was entirely general and could be applied to any gene for which transposon mutations were available. Since no requirement for *lux* gene expression was necessary in the course of the cloning procedure, we have not selected those recombinant plasmids which, because of mutation or manipulation, produced light in *E. coli*. *Lux* gene expression in *E. coli* as measured by light production was inefficient compared to that in *Vibrio* even though the *lux* genes in *E. coli* resided on a multicopy plasmid. If *lux* gene dosage were taken into account, the luminescence at 30°C in *E. coli* containing pBB123 was at least three orders of magnitude lower than *Vibrio*. It is difficult to assess the contribution of *lux* gene promoter (or promoters) to this low level of expression in *E. coli* since plasmid promoters can initiate transcription of cloned genes (*14*). Since luciferase activity could be greatly enhanced by the provision of bacteriophage λ promoters as in plasmid pBB123 and pBB128, *lux* gene expression in *E. coli* was probably limited by transcription from the *lux* gene promoter (or promoters). Accessory regulatory factors probably were not cloned with the *lux* genes or, alternatively, do not operate in *E. coli*. Since *V. harveyi* luciferase could be synthesized in *E. coli*, it should be possible to eliminate the need to supply exogenous aldehyde by cloning genes whose products fulfill the aldehyde requirement. Other genes required for expression of luminescence, such as those involved in autoinduction, could be isolated as well.

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References and Notes

1. K. H. Nealson and J. W. Hastings, *Microbiol. Rev.* **43**, 496 (1979).
2. D. M. Karl and K. H. Nealson, *J. Gen. Microbiol.* **117**, 357 (1980).
3. K. H. Nealson, *Arch. Microbiol.* **112**, 83 (1977).
4. D. Riendeau and E. Meighen, *J. Biol. Chem.* **254**, 7488 (1979); M. M. Ziegler and T. O. Baldwin, *Curr. Topics Bioenerg.* **12**, 65 (1982).
5. R. A. Rosson and K. H. Nealson, *Arch. Microbiol.* **129**, 299 (1981).
6. A. Keynan and J. W. Hastings, *Biol. Bull. (Woods Hole, Mass.)* **121**, 375 (1961).
7. R. Belas, A. Mileham, M. Simon, M. Silverman, unpublished results.
8. D. H. Cohn, R. C. Ogden, J. N. Abelson, T. O. Baldwin, K. H. Nealson, M. I. Simon, A. J. Mileham, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
9. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
10. M. J. Casadaban, J. Chou, S. N. Cohen, *J. Bacteriol.* **143**, 971 (1980).
11. J. Hedgpeth, M. Ballivet, H. Eisen, *Mol. Gen. Genet.* **163**, 197 (1978).
12. K. H. Nealson, *Methods Enzymol.* **57**, 153 (1978).
13. M. Z. Nicoli, thesis, Harvard University (1972).
14. D. Strüber and H. Bujard, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 167 (1981).
15. J. H. Miller, *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1977), p. 433.
16. N. E. Murray, W. J. Brammar, K. Murray, *Mol. Gen. Genet.* **150**, 53 (1977).
17. J. W. Hastings and G. Weber, *J. Optical. Soc. Am.* **53**, 1410 (1963).
18. M. Silverman, J. Zieg, M. Hilmen, M. Simon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 391 (1979).
19. Supported by contract ONR N00014-81-K-0343 from the Office of Naval Research. We thank Drs. T. Baldwin, K. Janssen, G. Mandel, and J. Engebrecht for strains and helpful advice; and Dr. K. Nealson for the use of equipment and other valuable assistance.

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Complex Venation Patterns in the Leaves of *Selaginella*: Megaphyll-Like Leaves in Lycophytes

Abstract. *Venation patterns of the leaves of two lycophytes,* Selaginella adunca *and* Selaginella schaffneri, *do not fit the definition of microphylls as having a single, unbranched vein. Although* S. adunca *has a simple pattern,* S. schaffneri *has a complexity matching that of many megaphylls, with numerous branching veins. The veins of* S. schaffneri *undergo an average of 13 branchings (range, 8 to 21), and reticulation between veins is frequent. The discovery of this radical departure from the familiar microphylls of lycophytes indicates that complex venation patterns in leaves do not necessarily arise from fusion of whole branches. The microphyll may not be as structurally stable as formerly believed.*

The two leaf categories traditionally recognized in vascular plants, microphylls and megaphylls, are separated on the basis of venation patterns. These categories have been of major importance in plant morphology and phylogeny for distinguishing major evolutionary clades. A microphyll has a single unbranched vein running more or less medially through the blade; a megaphyll has a complex, branching venation pattern. Only by reduction can the megaphyllous pattern be simplified to resemble that of a microphyll. Such convergence can be readily recognized by comparisons with near-relatives or other leaves on the same plant. Most plant morphologists regard the single vein as a primitive condition that has remained static since the Devonian (*1*). Microphylls have been valuable for identifying living and fossil members of the large group of plants generally known as Lycophyta or Microphyllophyta.

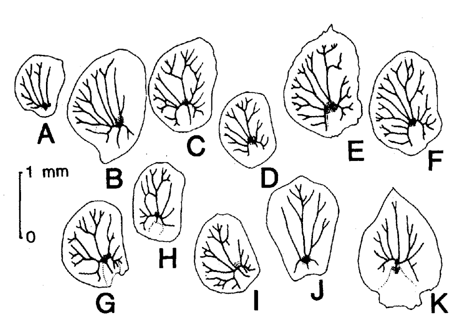

Fig. 1. Leaves of *Selaginella schaffneri*, showing complex venation patterns. (A to I) Lateral leaves. (J) Leaf at stem branching. (K) Sporophyll [specimens collected by J. G. Schaffner in 1879 in San Luis Potosí, Mexico (University of Michigan Herbarium)].

The microphyll is believed to have originated separately from the megaphyll. According to the enation theory, which is accepted by most morphologists, the microphyll arose as a nonvascularized superficial outgrowth of the stem (*1, 2*). The single vein of the microphyll arose de novo, as an extension of the vascular cylinder of the stem. The megaphyll arose by amalgamation of numerous stems, each with a vascular strand. According to the telome theory, branches, or telomes, aggregated and became modified to form leaves (*2*). Thus the veins of a megaphyll are traces of ancient stems once separate but now assembled into a united photosynthetic lamina, flattened for maximum efficiency (*3*).

Lycophyta have a long and complex history, for which there is extensive fossil documentation, including the remains of several orders now extinct (*1*). The group is represented today by three orders: Lycopodiales, the club mosses (about 400 species); Selaginellales, the spikemosses (about 800 species); and Isoetales, the quillworts (about 50 species). We describe here two species of *Selaginella* which show branching venation. One species possesses a ternate venation pattern with a midrib and two laterals. The other has a much more complex pattern with many branches, some of which form networks—a venation as complex as that of many unquestioned megaphylls. We studied fragments of dried herbarium specimens cleared for 24 hours in 5 percent sodium hydroxide solutions and bleached in dilute solutions of sodium hypochlorite.

The simpler pattern is shown by *Se-*

laginella adunca A. Br. ex Hieron., known from the western Himalayas (*4*). The veins are ternate, with a long central midrib and two shorter branches opposite each other and arising at the base (*5*). Some leaves have only one branch. The leaves that arise at the stem dichotomies contain only one vein, as do the sporophylls. Although leaves with two or more veins have been described in *Selaginella willdenowii* Baker (*6*) and *S. martensii* Spring (*7*), these are evidently abnormalities; the vein branchings occur below the blade in the cortex of the stem. Also, the expression of multiple veins is sporadic, unlike that in *S. adunca*, in which the majority of leaves have ternate venation. The rare microphylls that have lobes apparently retain the condition of a central, essentially unbranched, vein (*7*).

We report that the leaves of *Selaginella schaffneri* Hieron. have a venation pattern as complex as that of certain ferns and gymnosperms. All leaves sampled from three localities have this pattern. To our knowledge, no species has been described in this or any other lycophyte genus that even approaches such a complex venation pattern. Schaffner's spikemoss occurs in the Central Mexican Plateau in the states of San Luis Potosí, Jalisco, and México (*8*). The plants form sprawling or hanging masses of narrow wiry branches on cliff faces, ledges, and bluffs. The lateral branches are densely covered with overlapping leaves 0.9 to 1.8 mm long and 0.7 to 1.5 mm broad—remarkably small for leaves with such complex venation. The blades have a laminar flap extending below the area of attachment. They point forward and lie flat along the stem, their outer margins broadly rounded and their inner margins nearly straight.

The vascular system of the stem is distelic, and the plane of the steles is horizontal and parallel to the dorsiventral arrangement of the leaves. A single departing vascular strand branches near the leaf base into a venation pattern that is pedate and palmate. Instead of being strictly palmate from a point, the branches run out from the center and sides of a transverse vein axis extending to either side of the leaf base (Figs. 1 and 2). Most of the veins are developed on the outer side of the leaf. The inner, more thinly textured side of the blade sometimes lacks veins (Fig. 1B).

The pattern of the branchings of individual major veins is subpinnate (Fig. 1D), dichotomous (Fig. 1G), or trichotomous (Fig. 1I). Reticulations are found in roughly one-third of the leaves. There may be two or three areoles per blade, and these may involve major veins (Fig. 1, C and H) or minor veins (Fig. 1, F and I). The total number of branchings in a random sample of excised leaves ranged from 8 to 21 and averaged 13 (not including the dilated vein tips that may sometimes be slightly divided). Both the leaves at the stem branchings (Fig. 1J) and the sporangium-bearing leaves (Fig. 1K) also exhibit multiple veins. The veins are composed of mainly annular tracheids, one to five in number except at the vein bases and in the dilated vein tips, where the number of tracheids is greater. Figure 2 shows a representative leaf.

The leaves described here have fundamental significance for the understanding of foliar morphology in relation to the phylogeny of higher plants. By definition the leaves of *Selaginella adunca* and *S. schaffneri* are megaphylls, the former simple, the latter complex. This discovery of truly complex venation in a lycopod has a number of implications. Outgroup comparison with other lycopods, extinct and extant (*1, 2*), supports the conclusion that the foliar organs of spikemosses are primitively microphyllous, possessing only a single unbranched vein. We therefore conclude that the venation patterns of the spikemosses described here are derived directly from the single vein of the microphyll. The veins of *S. adunca* show an incipient stage and those of *S. schaffneri* an advanced stage of convergence toward the branched patterns of typical megaphylls. The vein systems of *S. adunca* and *S. schaffneri* probably did not arise from concrescence of branching stem systems but rather by elaboration of vascular strands within single microphyll blades; complex venation can thus apparently arise without telomic fusion. This does not mean, however, that the traditional interpretation of the origin of megaphyllous leaves is incorrect; it simply means that other evolutionary pathways are possible. It also signifies that the vascular pattern may be labile in evolution and that surprisingly radical changes can take place even in a tiny microphyll. The concept of stasis in the foliar architecture of lycophytes is challenged by the radical departures reported here.

Fig. 2. Leaf of *Selaginella schaffneri*, showing an approximately average amount of vein branching. Note dichotomous, trichotomous, and lateral branchings [specimen collected by C. G. Pringle near Guadalajara, Mexico (No. 15630, Michigan State University Herbarium)].

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References and Notes

1. T. N. Taylor, *Paleobotany* (McGraw-Hill, New York, 1981), pp. 118 and 121.
2. H. C. Bold, D. J. Alexopoulos, T. Delevoryas, *Morphology of Vascular Plants and Fungi* (Harper & Row, New York, 1981), p. 751; A. S. Foster and E. M. Gifford, Jr., *Comparative Morphology of Vascular Plants* (Freeman, San Francisco, 1974), p. 39; R. F. Scagel, R. J. Bandoni, G. R. Rouse, W. B. Schofield, J. R. Stein, T. M. C. Taylor, *An Evolutionary Survey of the Plant Kingdom* (Wadsworth, Belmont, Calif., 1966), p. 603; D. W. Bierhorst, *Morphology of Vascular Plants* (Macmillan, New York. 1971), p. 212.
3. Microphylls are associated with simple cauline vascular cylinders (protosteles) that lack parenchymatous gaps, and megaphylls are associated with more complex systems (dictyosteles and eusteles). Stelar gaps are not diagnostic for megaphylls, and some, including the largest known (*Gleichenia, Lygodium*), are connected with protosteles and have simple leaf traces [Y. Ogura, *Comparative Anatomy of Vegetative Organs of the Pteridophytes* (Borntraeger, Berlin, 1966)].
4. A. H. G. Alston, *Proc. Natl. Inst. Sci. India* **11**, 216 (1955). In an earlier report on the *Selaginella adunca* leaf, R. N. Mukherjee and U. Sen [*Fern Gaz.* **12**, 175 (1981)] stated that the main vein undergoes two successive dichotomies, but we find a single trichotomy. What they labeled as vein is actually the cortical trace in the stem.
5. L. Grambast and S. Rosello, *C. R. Acad. Sci.* **261**, 5183 (1965).
6. T. R. Webster, *Am. Fern J.* **60**, 1 (1970).
7. The single vein of the lobed leaf of the Devonian *Leclercqia* extends to the tip of the median segment. Although two small laterals run to the bases but do not enter the lateral segments, tracheids are confirmed only in the main strand [H. P. Banks, P. M. Bonamo, J. D. Grierson, *Rev. Palaeobot. Palynol.* **14**, 19 (1972)]. Lateral segments similar to *Leclercqia* in size and shape occur in the extant *Lycopodium pecten* Baker, but they are also unvascularized.
8. A. H. G. Alston, *Bull. Br. Mus. Nat. Hist. Bot.* **1**, 240 (1955).
9. Supported by NSF grant DEB75-03550. We acknowledge the help of W. W. Thomas, D. C. Wight, and M. R. Mayfield. Specimens for this study were loaned by the University of Michigan and Michigan State University herbaria.

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