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ROLE OF PHENOLICS IN PLANT EVOLUTION

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Key Word Index—Swain; plant evolution; flavonoids; phenolics.

Abstract—The biosynthesis of different flavonoids in plants has evolved in response to changes in the external environment. Phenolics have been implicated in diverse functional roles such as antioxidants and metal chelators, as UV-B light screens and as signaling agents both above and below ground between plants and other organisms. Many individual classes of flavonoids have multiple roles which have changed over the course of evolution. Tony Swain was in the forefront of such studies and applied his extreme originality and enthusiasm to show that biochemical diversity, engendered by species interactions, holds the key to understanding the evolution of plants. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

"The evolution of any major phylum of higher organisms is normally discussed as though it existed in complete isolation or, at the most, in competition with only one or two cognate groups." So begins Tony Swain's seminal paper on Angiosperm-reptile co-evolution [1]. Original and somewhat outrageous was Tony's engagement of Her Majesty the Queen's veterinarian to come weekly to the Royal Botanic Gardens, Kew, to inspect ten tortoises (*Testudo graeca*) and a few green lizards (*Lacerta viridis*). Through testing these reptiles' taste responses to lettuce leaves dipped in varying concentrations of tannins and alkaloids, Tony concluded that the dinosaurs died out because they could not detect by taste the toxic alkaloids present in early flowering plants. In other words, it was the chemical changes in the Cretaceous flora, that led to the demise of the dinosaurs! In all his many papers on plant evolution [2–6], the overall message he emphasized was always the same: "species do not evolve in isolation".

Being trained as an organic chemist, Tony Swain stressed the importance of biochemistry and biochemical interactions in the evolution of land plants [6]. He believed that the ability of plants to cope with the ever changing environmental challenges over evolutionary time was because plants can modify the rates of synthesis, delivery and turnover of chemical compounds which control growth and development. Plants also have the ability to synthesize specific chemical compounds which can act as toxins and deterrents to pathogens, herbivores and other competitors and are also able to attract needed symbionts for procreative purposes [6]. This chemical response to changing environments has led to the enormous structural variation in the major groups of secondary metabolites such as alkaloids, terpenoids and phenolic compounds, which are evident in plants today [7]. Tony's heart-felt belief in the prime importance of biological chemical interactions on evolution made him extremely intolerant of molecular biologists who were publishing in the early part of the 1980s. The molecular biologists, were, in his view, regarding evolution and evolutionary advancement as being concerned only with "variations in the sequences and structures of nucleic acids and proteins in different organisms"—a view, which according to him [6]: "subsumes macroevolution to some unimaginative biochemical trashcan!"

EVOLUTION OF FLAVONOIDS

Flavonoids are a class of phenolic compounds which lend themselves particularly well to studies on plant evolution [7]. Based on their known biosynthetic pathways (see Fig. 1), it is assumed that different flavonoid groups have appeared sequentially during plant evolution. This assumption presumes that the simplest structural compounds or groups of compounds (e.g. flavanones) which appear early in the biosynthetic pathway, evolved in the first photosynthetic plants, whereas, compounds synthesized later in the biosynthetic pathway (e.g. anthocyanins)

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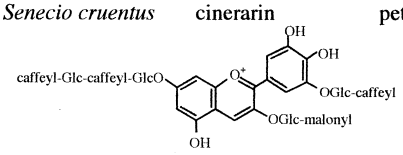
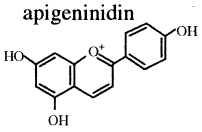
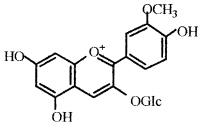
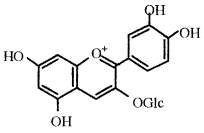
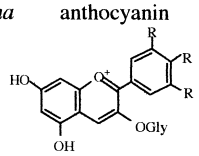
Plant	Compound	Organ	Function / Ref.
ANGIOSPERMS			
Senecio cruentus	cinerarin 	petals	pollination [45]
Sorghum	apigeninidin 	leaf sheath	phytoalexin [50, 51] anti-microbial [52] antioxidants [13, 14]
GYMNOSPERMS			
Abies concolor	petunidin-3-glucoside 	cones	? [55]
Pinus contorta	cyanidin-3-glucoside 	leaves	cold tolerance [58]
Pinus banksiana	anthocyanin 	seedlings	photoinhibition tolerance [56, 57]
FERNS			
Davallia divaricata	pelargonidin-3-*p*-coumaryl-glc-5-glc (monardein)	young leaves	? [48]
Fern species		leaves	? [48]
MOSSES			
Bryum, Splachnum	luteolinidin-5-glc	leaves	? [9]
LIVERWORT			
Cephaloziella exilifolia	anthocyanin-like	thallus	? [60]

would occur in the most recently evolved plants or extant plant taxa [8].

Flavonoids as end products

In 1986, Tony Swain [6] proposed an evolutionary scheme for the different flavonoid groups and their biosynthesis as they are related to four major events in plant evolution (see Table 1).

Dehydrogenation of the C_2–C_3 bond in flavanones led to the synthesis of flavones, which first appeared in the green algae. The evolution of cinnamic acids and cinnamyl alcohols in the early vascular plants, led to the biosynthesis of lignin and its role as important components of cutin, suberin and sporopollenin (G. A. Cooper-Driver, in press). The evolution of the ferns and the introduction of 3-hydroxylation to the flavanone C-ring led to the synthesis of flavonols and the corresponding flavan-3-ols (catechins) and flavan-3,4-diols. The latter two are precursors of the pro-anthocyanidins or condensed tannins, important feeding deterrents to herbivores and pathogens. Evolution of the angiosperms was accompanied by the synthesis of colored pigments, anthocyanins, chalcones and aurones, important as pollinator attractants and fruit and seed dispersers [6].

Since Tony Swain published this scheme, some of the data existing at the time have been questioned and also new information has become available. First, Markham has questioned the presence of flavonoids in present day algae: "Until confirmation of the original finding is achieved, the ability of *Nitella*, or any algae, to biosynthesize flavonoids can only be accepted with reservation" [9]. There is also a great deal of additional information available on the evolution and chemistry of the bryophytes [9–11]. While approximately 50%

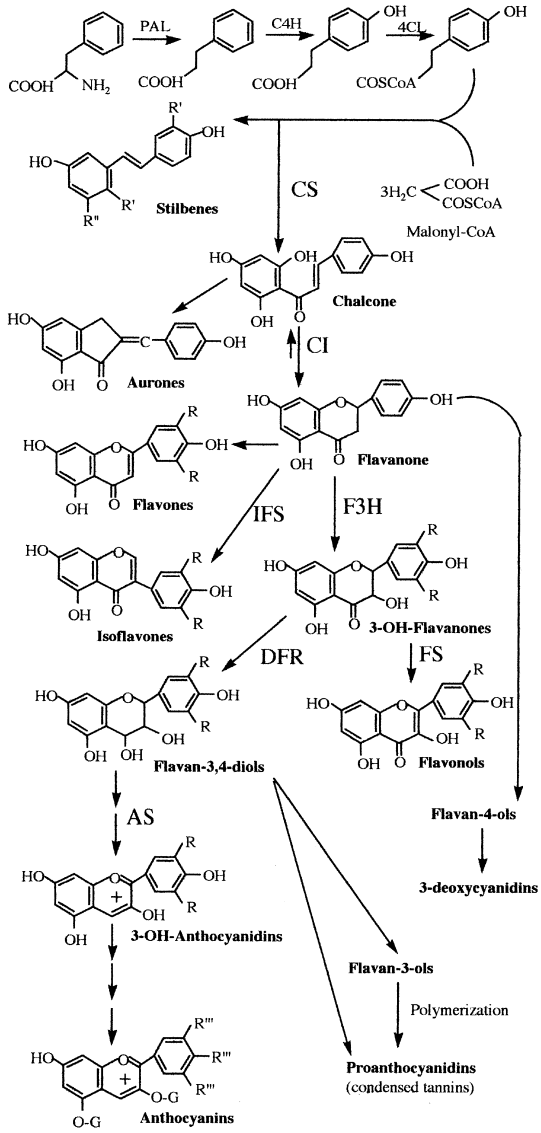


Fig. 1. Biosynthesis of phenolic compounds. Abbreviations: Enzymes—PAL: phenylalanine ammonia lyase; C4H: cinnamate 4-hydroxylase; 4CL: 4-coumaroyl-CoA ligase; CS: chalcone synthase; CI: chalcone isomerase; F3H: flavanone 3-hydroxylase; IFS: isoflavone synthase; DRF: dihydroflavonol reductase; FS: flavonol synthase; AS: anthocyanin synthase. Side groups: R = H, OH; R′ = H, OCH_3, COOH; R″ = OH, OCH_3, 0-Glu; R‴ = H, OH, OCH_3; O-G = *O*-Glycosidic groups. Adapted from [8, 49].

of bryophytes studied totally lack flavonoids, (the hornworts lack flavonoids altogether), those that do synthesize flavonoids show a high level of biochemical sophistication. For example, mosses in the order Bryales, synthesize flavone *C* and *O*-glucosides, biflavones, isoflavones, aurones and 3-deoxyanthocyanidins. Flavonoids are widely distributed in the liverworts as flavone *C* and *O*-glycosides, dihydroflavones, dihydrochalcones, flavonols, and aurones. On the basis of secondary metabolites it seems that both mosses and liverworts have originated from completely different ancestors [11].

Flavonoid enzymes

While Tony's phylogenetic scheme [6] recognizes the different biosynthetic steps in flavonoid biosynthesis and their role in evolution, more is now known concerning the enzymes involved [8, 12]. Many structural genes from the flavonoid pathway have now been sequenced and their gene products show homologies with enzymes from primary metabolism [8, 12]. For example, the condensation of *p*-coumaroyl CoA with malonyl CoA, which is catalyzed by chalcone synthase, is highly similar to condensation reactions in fatty acid biosynthesis and based on the sequence homologies between the proteins, the condensing enzymes of fatty acid biosynthesis and chalcone synthase. These might originate from a common ancestor [12]. Other homologies between flavonoid biosynthetic enzymes and primary metabolism include chalcone synthase and stilbene synthase [8]; and flavonoid 3′ hydroxylase and flavonoid 3′5′ hydroxylase, both P_{450} type enzymes which are localized in the endoplasmic reticulum, and presumably originate from P_{450} hydroxylases of primary metabolism. Dihydroflavonol-4-reductase, another enzyme involved in flavonoid biosynthesis, shows homologies with enzymes involved in steroid metabolism. All these example suggest that the enzymes involved in flavonoid biosynthesis were recruited from more ancient pathways (Table 2).

Functional evolution of flavonoids

Tony always emphasized that any biochemical changes in flavonoids, during the course of plant evolution, must be understood in relationship to their functional roles as anti-oxidants and metal chelators, as increased protection against excess UV-B; as protectants against herbivores and pathogens, and as attractants to plant pollinators and seed dispersers [6].

Flavonoids as antioxidants and metal chelators

Tony [6], predicted that future research would be based on such important functions of flavonoids as in their role as antioxidants and as metal chelators. A search for bioactive disease-preventing phytochemicals in food, has fuelled and renewed an interest in plant phenolics such as flavonoids, especially after studies using *in vitro* model systems have shown many of them have a higher antioxidant efficiency compared to vitamin C and vitamin E. Many phenolic compounds are being sought for their qualitative radical-scavenging (antioxidant) and antithrombotic properties, as potential agents against cancer and cardiac diseases [13, 14].

The antioxidant activity of flavonoids is based on the polyphenolic ($C_6C_3C_6$) structure, and the presence

Table 1. The evolution of phenolic compounds (1986). Adapted from [6]

Time (mya)	Taxa	Phenolic class	Enzymes
500	Green algae	cinnamic acids chalcones flavanone	PAL chalcone synthetase
		flavone	flavanone dehydrogenase
400	Psilophyta	hydroxy-flavones	B-ring hydroxylation
		cinnamyl alcohols/lignins	cinnamic acid reductase
370	Ferns	flavonols	flavanone 3-hydroxylase and dehydrogenase
		catechins flavan-3, 4-diols (proanthocyanidins)	dihydroflavonol 4-keto-reductase
120	Angiosperms	anthocyanidins aurones	dihydroflavonol dehydratase chalcone/aurone isomerase
		isoflavone	flavanone-2, 3 phenyl isomerase

Table 2. The evolution of phenolic compounds (1997). Adapted from [8, 12]

Time (mya)	Taxa	Phenolic class	Enzymes
500	Bryophytes	chalcones flavanone flavone biflavone isoflavone 3-OH flavanone flavonols aurones 3-deoxyanthocyanidins	chalcone synthase (CS) chalcone-flavanone isomerase (CI) flavone synthase isoflavone synthase (IFS) flavanone-3-hydroxylase (F3H) flavonol synthase (FS) synthase
370	Ferns	flavan-3, 5-diol flavan-3-ol 3-OH proanthocyanidins	dihydroflavonol-4-reductase (DFR) synthase
120	Gymnosperms Angiosperms	3-OH anthocyanidins	synthase (AS)

of both 4-carbonyl (or 4-oxo), and 5- or 3-hydroxyl groups [6]. 2, 3 Unsaturation in the C-ring and 3′4′ orthodiphenolic structure of the B-ring also influence antioxidant activity [13] (see Fig. 2).

Antioxidant activity is dependent on the phenolic hydrogen donating ability of the flavonoid. The hydrogen ions hinder or prevent the auto-oxidation or oxidation of substrates like low density lipoproteins, by damaging free oxyl radicals by their scavenging action [13]. When measured relative to TROLOX (water soluble analog of vitamin E), the main criteria determining radical scavenging activity also depends on the stability of the antioxidant generated aryloxyl radical, the ability of the flavonoid to protect other antioxidants and its potential as a metal chelator [14].

Recent advances in methods have helped to standardize and assess the antioxidant property of flavonoids and phenylpropanoids. There is new evidence to show anthocyanins provide additional protection to known antioxidants such as ascorbic acid by binding to form an ascorbic acid (copigment)-metal-anthocyanin complex [15]. Techniques like pulse radiolysis [16, 17] enable the measurement of relative radical-scavenging efficiency of flavonoids for radicals such as hydroxyl ($^{\bullet}OH$), azide ($N_3^{\bullet}$), superoxide ($O_2^{\bullet}$-) lipid

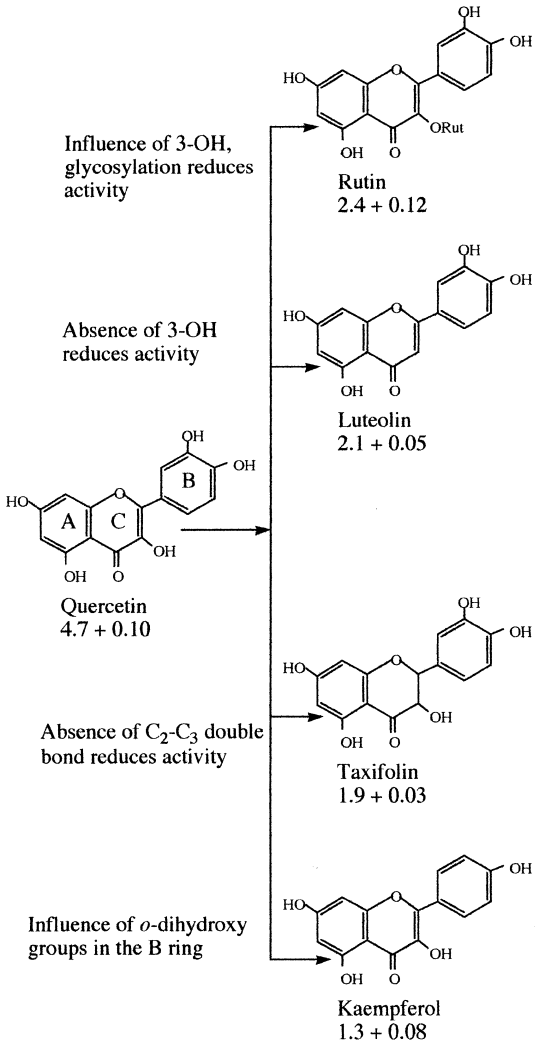


Fig. 2. The influence of chemical structures on the relative anti-oxidant values of flavonoids. Adapted from [13].

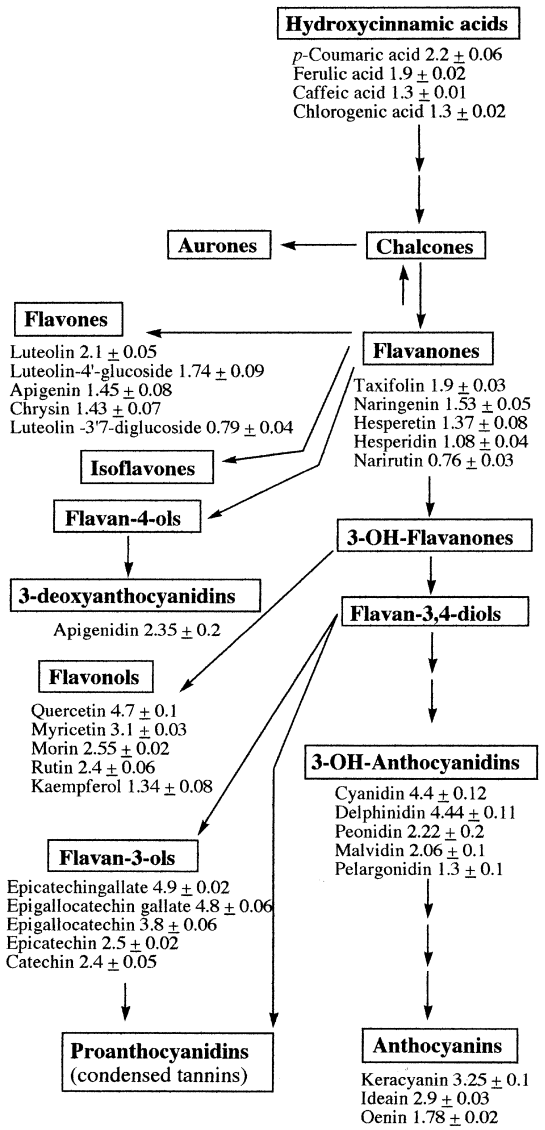


Fig. 3. Anti-oxidant values of different groups of flavonoids relative to TROLOX. Adapted from [13, 14].

peroxyl ($LOO^{\bullet}$) and tertiary butyl alkoxyl radical ($tBuO^{\bullet}$). Spectrophotometric analysis following ferrylmyoglobin/ABTS assay has facilitated the study of antioxidants from diverse sources like plant extracts, body fluids (salvia, serum/plasma) and pure solutions of possible antioxidants [18, 19]. A hierarchy based on antioxidant activity can be generated within each flavonoid group, which can be used to correlate the important structural contributions of the C, B and A rings that are critical to and influence the antioxidant activity of flavonoids [13]. When measured relative to TROLOX, the antioxidant activity of the different phenolic groups can be compared (see Fig. 3).

Overall, those phenolic groups that occur earlier in the biosynthetic pathway have lower values than groups that occur later, suggesting that over the course of plant evolution, plants may have required phenolics with higher antioxidant values to combat not only the free radicals generated from UV light action, but also those generated by their own metabolism. All this is highly speculative as an antioxidative function in plants themselves is still a matter of debate (Harborne 1986) [7].

Flavonoids as screens against destructive UV light

In recent years, there has been renewed interest in UV-B due to the deterioration of the earth's ozone layer. UV-B causes a number of detrimental effects to plants causing damage to DNA, Photosystem II and also to plant phytohormone (IAA) levels [20]. It is known that damage can be largely avoided by absorption of UV-B (280–320 nm radiation) by flavonoids and anthocyanins that are localized in epidermal cells.

However, the first direct evidence for the role of flavonoids in UV protection has come from studies with flavonoid deficient mutants of *Arabidopsis thal-*

iana [21, 22]. Work with *Arabidopsis* mutants has confirmed that flavonoids shield DNA from UV-B induced damage. The biosynthesis of flavonoids is rapidly and strongly induced by exposure to UV-B radiation at the level of the transcription of flavonoid biosynthetic genes for the enzymes PAL, 4-coumarate-CoA ligase, chalcone synthase, chalcone flavanone isomerase, and dihydroflavonol-4-reductase [23]. *Arabidopsis* mutants defective in the genes for ferulate hydroxylase, the first enzyme in the cinnamate pathway (cinnamate defective mutants), are even more sensitive to UV-B than the chalcone isomerase deficient mutants (flavonoid defective mutants). This suggests that hydroxycinnamic acids may be significantly more important than flavonoids in UV protection [24]. It also appears that both cinnamic acids and flavonoids must have been particularly important during the period of early land-plant evolution, when atmospheric O_3 layer was lower and the solar UV-B radiation higher than at present [25].

Flavonoids and interactions in the soil

While studies on the biochemical interactions between insects and plants was gaining most of the attention and excitement during the 1970s and 1980s [26–30], in the last 10 years there has been a shift in focus from above ground to below ground and in particular to chemical signaling between microbes and plant hosts [31]. Flavonoids have been implicated in interactions between nitrogen-fixing symbionts and their plant hosts, between plant roots and fungi in mycorrhizal associations, between parasitic plant roots and their hosts and also between plants and microbial pathogens [32].

Flavonoids and nitrogen fixing symbiotic bacteria

Nitrogen-fixing cyanobacteria are able to colonize leaves of the aquatic fern, *Azolla*; thalli in liverworts and hornworts; and leaves of the angiosperm, *Gunnera* [33]. Phenolic compounds have been found in the vicinity of the symbiotic tissue of *Gunnera* and are thought to be responsible for signaling between the cyanobacterium and its host. In the nitrogen-fixing symbiont and related genera, flavones, flavanones and the isoflavone diadzein, released by the legume host plant roots, induce the expression of bacterial nodD (nodulation genes) which determine the specificity of the interaction [34]. It has been found that there is a homology between certain flavonoid-responding genes of *Rhizobium* and the genomic sequences of symbiotic cyanobacteria in *Azolla* [33].

Flavonoids and symbiotic fungi

Modification of gene expression during arbuscular mycorrhizal synthesis has also been shown to involve changes in flavonoid chemistry [35]. Colonization of *Medicago truncatula* (alfalfa) by a mycorrhizal fungus, *Glomus versiforme*, is accompanied by the cell specific induction of genes encoding phenylalanine ammonia lyase (PAL) and chalcone synthase (CS). The PAL and CS transcripts are localized in cells containing arbuscules, where they may be involved in the biosynthesis of non-defense related secondary metabolites, such as 4′,7-dihydroxyflavone, which are known to accumulate in endomycorrhizal roots. The function of these metabolites is unclear but flavonoids and some isoflavonoids have been shown to stimulate the growth of endomycorrhizal fungi [35].

Flavonoids also play a significant role in the interactions occurring between tree roots and soil borne ectomycorrhizas [36]. In larch, mycorrhization of the fine roots activates the phenylpropanoid pathway and many different phenolic compounds are synthesized.

Flavonoids and invading plant pathogens

Phenolic compounds have a role in the active expression of susceptibility and resistance to plant pathogens [37]. Glycosides of the flavonols, kaempferol and quercetin, have been shown to induce the *vir* (virulence) genes of *Agrobacterium tumefasciens* [38].

The accumulation of salicylic acid, a simple hydroxybenzoic acid, in noninfected tissue in several angiosperm species, has been shown to be associated with the development of greatly enhanced resistance to further pathogen attack—systemic acquired resistance (SAR).

Transgenic plants have been created containing a bacterial gene for salicylate hydroxylase, which reduces the levels of salicylic acid in plant tissues. Plants expressing the bacterial gene are being used to assess the rate of the development of the lesions and spread of the pathogen. Tissues exhibiting SAR have a mechanism for quicker induction and enhanced expression of defense genes [39].

Much of this work, which implicates phenolics being involved in microbial/plant interactions, has been done with only a few species of angiosperms and gymnosperms. However, plant-microbial interactions involving phenolic signaling may have evolved earlier in plant evolution. Taylor *et al*. [40] have shown unequivocal evidence for arbuscules being present in an endomycorrhizal symbiosis from fossils collected from the 400 million-year-old Rhynie chert and also stunning visual evidence for chytrids attacking plants [41, 42]. While such chemical interactions will probably always be impossible to document from the fossil record, it seems likely that such chemical interactions occurred.

Rosema *et al*. [20] have attempted to draw a phylogenetic tree of flavonoid evolution based on phenolic function. This has proved difficult because of the multiplicity of roles played by individual flavonoid compounds or groups of compounds across plant taxa. The same compound or groups of compounds may appear in apparently unrelated taxa. This can be well

illustrated with the anthocyanins whose primary function appears to have changed over the course of plant evolution.

Multifunctional roles of anthocyanins

The presence of anthocyanins in angiosperms and their role as signals in pollination and seed dispersal is well documented [43, 44]. There are seventeen known anthocyanidins, which may be glycosylated, or acylated with sugars, or with flavones as a dimagnesium complex [43, 44]. Acylation in nature with aliphatic dicarboxylic acid renders the cationic anthocyanin a zwitterion [45].

Much of the early work on anthocyanins as "visible color signals" can be credited to Dr Bate-Smith (or EC as he was always called), Tony's life long friend and mentor. As previously described by Jeffrey Harborne (this series), EC was an amateur grower of dahlias and he studied the pigments of the dahlia petals growing in his garden and isolated anthocyanins many of which are still in existence [46, 47]. Other colored pigments, 3-deoxyanthocyanidins, occur occasionally in angiosperm flowers as is shown by the characteristic orange and yellow flowers of the New World Gesneriaceae [48]. Mutations arising from spontaneous events or the movement of transposable elements can quickly alter the expression, activity or substrate specificity of individual biosynthetic enzymes, resulting in new color hues or patterns. This is contingent with the ability of flavonoid metabolism to adjust quickly and easily to new environmental pressures [49].

Anthocyanins and 3-deoxyanthocyanidins have roles in flowering plants other than as attractants and are also present in other members of the plant kingdom (see Table 3).

Anthocyanins and 3-deoxyanthocyaninidins in flowering plants can also act as antioxidants [13, 14], phytoalexins [50, 51] or as antibacterial agents [52].

In gymnosperms the production of anthocyanins is largely confined to the reproductive structures. Glycosides of cyanidin and in several cases, of delphinidin, have been reported from conelets in Pinaceae and from several species of *Chamaecyparis* [53]. Pelargonidin glycosides and anthocyanins with complex sugars, such as neohesperidosides, have also been isolated from the Podocarpaceae [54]. More complex *O*-methylated anthocyanidins occur in *Abies*, namely malvidin, petunidin and peonidin-3-glucosides. There is, however, no evidence for acylation which is common in the angiosperms [55]. Their exact function in gymnosperms is still speculative. Most species of Pinaceae and other gymnosperms are wind pollinated and their seeds are also wind dispersed, so it is unlikely that either anthocyanins or 3-deoxyanthocyanidins play a direct role in seed dispersal. There is some evidence for alternative roles. Increased tolerance to photoinhibition in *Pinus banksiana* seedlings is stimulated by anthocyanins [56–57]. Also, they may play a role in protection against the cold. Cold-induced foliar accumulation of anthocyanin ("purpling") has been seen in many species of pine seedlings [58].

While anthocyanins and 3-deoxyanthocyanidins appear to be completely absent from the Lycopodiales, Selaginellales, Isoetales, Equisetales and Psilophytales, they are present in a number of species of ferns. The presence of anthocyanins has been reported in the leaves of 19 taxa of thelypteroid ferns of the Western Ghats of South India but no structures were given [59]. While most ferns that have been examined have 3-deoxyanthocyanidins there is one report of anthocyanins in ferns and that is in the sori of *Davallia divaricata* [48]. While their specific function in ferns is unknown, it is interesting that once again, as in the gymnosperms and angiosperms, they are found associated with the reproductive structures. Markham reports the presence of 3-deoxyanthocyanidins as reddish colored pigments in mosses [9]. These are present in *Bryum* cell sap. In *Sphagnum* the phenolic pigment, sphagnol may be bound to the cell wall. Anthocyanin-like pigments have been reported in the Antarctic liverwort, *Cephaloziella exilifolia* [60].

Thus it appears that the ability to synthesize anthocyanins and 3-deoxyanthocyaninidins is an ancient one, and over the course of evolution these compounds have developed varied functions in the biology of plants. Chemical compounds, which may have arisen early in the evolution of plants, have gradually taken over new functions.

Anthocyanins could best be interpreted as an exaptation, having arisen before the performance advantage which they later became associated with, namely flower color.

CONCLUSION

Much of the continuing work on flavonoids fits in with Tony Swain's views as expressed in his 1986 paper [6]. Flavonoids have appeared sequentially during plant evolution. The different genes and enzymes involved with phenolic synthesis have also appeared sequentially, many of which have been shown to be homologous with primary enzymes. New work with bryophytes suggests that the enzymes involved with the biosynthesis of individual groups of compounds, including the anthocyanins, are much more ancient than we first thought.

Over the course of evolution there have been changes in the functions or elaboration of existing functions and it is apparent that the same compounds can assume a variety of roles. It therefore appears that flavonoid metabolism is an extremely malleable biosynthetic system which is capable of adjusting quickly and easily to new environmental pressures [49].

Over the last 10 years there has been much more emphasis on the mechanisms underlying the successful adaptation of plants to changing environmental conditions [49] a trend which Tony encouraged. "We need

Table 3. The role of anthocyanins and 3-deoxyanthocyanidins in plants

Plant	Compound	Organ	Function/Ref.
Angiosperms			
Senecio cruentus	cinerarin	petals	pollination [45]
Sorghum	apigeninidin	leaf sheath	phytoalexin [50, 51] anti-microbial [52] antioxidants [13, 14]
Gymnosperms			
Abies concolor	petunidin-3-glucoside	cones	? [55]
Pinus contorta	cyanidin-3-glucoside	leaves	cold tolerance [58]
Pinus banksiana	anthocyanin	seedlings	photoinhibition tolerance [56, 57]
Ferns			
Davallia divaricata	pelargonidin-3-*p*-coumaryl-glc-5-glc (monardein)	young leaves	? [48]
Fern species	apigenidin	leaves	? [48]
Mosses			
Bryum, *Splachnum*	luteolinidin-5-glc	leaves	? [9]
Liverwort			
Cephaloziella exilifolia	anthocyanin-like	thallus	? [60]

to know more at the physiological, and ultimately at the molecular level about the ways in which phenolic compounds interact with or affect other organisms'' [60]. Molecular genetic studies are providing new insights into the mechanism of flavonoid evolution and new information on the evolution of flavonoid enzymes and their relationship to enzymes involved in primary metabolism. Additional clues regarding the evolution of flavonoid function are emerging from studies on flavonoid gene regulation. There is evidence for the possible existence of different regulatory constraints within different plant lineages. Overall, molecular biology is beginning to have an enormous impact on the study of evolution generally and on our understanding of flavonoid evolution in particular. Tony recognized the enormous contribution that this field was going to play [61] and would have been excited at the results.

Tony Swain's message that ''species do not evolve in isolation'' has been acknowledged and is not falling on deaf ears. *Phytochemistry* and *Biochemical Systematics and Ecology*—both journals which Tony started—and other journals, are publishing papers on chemical interactions and new information is constantly emerging. Thank you to a friend, the ultimate mentor stimulating a life-long interest in plants and their chemistry—we miss your ideas, your belligerence and your great gift for making us all care about each other.

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