However, owing to difficulties in isolating purified nuclei in high yields, and problems with proteolytic degradation, progress in this area has been slow and many conflicting reports exist (*33*). In our report, the low ratio of DNA to RNA of the chromatin indicates that ribosomal contamination is insignificant, and the gels show no indication of proteolytic activity if PMSF is included in the isolation medium.

Our results show that the eukaryotic nucleus of the binucleate dinoflagellate *Peridinium balticum* contains basic proteins with the same gel mobilities as four of the five histones from calf thymus. The question then arises as to whether these proteins should be called histones. Caution should be used in this regard because of the possibility that low-molecular-weight basic proteins arising from ribosomal or other cytoplasmic contamination can migrate in the same region of a gel as histones (*33, 34*). Furthermore, there are histone-like nonhistone chromosomal proteins which have gel mobilities nearly identical to those of known histones (*13, 35*). Thus the co-migration of a particular low-molecular-weight basic protein with a known histone could be coincidental. However, it is unlikely that the co-migration of all four of the bands detected in our study with four calf thymus histones is purely coincidental. It is suggested, therefore, that the proteins detected in our study are histones, although definite proof will require characterization of the individual bands. If these proteins are indeed histones, the apparent absence of H3 requires explanation. If just one histone is absent, one would not expect it to be a histone as highly conserved as H3 (*36*). It should be kept in mind that H3 could be present but may migrate in an anomalous manner. For example, the existence of histones in *Euglena* has recently been confirmed (*37*), and H1 was found to migrate in an anomalous manner. It is possible that H3 from *Peridinium balticum* has a slightly higher mobility than H3 from calf thymus and merges with the band of next highest mobility.

It is suggested that mitotic chromosome morphology is a result of further compaction of the nucleosomes, the DNA-histone complexes which constitute the basic structure of interphase chromatin (*38*). Thus one would expect histones to be present in organisms possessing morphologically distinct chromosomes, but absent in organisms whose DNA does not condense into chromosomes, and this is usually the case. A unique situation may therefore exist in binucleate dinoflagellates where we find a nucleus containing condensed chromosomes but no histones, and a nucleus containing histones but lacking condensed chromosomes.

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Chemical Evidence for Separating the Psilotaceae from the Filicales

Abstract. *The distribution of flavonoid compounds in* Psilotum, Tmesipteris, *and primitive filicalean ferns shows that it is unlikely the two groups are closely related. The Psilotaceae synthesize only amentoflavone and related biflavonyls which are totally absent from ferns. The primitive ferns contain the full complement of flavonols and proanthocyanidins, as found in all other filicalean families.*

The two living psilophyte genera, *Psilotum* and *Tmesipteris*, have been regarded as relics of ancient Devonian Rhyniophytes. As such, they are considered to be living fossils although there is no direct evidence regarding the origin and early evolution of the Psilotaceae and no *Psilotum*-like remains have been identified with certainty from intervening geological periods (*1*). Bierhorst has suggested, on the grounds of the similarity of the gametophytes and other morphological and anatomical features, that the primitive fern family Stromatopteridaceae and the Psilotaceae are very closely related and that the Psilotaceae should be transferred to the order Filicales and hence be regarded as primitive ferns (*2*). This proposal has, however, met with some degree of opposition (*3*), but the problem has not, so far, been resolved. This report presents information on the distribution of flavonoids in the two groups which strongly suggests that they are unrelated.

An analysis of the distribution of sec-

ondary plant compounds has often provided data concerning the phylogenetic and interrelationships of plant taxa (*4*). This has also been true for the ferns and fern allies and a large body of chemical information has been accumulated (*5*). Flavonoids are particularly useful in this respect because, along with an evolutionary progression in the complexity and variety of flavonoid compounds in the plant kingdom (*6*), there is a more or less exclusive ability of the plant divisions to produce only certain classes of flavonoids (*7*). With this in mind, I investigated the flavonoid chemistry of *Psilotum*, *Tmesipteris*, *Stromatopteris*, and members of the primitive fern families Schizaeaceae and Gleicheniaceae in an attempt to provide chemical information on the proposed affinities between these groups of plants.

Fresh plant material, with the exception of *Stromatopteris moniliformis* for which a herbarium specimen was used, was extracted with boiling alcohol. The extracts were concentrated, and the compounds were separated by two-dimensional chromatography (*8*). Part of the extract was hydrolyzed with 2*N* HCl at 100°C and further extracted with *n*-amyl alcohol, and the individual compounds were isolated and identified by a combination of thin-layer chromatography and ultraviolet spectroscopy (*9*) and by comparison with authentic markers. The distribution of the major flavonoid classes in the species analyzed, the biflavonyls, flavonols, and proanthocyanidins, is shown in Table 1.

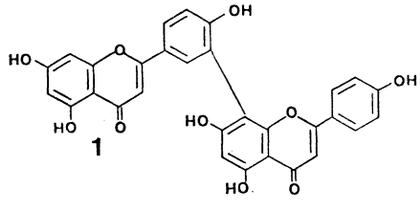
Biflavonyls were the only group of flavonoids present in the two genera of the Psilotaceae, and the biflavone amentoflavone (**1**) was identified and isolated from all species. The formation of biflavonyls appears to be a feature that was developed early in the evolution of vascular plants (*7, 10*). Amentoflavone and hinokiflavone have been isolated from *Psilotum triquetrum* Sw. (*11*) and amentoflavone has been isolated from three species of *Selaginella* (*12*). No biflavonyls have been reported to occur in the Lycopodiaceae, Isoetaceae, or the ferns, although they occur in gymnosperms and some angiosperms (*10*).

As was expected, biflavonyls were absent from the primitive ferns studied (Table 1). Instead, all contained the flavonols kaempferol (**2a**) and quercetin (**2b**) and the proanthocyanidins procyanidin (**3a**) and prodelphinidin (**3b**), as were found in the more advanced members of the Filicales. Flavonoids present in ferns show advanced features over the biflavonyls of the primitive tracheophytes in the introduction of the 3-hydroxy group leading to the synthesis of flavonols and the elaboration of the proanthocyanidins. Proanthocyanidins have been reported in 91 percent of fern genera and flavonols quercetin and kaempferol have been found in 69 and 48 percent of fern species examined, respectively (*13, 14*).

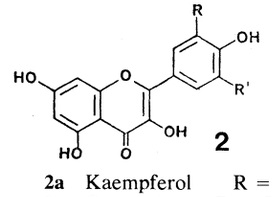
Table 1. Distribution of flavonoids in the psilotaceae and primitive filicales.

Taxa	Compounds present		
	Biflavonyls*	Flavonols†	Proanthocyanidins‡
Psilotaceae			
Psilotum complanatum Sw.	+	–	–
P. nudum (L.) Beauv.	+	–	–
P. triquetrum Sw.§	+	–	–
Tmesipteris tannensis Bernh.	+	–	–
Filicales			
Stromatopteris moniliformis Mett.	–	+	+
Gleichenia linearis (Burm. F.) Underwood	–	+	+
Aneimia dregeana Kze.	–	+	+
A. phyllitides (L.) Sw.	–	+	+
A. tomentosa (Sav.) Sw.	–	+	+
Mohria caffrorum (L.) Desv.§	–	+	+
Lygodium japonicum (Thbg.) Sw.	–	+	+
Schizaea pectinata Sw.	–	+	+

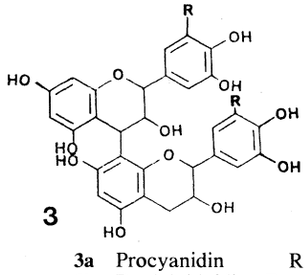
*Amentoflavone. †Kaempferol and quercetin. ‡Procyanidin and prodelphinidin. §Data from Voirin (*14*).



1 Amentoflavone



2a Kaempferol R = R′ = H
2b Quercetin R = OH, R′ = H



3a Procyanidin R = H
3b Prodelphinidin R = OH

Thus, there is a primary distinction between the Psilotaceae and primitive ferns in their ability to synthesize the two distinct groups of flavonoids. The chemical distinctness of the psilophytes is also supported by work on the components of the cutin (*15*). Caldicott *et al.* found unique trihydroxy hexadecanols in the stem cutins of both *Psilotum* and *Tmesipteris*, whereas all higher plants including ferns have dihydroxyhexadecanoic acids (*15*).

From the chemical evidence presented here, it seems that *Psilotum* and *Tmesipteris* should be retained in the Psilotales and should not be regarded as primitive members of the Filicales. It seems likely, therefore, that *Stromatopteris* and the primitive fern genera represent an evolutionary continuum, but that *Psilotum* and *Tmesipteris* are on a different branch of the line of vascular plant evolution.

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Spiroplasmavirus citri 3: Propagation, Purification, Proteins, and Nucleic Acid

Abstract. *SVC3 is a short-tailed polyhedral virus particle morphologically detectable in many spiroplasmas. It was isolated from two different spiroplasmas (*Spiroplasma citri *and the suckling mouse cataract agent) by infecting lawns and broth culture of another strain of* Spiroplasmavirus citri. *Virions from either donor strain had a buoyant density of 1.26 grams per cubic centimeter (metrizamide) or 1.45 grams per cubic centimeter (cesium chloride), and contained five proteins and linear double-stranded DNA with a molecular weight of 14 × 10^6. Other spiroplasmaviruses have not been propagated, and the molecular weights of double-stranded DNA from other mycoplasma* (Acholeplasma) *viruses are unknown.*

Spiroplasmaviruses of three different morphologic types are detectable by electron microscopy in the plant-pathogenic mycoplasma, *Spiroplasma citri*, and some other spiroplasmas (*1, 2*). However, unlike viruses of certain other Mollicutes [such as *Acholeplasma laidlawii* (*3*)], spiroplasmaviruses have not been previously propagated nor characterized. We report here the propagation and some properties of *Spiroplasmavirus citri* 3 (SVC3), a short-tailed polyhedral virus particle (*1*).

To isolate virus, lawns of 21 strains of spiroplasmas (*4*) were inoculated by spotting (*5*) with donor broth cultures of each other. Although, in more than 500 trials, various combinations were occasionally successful, plaques were consistently produced by strain 608 of *S. citri* and by a strain (SMCA) of the suckling mouse cataract agent (*4, 6*), when either was spotted on lawns of *S. citri* strain 750. Samples of these donor cultures in SMT broth (*5*), incubated at 32°C for different durations (2 to 5 days), were tested on SMT agar lawn inoculums of strain 750 that were kept at 32°C for 3 to 24 hours after seeding and before spotting. The best results were found by spotting 48-hour donor cultures on lawns incubated for 6 hours, and then by incubating further at 32°C in an atmosphere of 5 percent CO_2 and 95 percent N_2. Large clear plaques were seen after 5 days of incubation. Washings (*7*) of these plaques examined by electron microscopy showed only SVC3 particles (frequently adsorbed to the few spiroplasmas present). Millipore-filtered (0.22 μm) samples, spotted on fresh lawns of strain 750, reproduced the cycle of plaques and infectious washings repeatedly. Washings from single plates contained 1 × 10^8 to 1 × 10^9 plaque-forming units (PFU) per milliliter, as determined by terminal dilutions showing individual clear plaques about 1.5 mm in diameter (Fig. 1a). Plaques were produced by supernatants of donor cultures (*8*) as well as by whole culture, and by Millipore (0.22 μm) filtrates thereof.

No plaques appeared spontaneously on control (unspotted) lawns or on those spotted with donor cultures heated at 90°C for 30 minutes, although electron microscopy indicates that SVC3-like particles can sometimes be detected in strain 750. The situation is analogous to the use of known virus-carrying strains as indicators for viruses of *A. laidlawii* (*3*).

Both viruses (SVC3/608 and SVC3/SMCA) were propagated in broth cultures of strain 750 to provide better yields for purification, and virions were banded by isopycnic centrifugation in

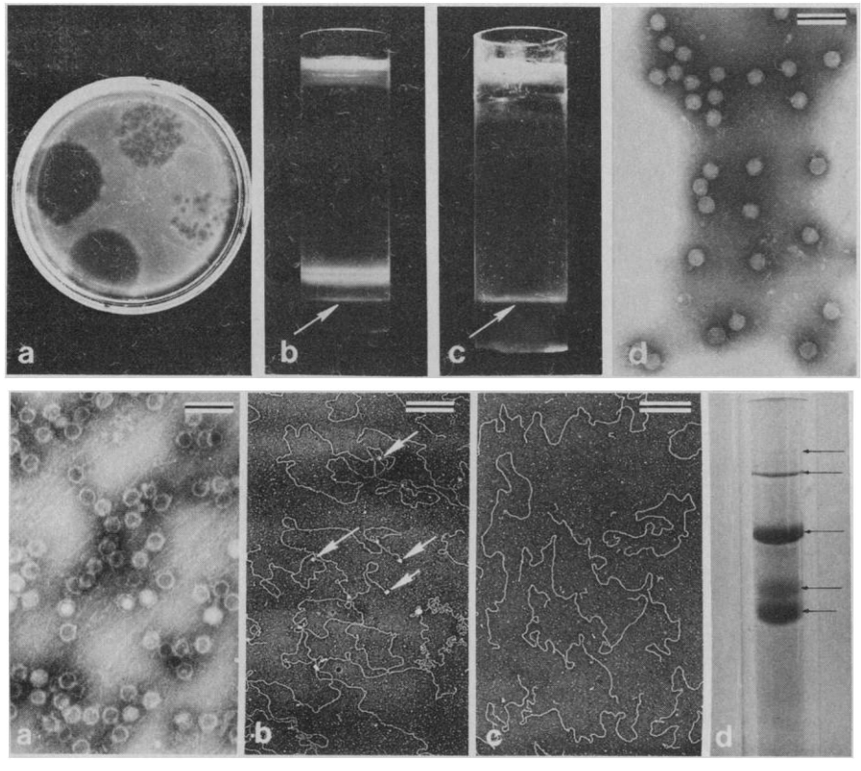

Fig. 1 (top). (a) Plaques produced on *S. citri* 750 lawn by SVC3/608: tenfold dilutions to 10^{-6} of filtered plaque washings. (b) Virus band (arrow) at 1.26 g/cm³, from isopycnic centrifugation in metrizamide (*9*) of PEG-sedimented material from infected broth culture supernatant fluid. (c) some band material, rerun in metrizamide and banding (arrow) at same density. (d) Intact SVC3 virions comprising such bands; electron micrograph (marker = 0.1 μm) of material negatively stained by 2 percent ammonium molybdate. Fig. 2 (bottom). (a) Empty SVC3 virions and stranded material in preparation from isopycnic banding (*10*) in CsCl; electron micrograph (marker = 0.1 μm) of material negatively stained by 2 percent uranyl acetate. (b) Metal-shadowed aqueous Kleinschmidt preparation (*11*) of material from (a), showing strands extruding from virions (arrows): electron micrograph (marker = 1 μm). (c) Similar preparation of purified SVC3 DNA, showing extended linear molecules: magnification same as in (b). (d) Pattern of five structural proteins of SVC3, obtained by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (*11*).