



BIOSYNTHETIC INTERDEPENDENCE OF LIGNINS AND SECONDARY METABOLITES IN ANGIOSPERMS*

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Key Word Index—Angiosperms; macrosystematics; cell walls; lignins; Sporne indices; woodiness indices; herbaceousness indices; metabolic replacements; replacement indices; polyphenols; gallotannins; caffeoylegtannins; shikimate derivatives; acetate derivatives.

Abstract—The morphology and metabolism of angiosperms are connected to lignin production. Morphology depends on the structure of genetically controlled cellulose templates for lignin deposition; metabolism is the consequence of lignin-requirement directed biosynthesis of secondary metabolites. Hence, it is possible to correlate morphological features (such as evolutionary replacement of woodiness by herbaceousness) with general chemical features (such as evolutionary replacement of aromatics by aliphatics) and with particular chemical features (such as oxidative diversification of biosynthetic categories of metabolites). The first correlation is useful for the systematic classification of plants at higher levels (e.g. orders). The last correlation is useful for the evolutionary assessment of plants at lower levels (e.g. families). These concepts allow the construction of an integrated system of angiosperm classification based on morphology for the circumscription of taxa and on chemistry for the evolutionary assessment of plant groups.

INTRODUCTION

From the primordial prokaryotes to the modern eukaryotes and their organelles, membranes are among the most important cellular structures. 'Life' might not have originated earlier than the moment that the first ionic or concentration gradient was actively established over the limiting membrane of the first cell [1]. Without membranes, cell contents would suffer dispersion, information-bearing molecules would be lost by diffusion and metabolism would tend toward thermal equilibrium. All these phenomena are known to lead to the death of a living system [2]. The specific components, but not their general biosynthetic pathways, may vary somewhat from one type of membrane to another. These pathways involve carbohydrate-centred routes towards acetate derivatives (lipids, terpenoids and Krebs cycle-derived products), and towards shikimate derivatives (up to phenylalanine) (Fig. 1). Prior to the advent of plants, all classes of secondary metabolites were derived from the same pathways and may constitute 'overflow' [3] of the pertinent routes in search of optimization of membrane structure. Seemingly, morphology and metabolism are

manifestations, at this stage almost entirely acetate derived, of identical biosynthetic pathways.

The particular features that ultimately provide a plant with its form are the cell walls. Each cell wall structure is under strict genetic control, operative only during the construction of its primary cellulosic part [4]. After completion of cell division, growth and extension, secondary polyphenolic lignin-type material fills in this primary matrix. As a consequence, localization and quantity of lignins imply a degree of control in spatial terms by the cellulosic template.

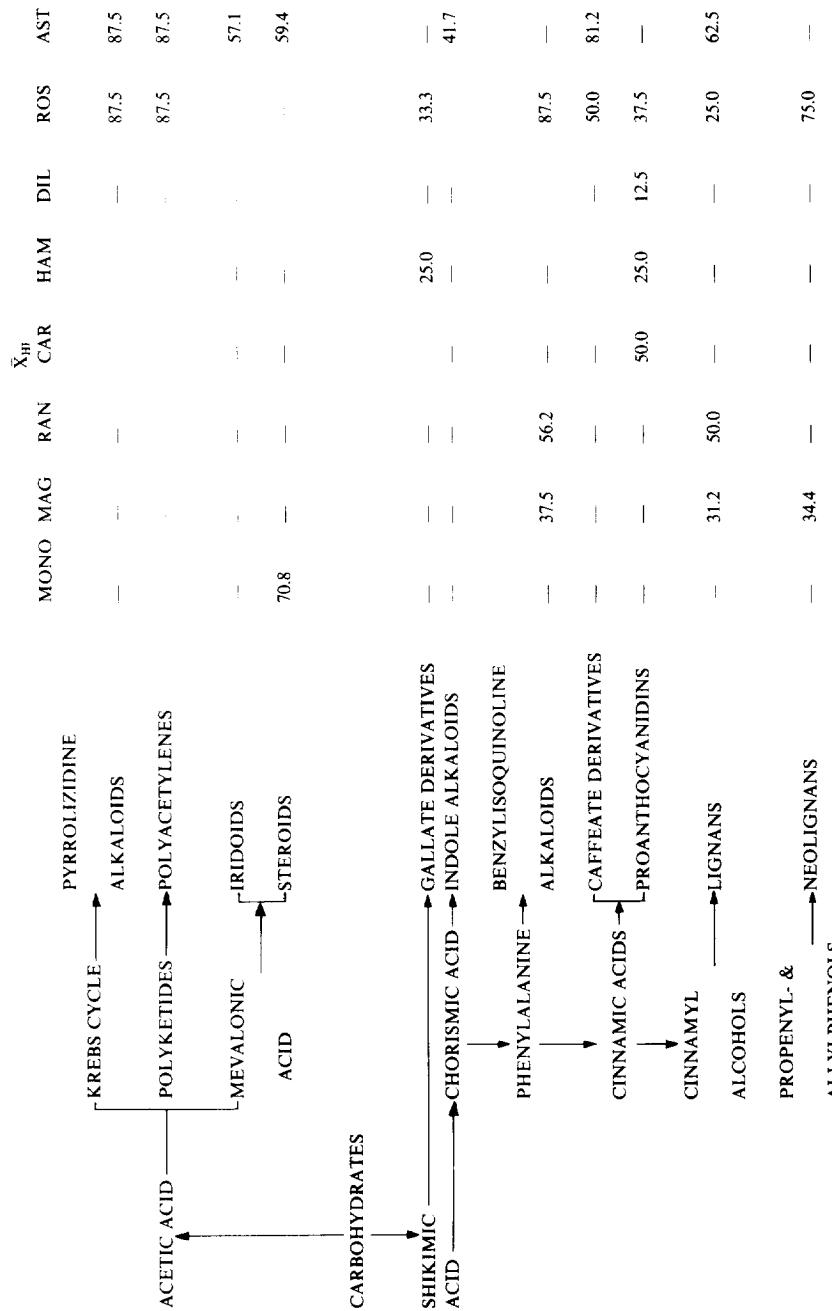
Lignin production requires expansion of the shikimate pathway beyond phenylalanine (Fig. 1), a process which, by analogy to the process described for membrane formation, could also occur by overflow to all categories of shikimate-derived secondary metabolites of plants.

This report provides an experimental basis for this conceptual framework with respect to angiosperms. For this purpose, the dicotyledons were divided into the subclasses Magnoliidae, Ranunculidae and Caryophyllidae (here collectively designated Magnoliidae *s.l.*), Hamamelidae, Dilleniidae and Rosidae (here collectively designated Rosidae *s.l.*) and Asteridae [5, 6].

RESULTS

Sporne [7] quantified evolutionary, mainly morphology-based, features of angiosperm families by percentage

*Based on a contribution to the DOE workshop on lignin biosynthesis/biodegradation at Asilomar, California, 24-27 May 1994.



The mean herbaceousness indices (HI) refer to families of the indicated angiosperm groups (MON, Monocotyledoneae; MAG, Magnoliidae; RAN, Ranunculidae; CAR, Caryophyllidae; HAM, Hamamelidae; DIL, Dilleniidae; ROS, Rosidae; AST, Asteridae) possessing > 5% of the universe of occurrence of the corresponding biosynthetic category of metabolites (see Table 1 and text).

Fig. 1. General biosynthetic scheme for the derivation, via primary and intermediate metabolism, of selected categories of secondary metabolites.

advancement indices (Sporne indices, SI). These indicate how infrequent the presence of 30 characters (woodiness included), regarded as primitive, is in each of 291 families. In turn, we have defined percentage indices for the measurement of herbaceousness (previously designated herbacity, HI = 100 – woodiness index) of angiosperm families of plants [8, 9]. While HI should in future involve direct measurement of mechanical, anatomical or chemical properties of species, in this report HI is again deduced from indications in the literature [6] for the predominant habits of species in families.

Furthermore, we represented micromolecular categories in plant groups by frequency (= number) of occurrences (NO), and by evolutionary advancement indices based on oxidation levels and on skeletal modification of the constituent micromolecules [8, 9]. The NO of a selected biosynthetic category known to occur in a family is established by the total number of compounds registered for their species. Only NOs of more than 5% (numbers

given in bold in Table 1) of the total NO values per category were considered systematically relevant for a family. Elimination of background noise sharpens the resolution sufficiently for the perception of evolutionary trends. These trends point precisely in the expected direction (Fig. 1).

Shikimate-derived chemistry characterizes plant groups of low herbaceousness (mean HI = 45.5, lower part of Fig. 1), in opposition to acetate-derived metabolism, which predominates in plant groups of high herbaceousness (mean HI = 78.8, higher part of Fig. 1). Compare, particularly, the metabolic categories in Rosidae and Asteridae. In the less herbaceous Rosidae, shikimate-derived compounds (six categories in plant groups characterized by a mean HI of 51.4) predominate over acetate-derived compounds (two categories in plant groups characterized by a mean HI of 87.5), and in the more herbaceous Asteridae, shikimate-derived compounds (three categories in plant groups characterized by a mean

Table 1. Herbaceousness indices (HI), Sporne indices (SI) and numbers of occurrences (NO) of neolignans (NLG), lignans (LGN) [26, 27], caffeoyletannins (CAF), proanthocyanidins (PRO) [28], benzylisoquinoline alkaloids (BIQ) [29], indole alkaloids (IND) [30], gallo- and ellagitannins (GAL) [28], pyrrolizidine alkaloids (PYR) [Ferreira, Z. S., unpublished data], steroids (STE) [1], iridoids (IRI) [31] and polyacetylenes (POL) [23]; as well as A1, A2 and A3 for families of angiosperms, given as per cent values of A1 + B1, A2 + B2 and A3 + B3, respectively*

Orders	Families (aceae)	HI	SI	Numbers of occurrences (NO)													
				NLG	LGN	CAF	PRO	BIQ	IND	GAL	PYR	STE	IRI	POL	A1	A2	A3
Alismatidae																	
Hyd	Hydrocharit	100.0	28			8				1					100	11	100
Naj	Zoster	100.0	50			1				1					100	50	100
Naj	Cymodoce	100.0	50			4									100	0	
Arecidae																	
Are	Arec	25.0	51			5	16			1		18			55	17	49
Ara	Ar	87.5	55	1		4	2	3							100	0	33
Ara	Lemn	100.0	88			2									100	0	
Commelinidae																	
Com	Commelin	100.0	50			1				1		2			50	50	33
Cyp	Po	100.0	81	2		36	22			7	16	42			62	16	33
Cyp	Cyper	100.0	83			2				1		11			21	33	8
Typ	Typh	100.0	88			1	3					1			80	0	75
Zingiberidae																	
Bro	Bromeli	100.0	25							6					0	0	
Zin	Mus	100.0	49			2	1			1		10			29	33	17
Zin	Strelitzi		50.0	49								3			0	0	
Zin	Cost	100.0	49									11			0	0	
Liliidae																	
Lil	Amarillid		8							1					0	0	
Lil	Smilac	75.0	13							21					0	0	
Lil	Rusc	50.0	13							17					0	0	
Lil	Agav	50.0	13							275					0	0	
Lil	Alli	100.0	13			2				43					4	0	0
Lil	Tacc	100.0	17							5					0	0	
Lil	Dioscore	75.0	27			1	12				186				6	0	6
Lil	Trilli	100.0	26								62				0	0	
Lil	Lili	87.5	28			8		14		4	372				6	33	1
Lil	Irid	100.0	38							2		20			9	100	9
Orc	Orchid	100.0	33							54		29			0	0	

Cont'd overleaf.

Table 1. *Continued.*

Orders	Families (aceae)	Numbers of occurrences (NO)															
		HI	SI	•	NLG	LGN	CAF	PRO	BIQ	IND	GAL	PYR	STE	IRI	POL	A1	A2
Magnoliidae																	
Mag	Magnoli	25.0	25	51	28			74		1					100	100	1
Mag	Himantandr	1.0	38	6											100		0
Mag	Myristic	12.5	34	166	35			3		1			1		100	100	2
Mag	Canell	12.5	38		4										100		0
Mag	Annon	25.0	40	2				9	370						16	96	2
Mag	Eupomati	50.0	42	16					3						100		0
Mag	Austrobailey	50.0	37	5	2										100		0
Lau	Trimeni	25.0	34	2											100		0
Lau	Monimi	25.0	47		2				33						100		0
Lau	Calycanth	25.0	52										1		0	0	0
Lau	Laur	50.0	52	352	25		5	68	215				3		100	0	10
Lau	Hernandi	25.0	61		32				61						100		0
Pip	Saurur	100.0	58	8											100		0
Pip	Piper	50.0	60	16	45				4						100		0
Ari	Aristolochi	75.0	50	26	15		2		94				5		96	0	0
Ill	Schisandr	50.0	28	106	2										100		0
Ill	Illici	25.0	38				11								100		100
Nym	Nelumbon	100.0	43					19							100		0
Nym	Nymphae	100.0	48							16					100	100	100
Ranunculidae																	
Ran	Sargentodox	50.0	36	1											100		0
Ran	Berberid	50.0	45		42		5		204						100	0	0
Ran	Menisperm	25.0	47		2				585				12		98		0
Ran	Lardizabal	50.0	48		2										100		0
Ran	Ranuncul	75.0	53				10		331			2	78		81	0	0
Ran	Coriari	50.0	57							19					100	100	100
Pap	Papaver	75.0	48				2		998				3		100	0	0
Pap	Fumari	100.0	48				8		191						100	0	0
Caryophyllidae																	
Car	Aizo	75.0	45					3				2			60		0
Car	Phytolacc	50.0	50									6			0	0	0
Car	Cact	50.0	53									30			0	0	0
Car	Caryophyll	87.5	55				7	6				62			17	0	9
Car	Amaranth	50.0	63				1	4			1		82		7	50	6
Car	Chenopodi	87.5	70				3	2			1		44		12	25	6
Pol	Polygon	50.0	52				32	102			76		10		96	70	95
Plu	Plumbagin	75.0	60					3					15		17		17
Hamamelidae																	
Ham	Cercidiphyll	1.0	26								10				100	100	100
Ham	Hamamelid	25.0	37							22		4			85	100	85
Dap	Daphniphyll	25.0	38									3			0	0	0
Euc	Eucommi	1.0	40		18								10		64		0
Urt	Mor	25.0	48				1					37			3	0	0
Urt	Ulm	1.0	52		3	10	7				7				100	41	82
Urt	Urtic	50.0	52		4	1						4			56	0	0
Urt	Cannabin	100.0	51				1	6				9			44	0	40
Jug	Jugland	12.5	50				5				12				100	71	100
Myr	Myric	25.0	50					12			4				100	100	100
Fag	Betul	25.0	38		1			2			25		2		93	100	90
Fag	Fag	25.0	43		1	8	73				308		6		98	98	98
Fag	Coryl	25.0	41				2				9		8		58	82	53
Cas	Casuarin	25.0	50					8			13				100	100	100
Dilleniidae																	
Dil	Paeoni	87.5	37					1			20				100	100	100
Dil	Dilleni	50.0	37								5				100	100	100
The	Sarcolaen	25.0	31				1				1				100	50	100
The	Dipterocarp	12.5	38				2				21				100	91	100

Cont'd overleaf.

Table 1. *Continued.*

Orders	Families (aceae)	Numbers of occurrences (NO)															
		HI	SI	NLG	LGN	CAF	PRO	BIQ	IND	GAL	PYR	STE	IRI	POL	A1	A2	A3
The	The	12.5	40		1	4	126			21		19			89	84	88
The	Clusi	50.0	42			9	15			5					100	36	100
The	Ochn	25.0	43				3								100		100
The	Caryocar	12.5	44							3					100	100	100
The	Actinidi	25.0	45				5			1			13		32	100	100
Mal	Elaeocarp	25.0	30			1				9					100	90	100
Mal	Bombac	1.0	42			1				2					100	67	100
Mal	Tili	25.0	42			6	2					14			36	0	12
Mal	Sterculi	25.0	45			4	29				3			2	87	0	85
Mal	Malv	75.0	48			8	14			4	5	6			70	33	62
Lec	Lecythid	12.5	48							2		2			50	100	50
Lec	Barringtoni	12.5	48							1					100	100	100
Nep	Nepenth	50.0	43									3			0	0	0
Nep	Sarraceni	100.0	48									3			0	0	0
Vio	Cist	75.0	45							4					100	100	100
Vio	Flacourti	25.0	35			13				1					100	7	100
Vio	Viol	50.0	43			2									100	0	
Vio	Begoni	75.0	46				1								100		100
Vio	Caric	25.0	50			1									100	0	
Vio	Cucurbit	50.0	54		5	3						97			8	0	0
Vio	Tamaric	25.0	45							6					100	100	100
Vio	Stachyur	25.0	24							16		4			80	100	80
Vio	Fouquieri	25.0	47									16			0	0	
Vio	Loas	87.5	64									70			0	0	
Sal	Salic	25.0	44			11	33				2				96	0	94
Cap	Capparid	25.0	52								2				0	0	
Cap	Resed	87.5	44		1										100		
Cap	Brassic	75.0	60			12				1		98			12	8	1
Eri	Clethr	25.0	42							2					100	100	100
Eri	Eric	50.0	45		1	18	31			12		1	13		82	40	74
Eri	Empetr	50.0	50			3									100		100
Eri	Pyrol	50.0	54			6				2			2		80	100	80
Ebe	Eben	25.0	45		1		2			6					100	100	89
Ebe	Styrac	25.0	48		1							2			33	0	
Ebe	Sapot	25.0	50			2					11	8			20	0	0
Ebe	Symploc	25.0	48			1	2			1			1		80	100	40
Pri	Myrsin	25.0	53							1		1			50	100	50
Pri	Primul	87.5	59			3	3			2		1			89	40	83
Rosidae																	
Ros	Davidsoni	1.0	37			1									100		100
Ros	Ros	50.0	43		5	105	211			181		4			99	63	98
Ros	Saxifrag	75.0	48			6	24			27					100	82	100
Ros	Crassul	75.0	55			10	8			24		21			67	71	60
Ros	Rhabdodendr	50.0	45							2					100	100	100
Ros	Pittospor	25.0	63											15	0	0	
Ros	Escalloni	1.0	52									2			0	0	
Ros	Montini	25.0	52									2			0	0	
Ros	Hydrange	50.0	52		2							22			8	0	
Fab	Mimos	25.0	48			1	61			23		11			88	96	88
Fab	Caesalpini	25.0	48		2		90			23		8			94	100	92
Fab	Fab	87.5	48		1	62	25	345		30	258	110		10	79	33	7
Pro	Elaeagn	37.5	50			17	9			14		1			98	45	96
Hal	Halorag	87.5	52							5					100	100	100
Myr	Thymelae	50.0	55		17	1	4					1			96	0	18
Myr	Myrt	25.0	45			8	8			113		2			98	93	98
Myr	Combret	25.0	47	2		1	4			75					100	99	98
Myr	Sonnerati	1.0	48							1					100	100	100
Myr	Punic	25.0	48							21					100	100	100
Myr	Trap	100.0	48							24		2			92	100	92
Myr	Onagr	87.5	55			47				65					100	58	100

Cont'd overleaf.

Table 1. *Continued.*

Orders	Families (aceae)	Numbers of occurrences (NO)															
		HI	SI	NLG	LGN	CAF	PRO	BIQ	IND	GAL	PYR	STE	IRI	POL	A1	A2	A3
Myr	Lythr	87.5	57			2			34		1			97	94	97	
Myr	Melastomat	75.0	57			1			34		1			97	97	97	
Myr	Crypteroni	1.0	64						3					100	100	100	
Rhi	Rhizophor	25.0	36			1	25			2	1			96	0	89	
Cor	Nyss	12.5	41						11					100	100	100	
Cor	Davidi	1.0	46					4			2			67	100	67	
Cor	Garry	25.0	50								5			0	0		
Cor	Alangi	12.5	52					1			1	2		25	0		
Cor	Corn	25.0	57			1			36		5	20		60	97	59	
San	Olac	25.0	46											16	0	0	
San	Santal	50.0	64			1	2			3	1			16	15	0	
San	Loranth	25.0	65		1		10			3		2		2	78	100	72
San	Visc	50.0	61		2	9									100	0	0
San	Balanophor	50.0	64		1	3									100	0	0
Raf	Rafflesi	50.0	55							1					100	100	100
Cel	Celastr	25.0	47			12	8			1	2	25			46	8	25
Cel	Aquifoli	25.0	57				10								100	0	
Cel	Icacin	25.0	55										4		0	0	
Eup	Euphorbi	25.0	37		30	11	15	38		250	5	24			94	96	73
Eup	Bux	100.0	36									3			0	0	
Rha	Rhamn	25.0	45		4	1	2	34				3			93	0	5
Rha	Lee	75.0	41							1					100	100	100
Rha	Vit	50.0	41			5	16			8		1			97	62	96
Lin	Lin	87.5	50		1	2				6		2			60	0	0
Pol	Polygal	50.0	55		13					2					100	100	13
Pol	Malpighi	25.0	57							1					100	100	100
Pol	Krameri	75.0	61	74			4								100	5	
Pol	Vochysi	25.0	62							1					100	100	100
Sap	Simaroub	25.0	40					4		3		5		1	54	100	23
Sap	Rut	25.0	45		82	10		140		8		8			97	44	3
Sap	Meli	25.0	47		1		5			2		23			26	100	23
Sap	Burser	12.5	48		7					2		9			50	100	11
Sap	Cneor	50.0	65								2			0	0		
Sap	Melianth	25.0	43							1		21			4	100	4
Sap	Sapind	25.0	45			3	5	8		3		4			83	50	40
Sap	Anacardi	25.0	52		4	21				59		1			99	94	99
Sap	Pistaci	25.0	52							3					100	100	100
Sap	Hippocastan	25.0	52			25						7			78	78	
Sap	Acer	12.5	52			1	3			60					100	98	100
Sap	Podo	75.0	50								1				0	0	
Sap	Zygophyll	50.0	55	30								31			49	0	
Sap	Balanit			51								14			0	0	
Ger	Oxalid	87.5	48							1					100	100	100
Ger	Gerani	87.5	53			6	12			49		2			97	89	97
Api	Arali	25.0	55		2	4	1			1				30	21	20	6
Api	Api	87.5	51		25	25				1		7		148	25	4	1
Asteridae																	
Gen	Apocyn	50.0	57		35	2		12	1325	3	30	310	60		79	60	1
Gen	Potali	25.0	61			1							12		8	0	0
Gen	Logani	50.0	61		6				175			3	42		80	0	
Gen	Asclepiad	50.0	62		1	11						291			4	0	0
Gen	Gentian	87.5	63			8				6		3	133		9	43	4
Sol	Convolvul	50.0	60			13						5			72	0	0
Sol	Solan	50.0	68		27	1				2		450			6	7	1
Sol	Cuscut	100.0	64			2									100	0	
Sol	Menyanth	100.0	69			3						2	8		23	0	0
Sol	Retzi	50.0	72										5		0	0	
Lam	Boragin	87.5	60			8		1	336						100	11	0
Lam	Ehreti	25.0	56			2				2					100	0	0
Lam	Verben	50.0	67	3	15	3	1	1				23	126		13	0	1

Cont'd overleaf.

Table 1. *Continued.*

Orders	Families (aceae)	HI	SI	Numbers of occurrences (NO)													
				NLG	LGN	CAF	PRO	BIQ	IND	GAL	PYR	STE	IRI	POL	A1	A2	A3
Lam	Lami	75.0	72		6	219	1			8		29	150		57	4	5
Cal	Callitrich	100.0	74										2		0	0	0
Cal	Hippurid	100.0	83										2		0	0	0
Pla	Plantagin	87.5	69			4						1	24		14	0	0
Scr	Ole	25.0	62		69	10				4			102		45	29	2
Scr	Myopor	37.5	63			6							7	1	43		0
Scr	Scrophulari	87.5	67		14	43				1	6	249	477		7	2	0
Scr	Globulari	75.0	69			2							25		7	0	0
Scr	Gesneri	87.5	70		1										100		0
Scr	Buddley	25.0	70										18		0	0	0
Scr	Pedali	87.5	70		1	2							13		19	0	0
Scr	Acanth	87.5	72		35	3				1		3	40		48	25	1
Scr	Martyni	100.0	75										5		0	0	0
Scr	Lentibulari	100.0	75										11		0	0	0
Scr	Bignoni	25.0	80		4	5	1			3		3	80		14	38	4
Cam	Campanul	50.0	63			5						4		25	15	0	0
Cam	Lobeli		61											15	0	0	0
Cam	Styliodi	87.5	62										11		0	0	0
Cam	Goodeni	100.0	63										16	1	0	0	0
Rub	Rubi	25.0	48		3	23	24	6	171			19	153		57	0	6
Dip	Adox	100.0	78										4		0	0	0
Dip	Sambuc		52			4					1		3		62	20	25
Dip	Caprifoli	50.0	60		1	11	7	1				10	137		12	0	4
Dip	Valerian	87.5	83			14							46		23	0	0
Dip	Dipsac	87.5	87			17		1					31		37	0	0
Cal	Calycer	100.0	67										1		0	0	0
Ast	Aster	87.5	72		35	239	3			7	628	75		2123	11	3	0

* A1 = % NO of NLG + LGN + CAF + PRO + BIQ + GAL, B1 = % NO of STE + IRI + POL; A2 = % NO of GAL, B2 = % NO of CAF; A3 = % NO of GAL + PRO, B3 = % NO of NLG + LGN + BIQ + PYR + STE + IRI + POL; determined for families (termination aceae), with reported chemical data, classified into orders (represented by their three initial letters) and subclasses according to Cronquist [6]. For the meaning of the numbers in bold see text.

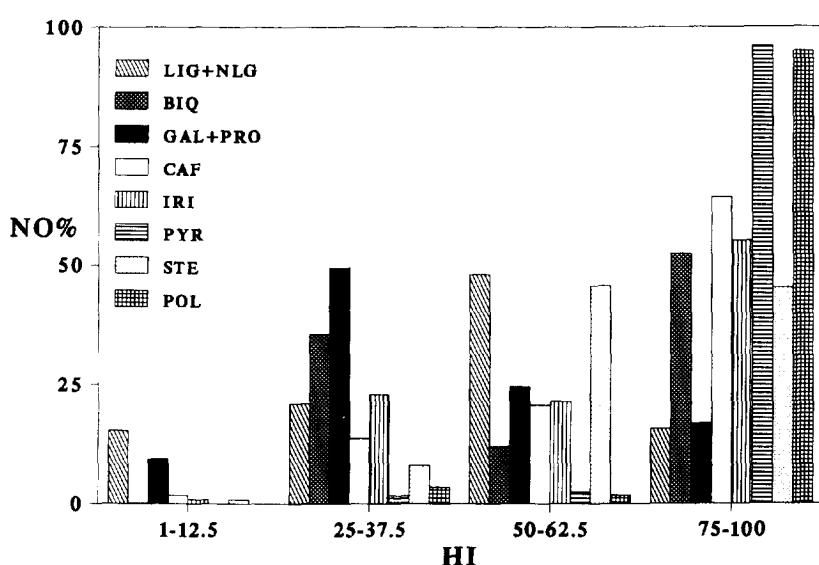


Fig. 2. Number of occurrences (NO, in % values of total NOs) for biosynthetic categories of chemical characters (see Table 1 and text) per families of dicotyledons arranged according to four ranges of herbaceousness indices (HI).

HI of 61.8) are less conspicuous than acetate-derived ones (four categories in plant groups characterized by a mean HI of 72.9). Moreover, within plant groups as defined here, contraction of reaction steps of the phenylpropanoid pathway in the sense of the evolutionary canalization theory [10] is accompanied by trends towards increasing herbaceousness.

More detailed experimental evidence is provided by the following cases. First, in comparison with HIs of dicotyledon families, included in four ranges of increasing values, NOs indicate progressive decrease of the distributional importance of gallotannins and proanthocyanidins and progressive increase of the importance of

caffeoylegtannins, iridoids, pyrrolizidine alkaloids, steroids and polyacetylenes (Fig. 2). As a consequence, when HI measurements are compared with the evolution of dicotyledons, they correlate positively with the replacement of gallotannins plus proanthocyanidins by caffeoylegtannins, and of aromatics by aliphatics.

Similarly, the measurement of NOs also indicates progressive decrease of lignoids (lignans plus neolignans) and benzylisoquinoline alkaloids, and progressive increase of caffeoylegtannins, iridoids, pyrrolizidine alkaloids, steroids and polyacetylenes (Fig. 3). These results indicate that the transition of dicotyledons from Magnoliidae *s.l.* over Rosidae *s.l.* towards Asteridae again corre-

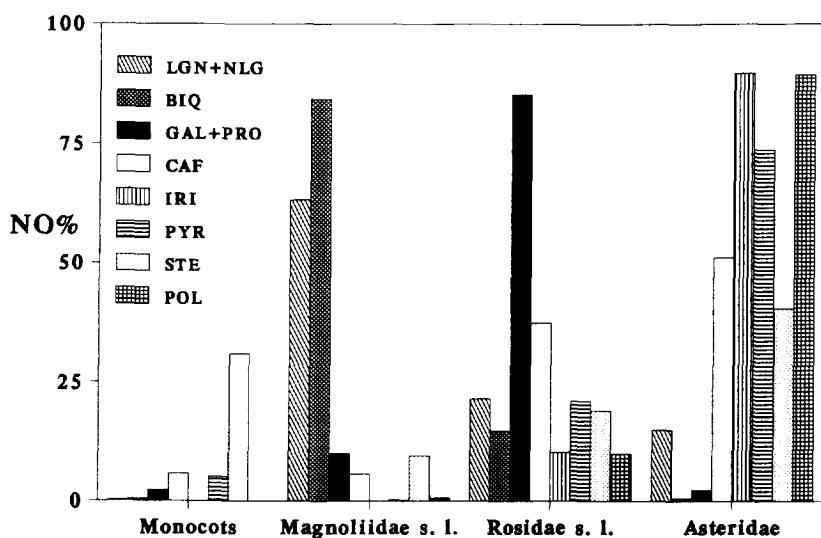


Fig. 3. Number of occurrences (NO, in % values of total NOs) for biosynthetic categories of chemical characters (see Table 1) per families of angiosperms arranged according to four groups (see text).

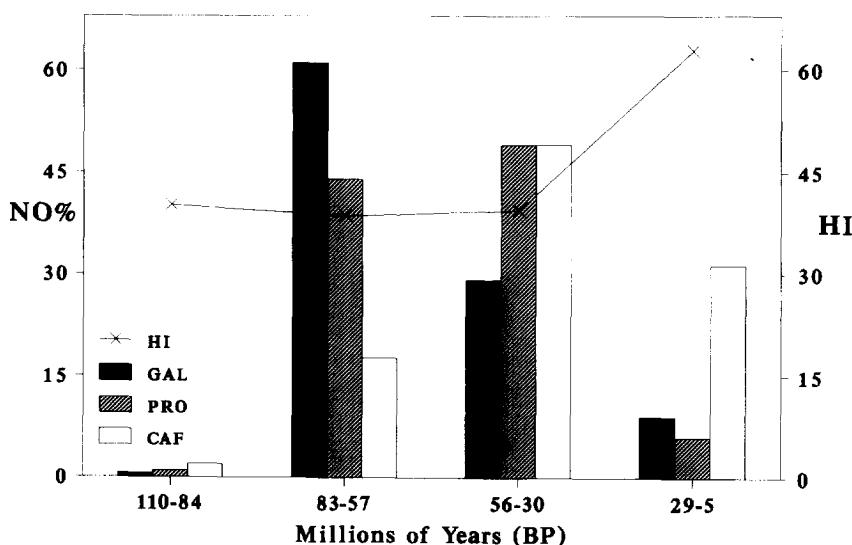


Fig. 4. Mean herbaceousness indices (HI) and number of occurrences (NO, in % values of total NOs) for three categories of polyphenols (see Table 1) per families of angiosperms arranged according to four ranges of ages (in millions of years before present).

lates well with replacement of gallotannins plus proanthocyanidins by caffeoylegtannins, and of shikimate-derived metabolic categories by those originating from acetate [11].

Finally, the age of angiosperm families (distributed in four ranges), as gauged by the age of the oldest known pollen fossils [12], was correlated with the pertinent mean HIs. Corresponding NO values for the three major classes of polyphenols show the expected distributional

trends (Fig. 4) found in Figs 2 and 3. At the rank of orders, the results indicate early and rapid diversification of gallotannins and proanthocyanidins in primitive plant families, whereas in more recent herbaceous groups the demise of these same categories of compounds is slow and gradual (Fig. 5a) together with a rapid increase of caffeoylegtannins (Fig. 5b). Indeed, presumed ages of the angiosperm groups correlate well with the reported replacements of the diverse tannin types (Fig. 6).

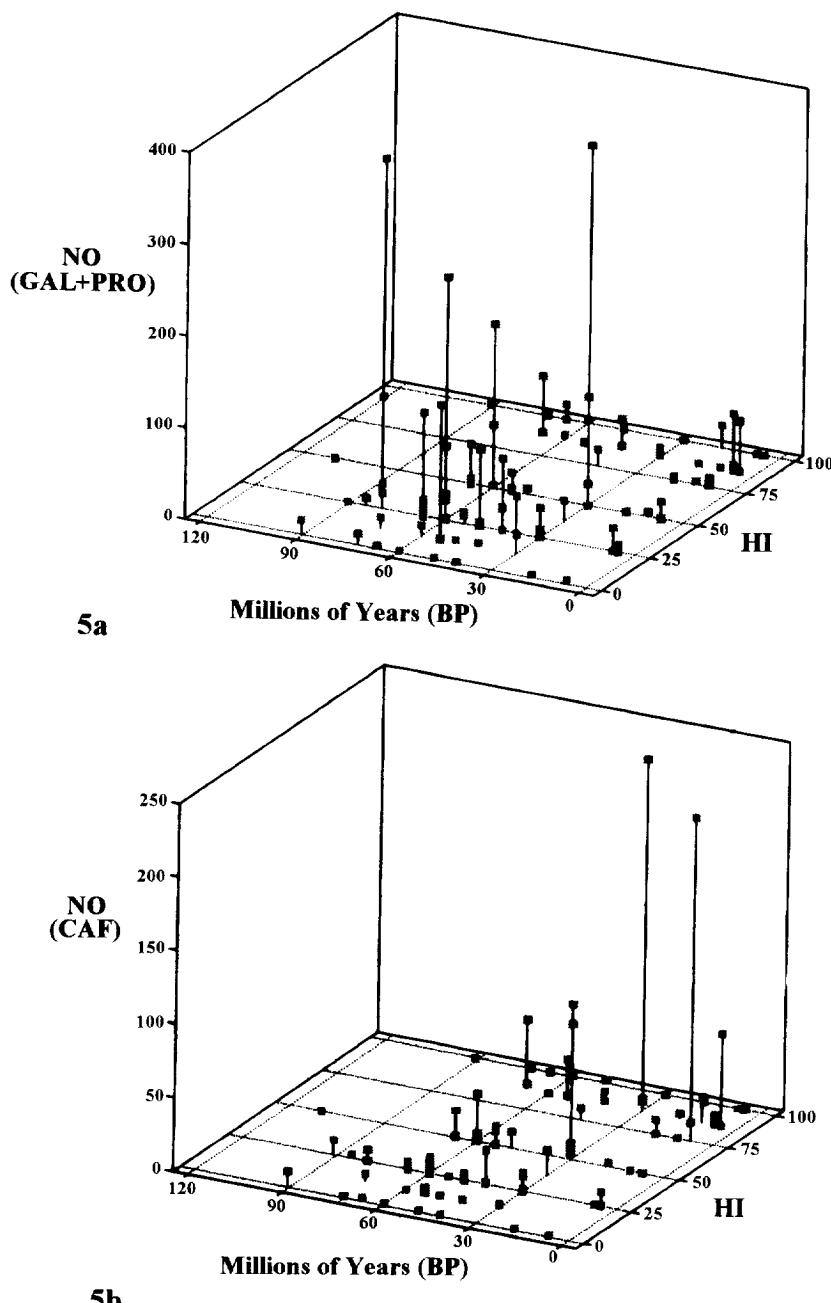


Fig. 5. Correlation of age (in millions of years before present), herbaceousness indices (HI) and number of occurrences (NO, in % values of total NOs) of (a) gallotannins + proanthocyanidins and (b) caffeoylegtannins for orders of dicotyledons.

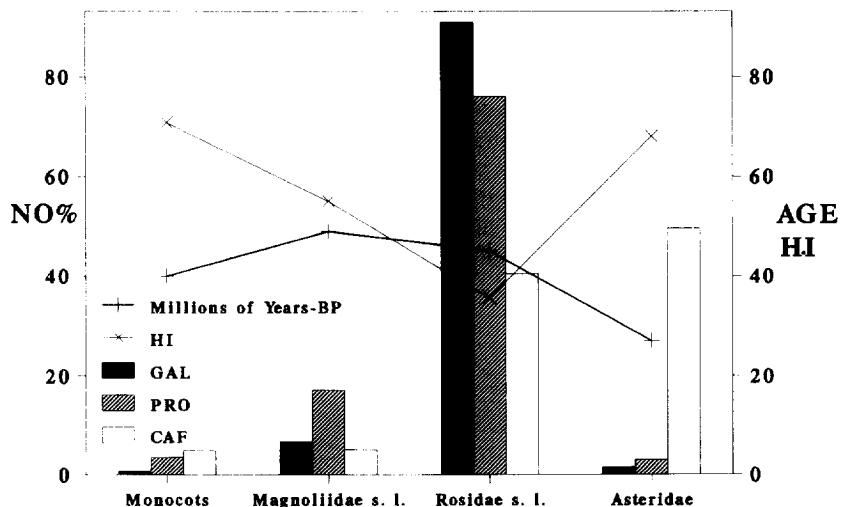


Fig. 6. Ages (in millions of years before present), herbaceousness indices (HI) and number of occurrences (NO, in % values of total NOs) for three categories of polyphenols (see Table 1) per families of angiosperms arranged according to four groups.

Based on such evidence, *general* (as opposed to *particular*) chemical criteria can be defined. Three systems were developed for the case of evolution of angiosperm metabolism, following careful consideration of the above results.

The first system concerns the evolutionary replacement of shikimate-derived aromatics by acetate-derived aliphatics. Lignans, neolignans, benzylisoquinoline alkaloids, proanthocyanidins, gallic acid derivatives and caffeic acid derivatives (designated A_1) were selected as models for the former group, and polyacetylenes, iridoids and steroids (designated B_1) were selected to represent the latter group. Indole alkaloids were omitted from this selection in view of their mixed aromatic-aliphatic nature. The second system involves replacement ratios of gallotannins versus caffeoylegtannins (A_2/B_2), whereas the third system involves replacement ratios of polyphenols (gallotannins plus proanthocyanidins) versus micro-molecules (here represented by lignans, neolignans, benzylisoquinoline alkaloids, polyacetylenes, iridoids, steroids, pyrrolizidine alkaloids) (A_3/B_3). The sums of NOs for the metabolic categories of corresponding types, A_n and B_n , were expressed in percentages for families (Table 1).

Correlations of general characters are presented for orders within subclasses (Fig. 7a-7c). When focusing on Magnoliidae s.l. (Fig. 7a) and Asteridae (Fig. 7e), two evolutionary modes can be discerned. One operates within these two plant groups, and one operates between them (from Magnoliidae towards Asteridae). Both modes are characterized by increasing herbaceousness of the pertinent taxa. However, while the former shows evolutionary variation by increasing oxidation level of the constituents [13], rather than variation of the aromatics-aliphatics ratio, the latter is accompanied mainly by variation from high to low A_1/B_1 values. The situ-

ation is somewhat less straightforward in the case of Rosidae s.l. (Fig. 7b-7d), possibly due to the general presence of gallic acid [11].

In all previous classification systems, secondary metabolites were used sparingly except in an auxiliary capacity. The present results allow the construction of systems in which both form and chemistry are conferred equal status. For example, morphological data were used for the circumscription of subclasses (*sensu* Cronquist [6]) with respect to families. Within each subclass, biosynthetic categories of metabolites were used as criteria for the lumping of families in which they possess significant NOs. Families are considered to be characterized by specific categories only if the respective NOs exceed 5% (Fig. 8a) or 2% (Fig. 8b) of the NO totals (Table 1). Biosynthetic categories were assigned precedence factors according to assumed evolutionary order. In angiosperms, acetate derivatives become important systematic markers only after the substantial demise of shikimate derivatives, which explains the precedence of the latter in the sequence. Within the shikimate-derived groups precedence is attributed in order of progressive shortening of the steps leading to the intermediates from which the particular categories are derived [10]. The acetate-derived categories are numbered in order of diminishing natural abundance.

The analyses of the chemistry-based correlations of SI/HI (Fig. 8) are consistent with all further verifications of the present work. Indeed, four equal areas of each plot are characterized by mean precedence factors (f) which increase from bottom to top (diagonally as well as vertically) and from left to right (Table 2). Furthermore, while low factors may appear in any of the areas, including the top ones (for reasons of unaltered inheritance of primitive characters by advanced taxa), high factors are found exclusively in areas situated in the top row at the right.

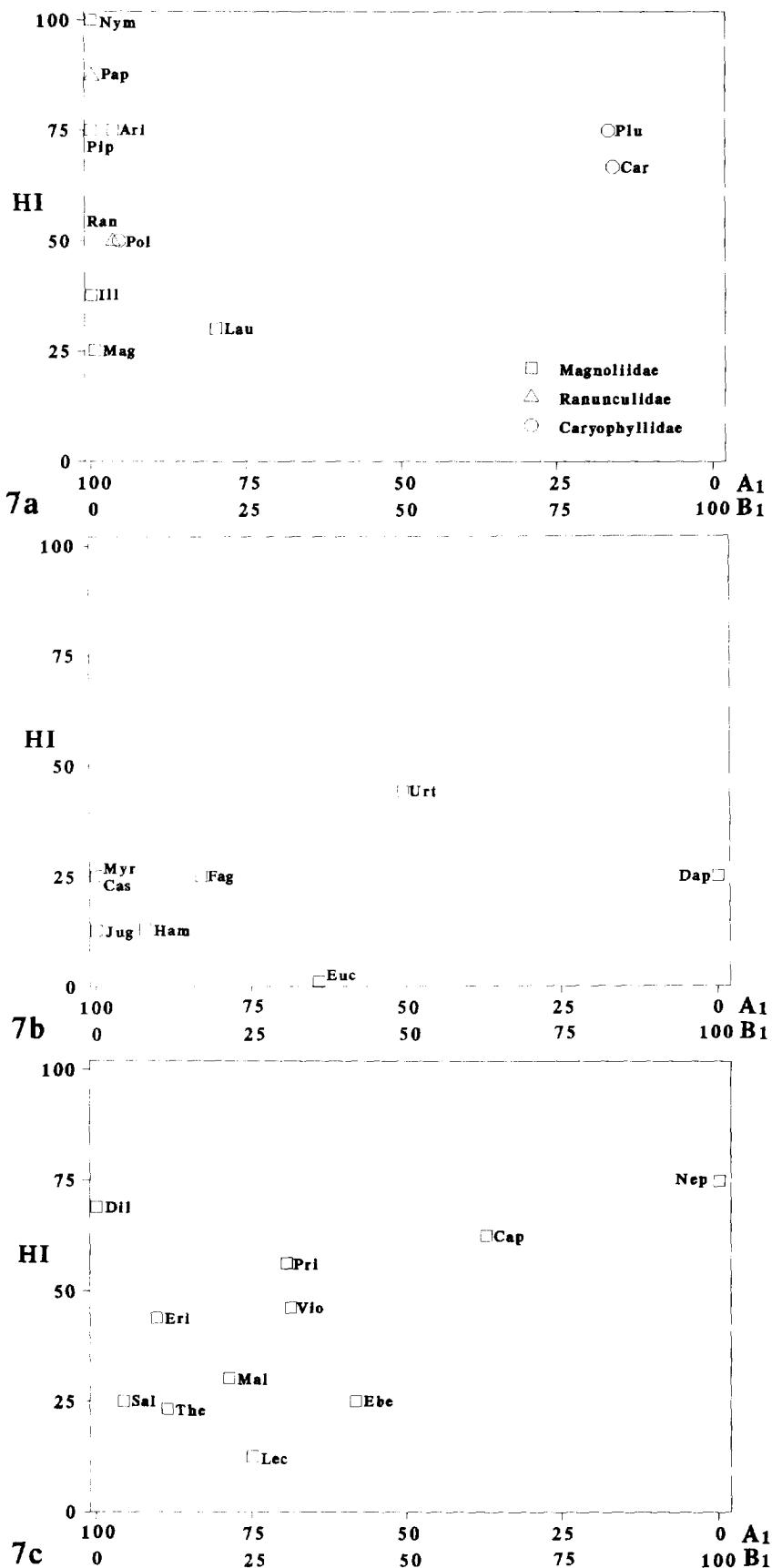


Fig. 7.

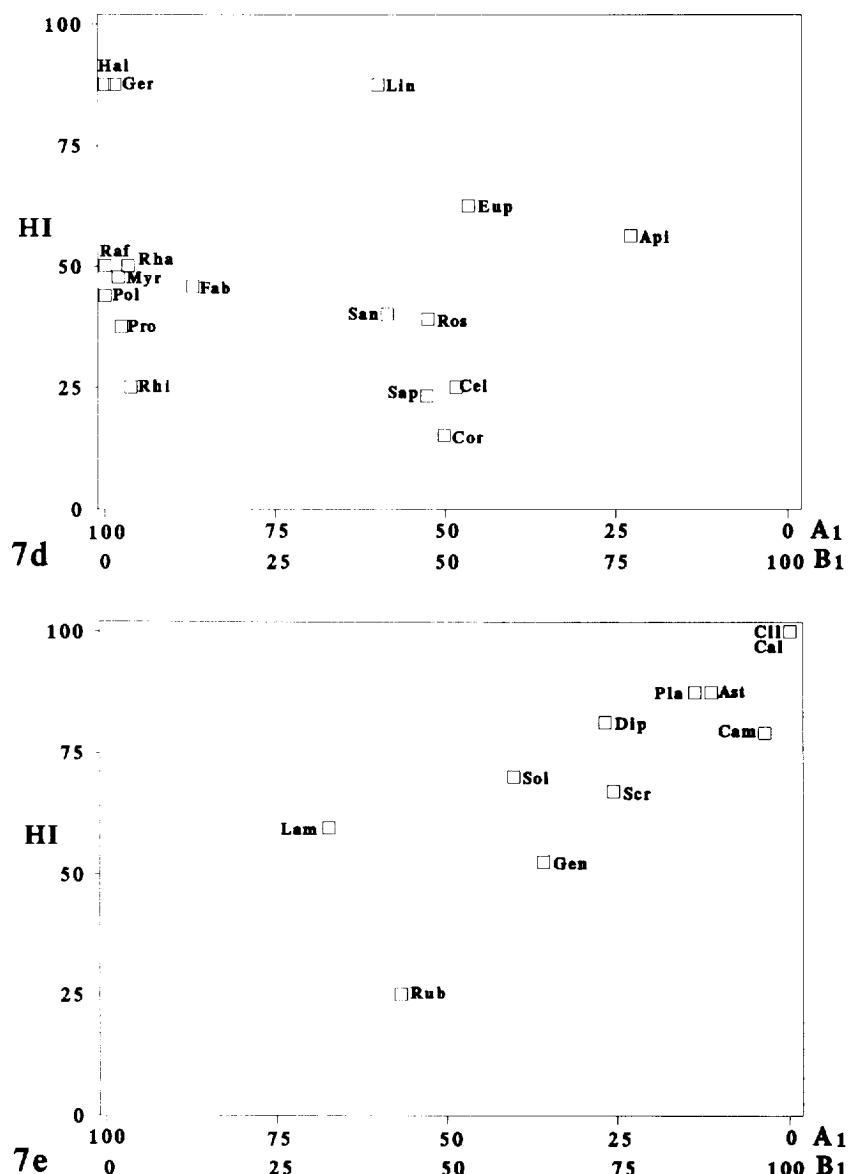


Fig. 7. Correlation of herbaceousness indices (HI) and aromatics/aliphatics (A_1B_1) for orders within replacement ratios (a) Magnoliidae s.l., (b) Hamamelidae, (c) Dilleniidae, (d) Rosidae and (e) Asteridae. The orders are represented by the three initials of their names (Table 1), except for Callitrichales (Cll in (e)).

DISCUSSION

At this stage a plausible sequence of chemical events, which may have accompanied, in active or passive form, the evolutionary history of the angiosperms, can be reconstructed. Indeed, all the most relevant data are now available and were calculated for the taxa as defined by Cronquist [6]. The present integrated system is well suited for attempts to solve classification problems. Nevertheless, such an analysis would go far beyond the scope of the present work and is restricted here to the following single example. According to our system, removal of Polygonales and Plumbaginales from the Caryophyllidae by Dahlgren [14] is plausible, since the over-

all evolutionary trends indicated by the quantified data become sharper. Such dynamic chemical data also corroborate Dahlgren's introduction of the two orders at widely diverging positions of his system (Table 3).

Table 2. Means of precedence factors for biosynthetic categories of metabolites distributed in the four equal areas of SI versus HI plots (Fig. 8a and 8b)

SI	HI	0-50	50-100
50-100		5.5/5.3	7.1/7.2
0-50		3.4/2.9	4.0/4.7

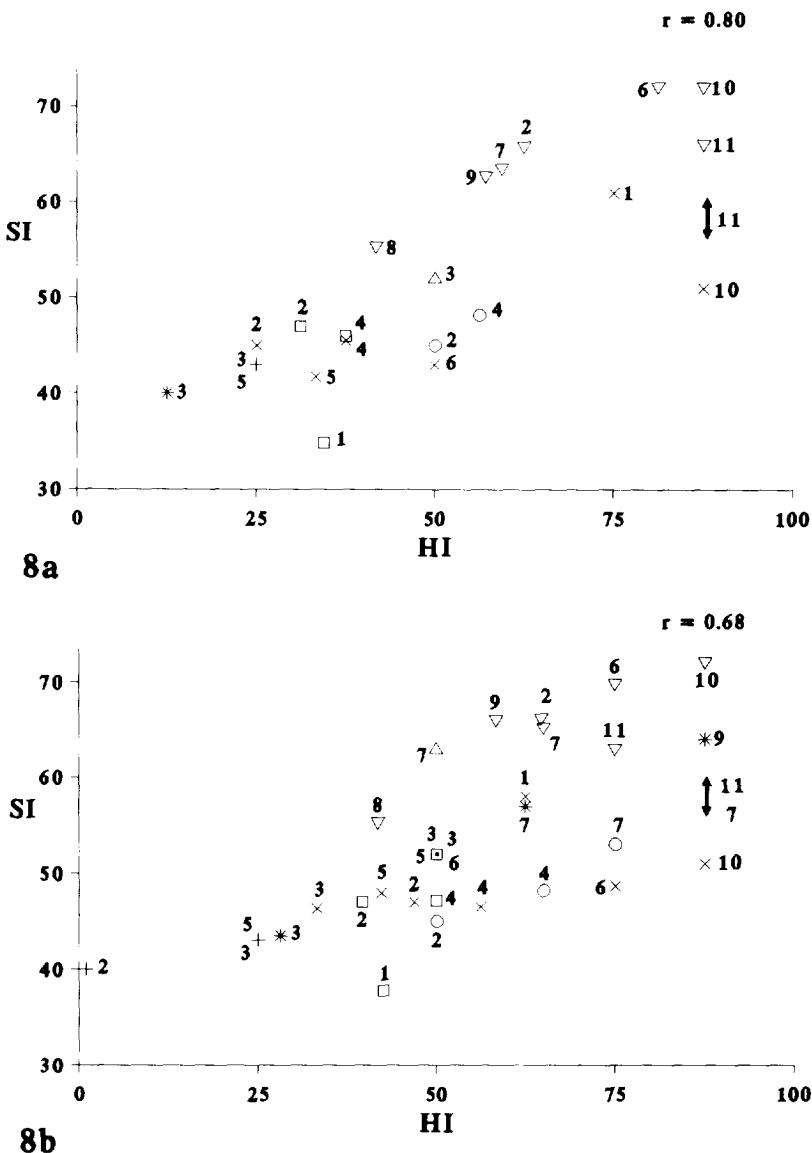


Fig. 8. Correlation of mean Sporne indices (SI) and herbaceousness indices (HI) for families belonging to Magnoliidae (□), Ranunculidae (*), Caryophyllidae (△), Hamamelidae (+), Dilleniidae (○), Rosidae (×) and Asteridae (▽). These subclasses are characterized by the indicated 11 biosynthetic categories numbered according to precedence factors: 1 NLG, 2 LGN, 3 PRO, 4 BIQ, 5 GAL, 6 CAF, 7 STE, 8 IND, 9 IRI, 10 POL, 11 PYR. The families are considered to be characterized by specific metabolic categories only if the respective NOs exceed 5% (a) or 2% (b) of the corresponding NO totals (Table 1). The correlation coefficients were obtained omitting the values for pyrrolizidine alkaloids (PYR) and steroids (STE) for Rosidae from the calculus. The omission is justified since HI refers to Fabaceae, the SI of which is unknown. The SI of Leguminosae (= 48) is of course not applicable to the particular case of one of its subfamilies (Papilionoideae = Fabaceae). More significant correlation indices are obtained if the chemistry of each subfamily is considered separately. Rosidae $r = 0.73$, Asteridae $r = 0.88$ (a); Magnoliidae $r = 0.54$, Ranunculidae $r = 0.97$, Rosidae $r = 0.43$, Asteridae $r = 0.86$ (b).

Magnoliidae $r = 0.54$, Ranunculidae $r = 0.97$, Rosidae $r = 0.43$, Asteridae $r = 0.86$ (b).

Obviously, gallic acid played a dominant regulatory role in this history. The compound must have been formed in primitive angiosperms, since it continued to appear in the magnolilean and the rosifloren blocks (in the terminology of Kubitzki and Gottlieb [15]) after they diverged. In the former block, it occurs sporadically in Magnoliidae and its offspring, the Monocotyledoneae,

Ranunculidae and Caryophyllidae. Gallic acid (oxidation level, O = 0.57) is well known as a powerful antioxidant [16], but in all these phyla its oxidative modification did not exceed the ellagic acid (O = 0.71) stage. In compensation [17], oxidative reactions led to the increasingly more pronounced oxidative diversification of other phenolic compounds of the shikimate pathway,

Table 3. Mean values of several parameters calculated for families within groups of angiosperms. The mean HI values refer only to the families for which A1/B1 ratios had been determined (see Table 1)*

	A1	A2	A3	HI (A1)	NO	GAL		AGE		
						NO/sp ($\times 10^{-3}$)	\bar{X}	EA ₀		
								Ranges	\bar{X}	
Monocots	13.2	19.8	5.8	87.5	19	0.4	0.57	—	40	67-7
Magnoliidae	98.7	72.0	5.6	43.5	18	2.2	0.62	0.57-0.71	60	110-30
Ranunculidae	96.2	43.2	0.8	59.4	19	5.0	0.63	0.57-0.71	12	18-7
Caryophyllidae	49.0	64.5	43.4	65.6	78	7.1	0.58	0.57-0.71	42	67-7
Caryophyllidae <i>sine</i>										
Poly & Plum	11.0	15.4	5.8	66.7	2	0.2	0.57	—	39	—
Polygonaceae	95.4	70.4	94.7	50.0	76	95.0	0.58	0.57-0.71	60	—
Plumbaginaceae	16.7	—	16.7	75.0	—	—	—	—	11	—
Hamamelidae	87.3	93.6	82.5	26.1	410	120.6	0.65	0.57-1.00	65	91-42
Rosidae	82.2	76.6	50.0	41.2	1211	20.9	0.64	0.57-0.86	42	91-5
Dilleniidae	58.0	59.6	50.8	40.2	149	6.0	0.64	0.57-0.74	41	67-5
Asteridae	15.8	5.2	1.1	69.7	37	0.6	0.63	0.57-0.71	27	60-5

* The data for the calculation of NO for gallic acid derivatives were taken from Table 1. Their normalization was effected by dividing these NO values by the numbers of species for which gallate content is registered in the literature. Oxidation levels were calculated for the gallic acid-derived portions of each gallotannin registered. Means and ranges of these values are considered to represent evolutionary advancement parameters based on gallate oxidation levels for the pertinent groups of angiosperms (EA₀). The mean ages and the ranges of appearance of angiosperm groups (in millions of years before present) were based on fossil pollen records [12].

such as tyrosine (into benzylisoquinoline alkaloids and betalains) as well as allyl- and propenylphenols (into neolignans) [9].

In the rosifloreal block, gallic acid assumed its role as antioxidant, as demonstrated by the strong structural diversification [18] into oxidized derivatives ($O_{max} = 1.00$) of widespread occurrence in the, relatively, very ancient Hamamelidae. In compensation, biosynthesis of compounds requiring oxidative steps is slowed down or inhibited [11]. The strong constraints placed on the micromolecular flexibility of the Hamamelidae may be a possible cause of the difficulty of adaptation encountered by this subclass, which contains only 1.5% of the total number of angiosperm species.

Evolutionary demise of gallate accumulation, and consequently of its role as antioxidant, occurs in Rosidae ($O_{max} = 0.86$) and even more perceptibly in Dilleniidae ($O_{max} = 0.74$). Culmination of this effect, as reflected in the return of the maximum oxidation level to the value corresponding to ellagic acid ($O = 0.71$), occurs in Asteridae. In compensation, oxidative diversification of acetate-derived compounds, such as diterpenoids [19], is enhanced.

The regulatory role of gallic acid is not limited to its control of the cellular redox potential. The HIs of existing flowering plants belonging to the Magnoliidae are rather high, seemingly confirming the herbaceous origin of angiosperms [20]. Magnoliidae contain gallates only sporadically and in traces (Table 3). The introduction of gallic acid perceptibly enhances lignin accumulation in Hamamelidae. Caffeic acid seems to act in the opposite direction, inhibiting steps of the shikimate pathway by

a feedback mechanism. Thus, it accompanies the gradual demise of lignins from Hamamelidae via Rosidae and Dilleniidae towards Asteridae (Table 3). Biosynthesis of gallic acid possibly involves evolutionary canalization of the shikimate pathway [13]. Caffeic acid must be accumulated (through deactivation of O-methylation to ferulic acid) on the occasion of the demise of the functionally advantageous, but energetically cumbersome, lignins. Indeed, it has been verified, if only on a limited experimental basis, that gallic acid favours the deamination of phenylalanine to cinnamic acid by activation of phenylalanine ammonia-lyase (PAL), while both caffeic and ferulic acids promote retroinhibition of this enzyme [21].

Even introspection into the chemical evolution of angiosperms at the level of families within orders may still require general criteria for the analysis of dichotomous cases (i.e. cases of two or more lineages of families characterized by different micromolecular categories [22]). However, consideration of a single lineage requires the use of less general classification criteria to avoid parallelisms. It is at this hierarchic level that particular marker systems become valuable, mainly if their dynamic evolutionary aspects are measured. As reported previously, oxidative diversification of benzylisoquinoline alkaloids in the Magnoliidae-Ranunculidae complex [13] and of polyacetylenes in the Rosidae-Asteridae complex [23], as well as of several other biosynthetic categories (for pertinent refs see [24]), are good evolutionary indicators in particular taxa.

Unless chemical dichotomies do occur again at still lower hierarchic levels, such as subfamilies, tribes or

genera within families, the use of such particular dynamic criteria can be valuable (cf. sesquiterpene lactones in Asteraceae [25]). Finally, comparative phytochemistry continues to supply auxiliary clues for the clarification of relationships amongst genera, species and populations.

CONCLUSIONS

An obvious consequence of the evolutionary interdependence of plant morphology and metabolism concerns the alternative use of either of these criteria for classificatory purposes. With the revelation of general chemical markers, equal status can be accorded to morphological and micromolecular systematics in an integrated system. This blueprint for an integrated type of plant systematics shuttles between morphology and chemistry, both anchored in herbaceousness-dependent trends. The understanding of these trends will not only illuminate systematics, but, since they reflect sophisticated mechanisms for sensing and reacting to environmental fluctuations, will also lead to a more exact ecology.

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