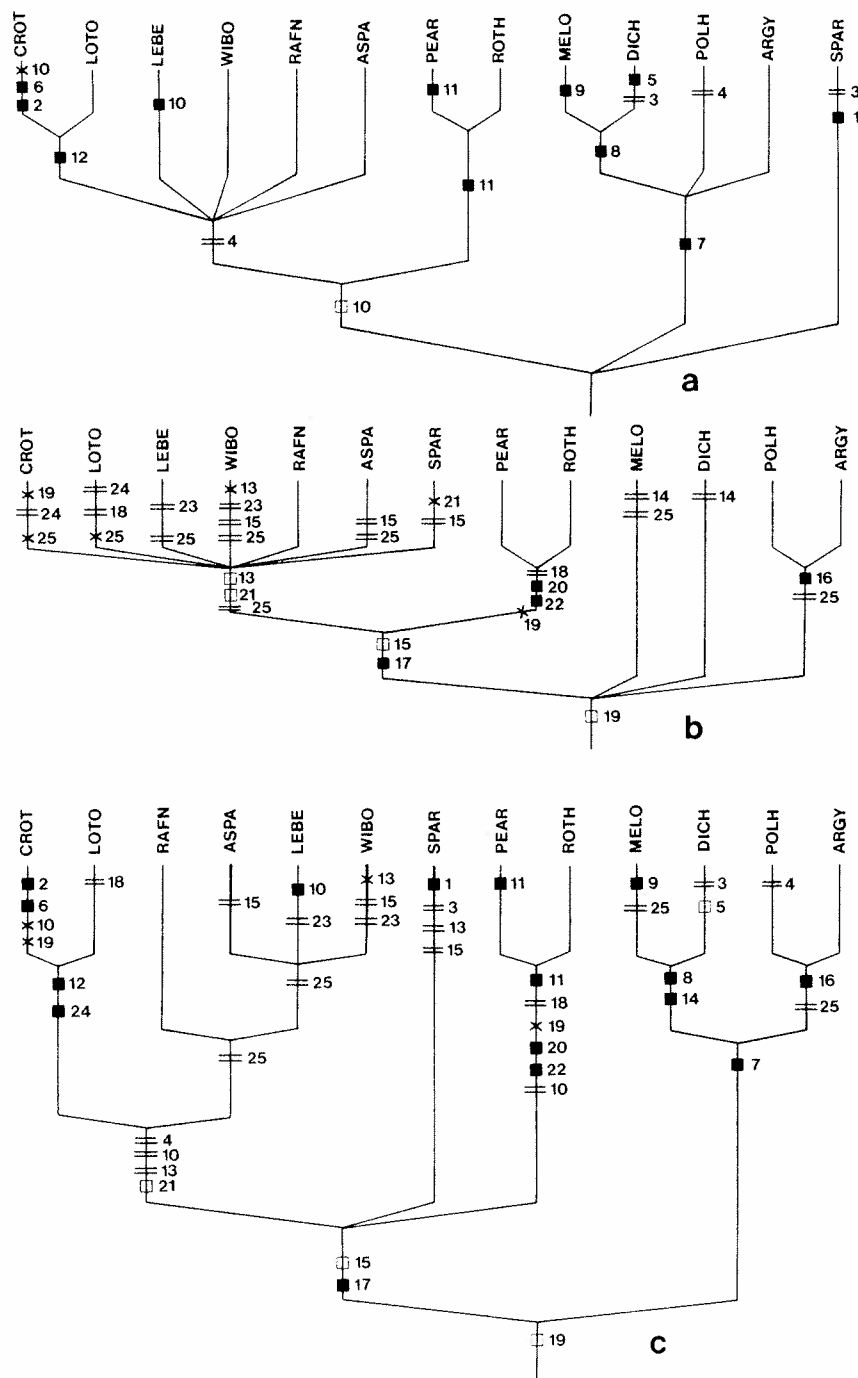


FIG. 3. PHYLOGENETIC RELATIONSHIPS BETWEEN 13 GENERA OF THE TRIBE CROTALARIEAE. (a) Cladogram based on alkaloid data only. (b) Consensus Tree based on morphological data and chromosome base number only. (c) Consensus Tree based on the complete data set as given in Table 2. (The four trees of minimum length which resulted from the combined data set are shown in Fig. 4.) Key to the symbols used: ■—apomorphies showing no reversal or convergence; □—apomorphies with reversal higher up in the cladogram; X—reversals; =—convergences. Characters are numbered as in Table 2.

trees (length 24, consistency index 62) with the "bb*" routine of HENNIG 86. A Consensus Tree of this result is shown in Fig. 3b. It shows the same terminal polychotomy ("Cape group") as the alkaloid cladogram (except that *Spartidium* is now included), the same position for *Pearsonia* and *Rothia* and also a basal position for the genera with bilabiate calyces. The latter, however, is no longer shown as a clade. The rather high consistency index of the 38 shortest trees indicates that the large number of trees is not entirely due to ambiguity in the data but rather to an imbalance between the number of characters and the number of genera. Interestingly, the morphological data also fail to resolve the Cape genera polychotomy, but it provides evidence that this group should in fact include *Lotononis* and *Crotalaria*, as was indicated by the alkaloid data.

Finally, the complete data set of 25 characters was analysed (autapomorphies again excluded), using the "mhennig*", "bb*" and "ie*" commands of HENNIG 86. The latter two options produced the same result, namely four shortest

trees with a length of 37 character state changes and a consistency index of 64. The four cladograms are shown in Fig. 4. This result is virtually the same as with the previous two analyses, except that the Cape genera polychotomy is now almost fully resolved. The only differences between the four cladograms in Fig. 4 are the position of *Spartidium* and the sequence of branching in *Lebeckia*, *Aspalathus* and *Wiborgia*. This is obvious from the Consensus Tree, which is shown in Fig. 3c (placed here to facilitate the comparison with previous results). The basic topology of all the cladograms shown in Fig. 3 is quite similar. Clearly, there is a remarkable congruence between the alkaloid data and the morphological data. When combined, the two data sets produced an almost fully resolved cladogram.

A large number of modifications to the matrix shown in Table 2 was analysed to study the effect of different polarizations on tree length and tree topology. When *Crotalaria* and *Lebeckia* were used as outgroups for the tribe as a whole, the consistency indices were generally much

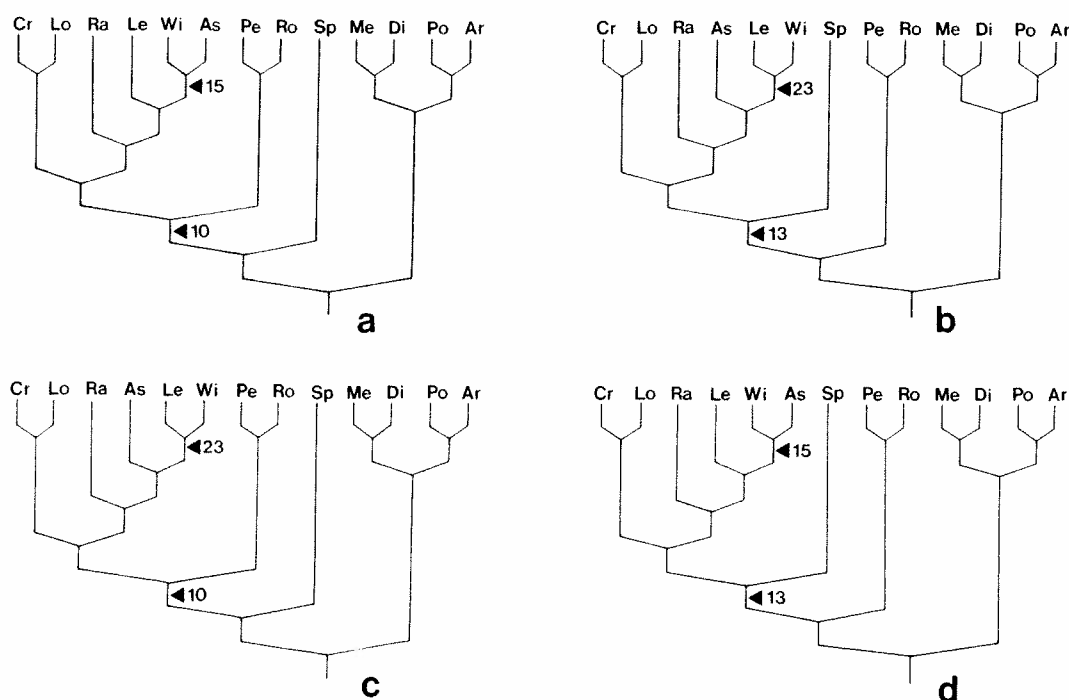


FIG. 4. PHYLOGENETIC RELATIONSHIPS BETWEEN 13 GENERA OF THE TRIBE CROTALARIEAE. (a-d) Four shortest cladograms produced by the complete data set in Table 2; the characters responsible for the lack of resolution are indicated.

lower. The respective topologies produced with these two genera as outgroups were often equally parsimonious but very different from one another. Chromosome base number invariably showed the highest number of changes and seems critically important, particularly because even minor changes in the polarity decision have a dramatic effect on the topology of the Cape group. When $x=7$ was polarized as the base number for the tribe as a whole (contrary to current belief that $x=9$ is basic [10]) the tree lengths and consistency indices were generally much improved and never resulted in longer trees. When $x=9$ is taken as basic, the character usually shows an early advance to $x=7$, with subsequent reversals back to 8 and 9. Except for *Crotalaria* and *Lotononis*, base numbers for the genera were taken from Goldblatt [10]. The base number for *Crotalaria* is given here as $x=8$, but it has been suggested [39] that $x=7$ in the subsection *Incanae* may be the plesiomorphic condition. Several counts for *Lotononis* [36] have shown the common occurrence of $2n=28$ and it is here suggested that $2n=18$ may be a secondary development in *Lotononis* (all annual species, for example, have $2n=18$). *Dichilus* ($2n=28$ in all five species) was recently shown to be tetraploid (Van Wyk and Schutte, unpublished observations) so that $x=7$ can be accepted as the base number for this genus. The presence of $x=7$ has been reported in a few species of *Aspalathus*, but it was argued that the condition is secondary and that $x=9$ is basic for *Aspalathus* [5, 40]. The chromosome number for *Spartidium* is not known and more counts, also for some of the other genera, are clearly needed. However, it seems that $x=9$ as a base number for the tribe as a whole may have to be reconsidered.

The Consensus Tree produced by the complete data set (Fig. 3c) shows a remarkable degree of conformity with the presumed evolutionary sequence suggested by morphological evidence (Fig. 1) and differs from the other two cladograms (Fig. 3a,b) only in that it is much better resolved. It reflects the intricate relationships between Polhill's "Cape genera" [9] (*Aspalathus*, *Lebeckia*, *Rafnia* and *Wiborgia*) and suggests that *Crotalaria* and *Lotononis* should be included here as sister taxa. The position of *Spartidium* shows the lack of evidence (other

than morphological similarity) to support a close relationship with *Lebeckia*. The *Pearsonia*-*Rothia* and *Argyrolobium*-*Melolobium* clades are defined by rather convincing and unambiguous apomorphies.

Of the four equally parsimonious trees in Fig. 4, the cladogram shown as b appears to be superior for two reasons. Firstly, *Spartidium* is morphologically virtually indistinguishable from *Lebeckia* [1] so that character 13 (simple leaves) appears to be a more convincing synapomorphy than character 10 (presence of hydroxylated lupanines). Secondly, character 23 (winged upper suture of the fruit) is clearly preferable to character 15 (total absence of stipules) because the latter synapomorphy also occurs in most species of *Lebeckia*. It should be noted that *Lebeckia* and *Wiborgia* are considered to be only marginally distinct at the generic level [5, 7]. Species of *Lebeckia* sect. *Wiborgioides* Benth. are morphologically almost identical to species of *Wiborgia* and the same is true for their alkaloids [14, 17]. The cladogram in Fig. 4b is therefore presented here as the most likely estimate of evolutionary relationships in the tribe Crotalarieae.

As a final presentation of available evidence, and to illustrate the predictive value of the hierarchy in Fig. 4b, the distribution of generic autapomorphies and other taxonomically useful characters was plotted on the chosen topology as shown in Fig. 5. The distribution of character states in Fig. 5 clearly shows that the chosen topology has considerable predictive value. (To us, predictivity means that new information about the taxa of a particular classification will tend to agree with their hierarchical arrangement as previously obtained from other information. It also means that the hierarchical pattern in a classification does not depend merely on one or more defining characters.) Examples are the presence of lupanine-type esters in the only two genera with resupinate flowers (*Pearsonia* and *Rothia*), the presence of pyrrolizidine esters in the only two genera which (at least occasionally) have rugose or tuberculate seeds, distinctly beaked keel petals and blue flowers, and the presence of α -pyridone alkaloids in all genera with bilabiate calyces. The position of *Lotononis* as a sister group of *Crotalaria* seems reasonable, and that both share a common ancestor with

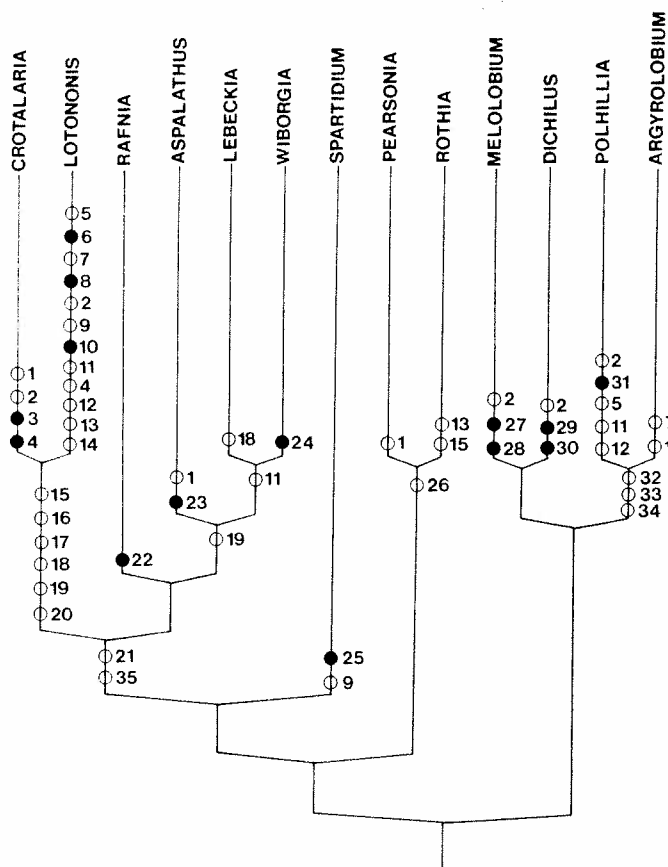


FIG. 5. CHOSEN CLADOGRAM (from Fig. 4b), SHOWING THE DISTRIBUTION OF TAXONOMICALLY USEFUL CHARACTERS (●) AND VARIABLE OR CONVERGENT CHARACTERS (○) THAT WERE NOT USED IN THE CLADISTIC ANALYSES. The character states are: 1=aneuploidy; 2=chromosome number; 3=style geniculate; 4=keel strongly beaked; 5=flexuous-plicate pods; 6=cyanogenesis; 7=dimorphic flowers; 8=polyploidy; 9=long funicles; 10=fruit with the upper suture verrucose; 11=fruit indehiscent; 12=absence of bracteoles; 13=single stipules; 14=geoxiphytic habit; 15=annual habit; 16=style hairy; 17=lobes and callosities on standard petal; 18=turgid fruit; 19=stem thorns; 20=flowers at least partly blue (not to be confused with purple); 21=keel petals often acute; 22=leaves totally glabrous; 23=leaflets sessile (i.e. petiole absent); 24=fruit winged; 25=seed orientation (at right-angle with the funicle); 26=resupinate flowers; 27=cordate (lobed) stipules; 28=epidermal glands; 29=spurred wing petals; 30=stipules minute; 31=internode below inflorescence elongated; 32=stipules often fused (with each other and/or with the petiole); 33=keel petals imbricate along lower side; 34=stamens fused into a closed sheath; 35=biramous epidermal hairs.

Rafnia, *Aspalathus* and *Lebeckia* is equally likely. Despite similarities between *Lotononis* and *Pearsonia* (notably the calyx structure), the data do not support a close relationship between these two genera but rather suggest a relatively early divergence of *Pearsonia* and *Rothia* from the rest of the *Lebeckia* clade. This agrees with the presence of *Pearsonia* in Madagascar (evidently separated from the African continent early in the geological history [41] and also the presence of *Rothia* in Asia and Australia (the latter perhaps a result of early dispersal when land masses were

somewhat less separated). It may be argued that *Crotalaria* has an even wider distribution, but an African origin was suggested for this genus [42] with secondary diversification outside Africa. The divergence of the *Argyrolobium* clade seems even earlier and this basal dichotomy is clearly the most obvious place for a tribal or subtribal division.

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