be associated with specific proteins (*17*). Calvet and Pederson have detected small amounts of dsRNA associated with ribonucleoprotein particles that were isolated from HeLa cells by sonic disruption of nuclei (*8*). These structures may have more accessible dsRNA in Vero and mosquito cells than in HeLa cells.

In the immunofluorescence method, cell disruption is not required; the only treatment before antibody exposure is fixation with cold acetone. Since little if any protein would be removed by this procedure, no opportunity exists for in vitro annealing or shearing of native structures. This assay, based on specific structural recognition, provides evidence that dsRNA does occur in some cells in their native state.

Discrete ribonucleoprotein particles may be derived from newly transcribed, nascent RNA fibrils that are associated with characteristic proteins in repeating subunits (*8, 20*). Regions of dsRNA in these particles could be important in further processing of the transcription product. Immunochemical studies with antibodies specific for dsRNA should be useful for the characterization of such regions, and for relating in situ observations to those made with isolated particles.

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Evidence of Paleozoic Chromosomes from Lycopod Microgametophytes

Abstract. *A Pennsylvanian arborescent lycopod cone,* Lepidostrobus schopfii, *has microspores that have been found to have intracellular features that are interpreted as nuclei and mitotic chromosomes. The cellularized gametophytes conform to the early stages of growth that occur in modern* Selaginella *microgametophytes. Since the megagametophyte of* L. schopfii *is similar in development to extant species of* Isoetes, *the fossil now is known to have portions of its life cycle in common with both* Selaginella *and* Isoetes.

Little is known of the internal contents of fossil plant cells, although there have been studies of fossil plant cell walls (*1*) and identification of residues such as chlorophyll and amino acids (*2*). Precambrian rocks have been discovered which contain fossil blue-green algae that have granular contents (*3*) and spherical green algal cells that have densely granular objects interpreted as nuclei (*4*). Cellular structures of vascular plants that have been reported include distinctive nuclei (*5*) and starch grains (*6*). Some Jurassic fern spores are reported to contain granular chromatin and chromosomes (*7*).

The opportunity to study gametophytes and nucleated spores in fossil vascular plants has been rare as a result of vagaries of preservation and random sampling of fossil floras. Recently, however, Paleozoic rocks have provided several well-preserved, structurally intact specimens from groups that include early Devonian plants (*8*), sphenopsids (*9*), lycopods (*10*), pteridophytes (*11*), and seed plants (*12*). Stewart (*13*) identified a monolete prepollen grain with sperm cells within a medullosan ovule. A germinated saccate gymnospermous grain discovered within a Pennsylvanian ovule has been observed to have a branched pollen tube similar to the ones produced by living species of *Ginkgo*, cycads, and some conifers (*14*). In other Pennsylvanian saccate pollen grains, Millay and Eggert (*5*) found three prothallial cells with nuclei and an antheridial initial. These features also occur in pollen of some extant conifers. Well-preserved gametophytes have been reported in megaspores of the arborescent lycopod fructification, *Lepidostrobus* (*15*). In one species (*L. schopfii*) rhizoids and archegonia in several developmental stages occur in abundance and compare closely to the same stages in modern *Isoetes* megagametophytes (*16*). Further examination of microspores from this same cone species now provides evidence for microgametophytic development complete with intracellular features that are interpreted to represent nuclei and mitotic chromosomes.

The most consistently well-preserved microgametophytes have come from in situ spores of Paleozoic plant fructifications. In the case of *L. schopfii*, cone specimens were discovered in coal balls (*17*) that were formed in a Pennsylvanian coal swamp, probably in an acidic environment. Cytochemical killing and fixing procedures for plant and animal tissues often prescribe the use of acidic reagents to preserve chromosomal material, because nucleic acids precipitate at low *p*H. During the early stages of fossilization, the highly acidic environment would minimize decomposition of submerged plant debris by bacteria and fungi. This environment was undoubtedly important in early stabilization of the structural details described here.

Within the microspores of *L. schopfii* there occur what appear to be nuclei and distinct mitotic figures in several stages of division. Nucleus-like bodies about one-fifth of the cell diameter are consistently observed. Mitotic figures were observed with differential interference contrast optics, which allow optical sectioning of intact spores, and by transmission and scanning electron microscopy. There appears to be an undetermined but small number (6 to 12) of chromosomes (1 to 2 μm long) in the fossil spores.

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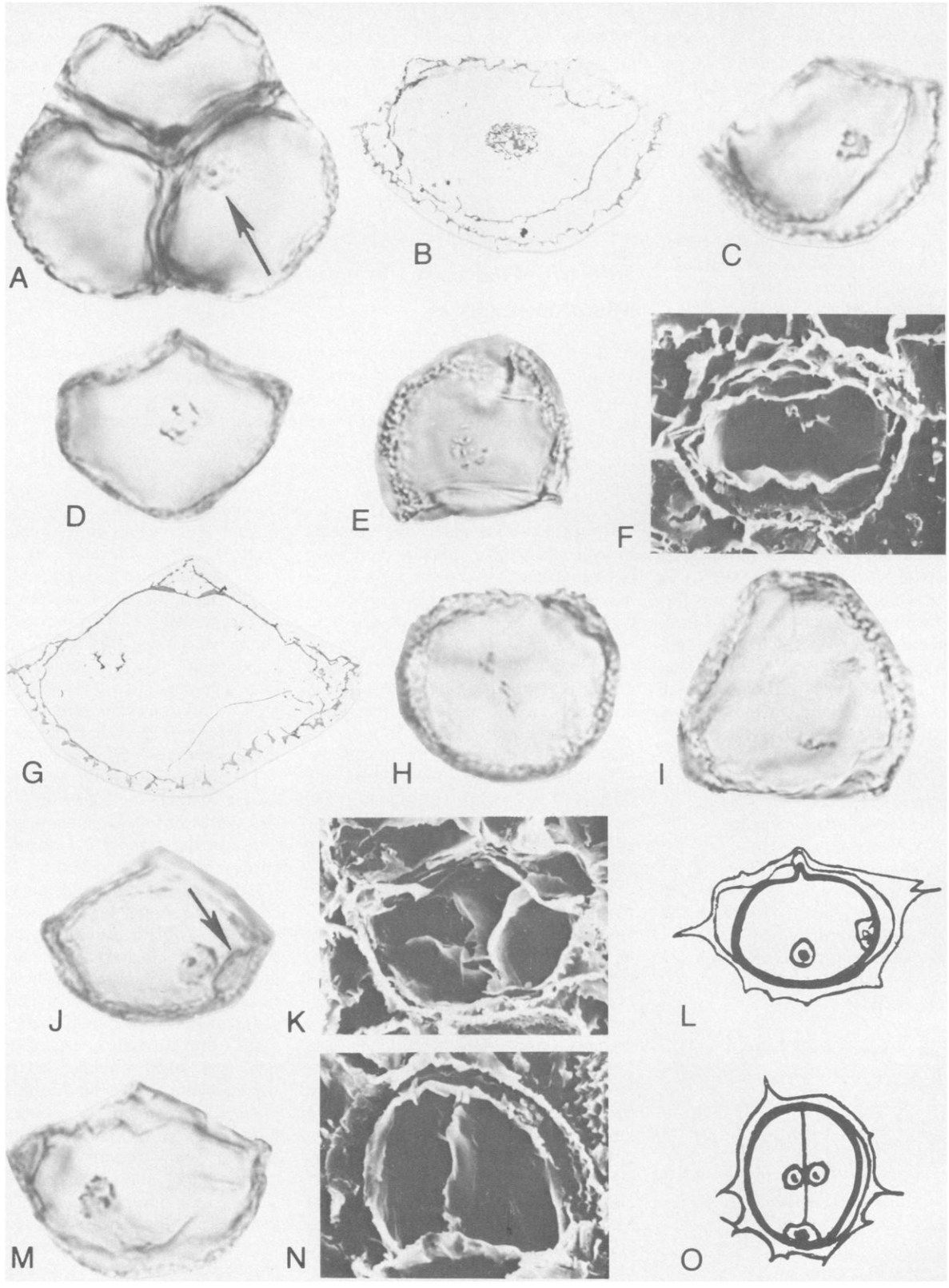

Fig. 1 (A to N). Fossil microspores (24 μm in diameter) from the Pennsylvanian cone, *Lepidostrobus schopfii*, and extant *Selaginella* microspores redrawn from Slagg (1932). Fossil microgametophytes (J, K, and N) are shown side-by-side to facilitate comparison with extant *Selaginella* material (L and O). (A) Tetrad of microspores with an interphase nucleus and nucleolus (arrow) in one spore (light optics). (B) Spore in early prophase (TEM). (C) Spore in prophase (light optics). (D) Spore in prophase (light optics). (E) Spore in prophase (light optics). (F) Spore with prophase chromosomes (SEM). (G) Spore in prophase with chromosome fragments (TEM). (H) Spore with metaphase plate arrangement of chromosomes (light optics). (I) Spore in late anaphase/telophase (light optics). (J) Two-cell stage of microgametophyte with large antheridial initial cell in interphase and small prothallial cell (arrow) near wall (light optics). (K) Two-cell stage of microgametophyte with large antheridial initial and small prothallial cell (SEM). (L) Two-cell stage of *Selaginella* microgametophyte. (M) Spore with prothallial cell near wall and antheridial cell in prophase (light optics). (N) Spore fragment with two cells of three-cell stage of microgametophyte, prothallial cell not present (SEM). (O) Three-cell stage of *Selaginella* microgametophyte.

Stages of mitosis that occur in the spores after tetrad formation—and which precede the production of an antheridial initial and a prothallial cell—include an interphase nucleus with nucleolus (Fig. 1A, arrow points to nucleus), early prophase where the nucleolus disappears and the chromatin condenses (Fig. 1, B and C), prophase with short, thick chromosomes (Fig. 1, D to G), metaphase (Fig. 1H), and late anaphase/telophase (Fig. 1I). Nearly 30 percent of the spores observed in a sampling of about 130 were in what we interpret to be interphase and nearly 60 percent were in prophase; the remainder observed resembled metaphase, anaphase, or telophase configurations. The described microspore features were abundant in several cone specimens of *L. schopfii*. With both light optics and electron microscopy, frequency, statistical data, and apparent sequence of mitotic stages, consistent size, configuration, proportion, and spatial arrangement within the spores, which conform well with such features in extant *Selaginella* microspores, compel us to propose that these structural features indeed represent nuclei and chromosomes rather than only condensed cytoplasmic remains as has been suggested by Knoll and Barghoorn (*18*) for cellular contents.

A second division within this material begins after the formation of the prothallial cell and antheridial initial (Fig. 1, J and K, compare with *Selaginella*, Fig. 1L). According to our interpretation of the fossil material, the interphase nucleus of the antheridial initial (Fig. 1J) goes into prophase (Fig. 1M) and produces two daughter cells, thereby forming a three-cell microgametophyte (Fig. 1N, compare with *Selaginella*, Fig. 1O). The prothallial cell becomes flattened and takes a position against the inner spore wall (arrow in Fig. 1J; Fig. 1, K and M). These early stages in microgametophyte development in trilete fossil lycopod spores are quite similar to those that occur in species of modern *Selaginella* microspores (*19*) (Fig. 1, L and O). It is significant that the microgametophyte generation of *L. schopfii* resembles *Selaginella* whereas megagametophyte development is like that of *Isoetes*. Perhaps *Isoetes* is a living relative of the Paleozoic arborescent lycopods. In addition, *Selaginella* may be related far more closely than previously thought.

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Pattern Formation by Cultured Human Epidermal Cells: Development of Curved Ridges Resembling Dermatoglyphs

Abstract. *In cultures made from disaggregated human epidermal cells, growth to a confluent cell layer is followed by the emergence of patterns resembling those of human dermatoglyphs. These patterns reflect intrinsic properties of keratinocytes. In vivo, only the epidermis of the volar surfaces forms patterns, but in culture, patterns are formed by epidermal cells from other sites as well. Patterns develop by a process of cell movement which first produces ridges and then curves the ridges into figures of increasing complexity, ultimately whorls.*

The skin of the palms and soles of primates becomes organized in embryonic life into patterns of ridges and grooves that are then maintained permanently. The patterns of the distal phalanges were designated by Galton as arches, loops, or whorls. At the conjunction of three adjacent ridge systems there is formed a triradiate structure converging to a single point (triradius). These features are the basis of classification of fingerprints for identification purposes. The patterns are thought to be the result of differential growth during embryonic life (*1*).

It is now possible to make serial cultivations of human epidermal keratinocytes under simple cell culture conditions (*2*, *3*). The epidermal cells depend for their multiplication on fibroblast products, and these products are supplied by including in the culture lethally irradiated fibroblasts (usually 3T3 cells, an established mouse fibroblast line). Epidermal growth factor (*4*) is added to each subculture beginning when the colonies are a few days old (*3*). Under these conditions, single epidermal cells give rise to colonies resembling a stratified squamous epithelium and consisting of multiplying and terminally differentiating cells (*5*). The colonies grow and eventually fuse to make a confluent layer (Fig. 1, 21 days). Some days later, numerous flattened cells begin to detach from the surface of the culture into the medium. These are terminally differentiated cells resembling the squames that become detached from the stratum corneum of intact skin, except that their nuclei have usually not yet been destroyed (*6*). At about this time, the cells remaining on the vessel surface begin to concentrate in thickened ridges, and by 30 to 40 days after inoculation, the ridges form patterns that can be described as arches, loops, and whorls. Transitional stages can also be seen.

Some well-developed patterns are shown in Fig. 2. Regions of organization are sometimes separated by triradials meeting at an angle of approximately 120°. Whorls are of either handedness. In some instances a whorl seems related to two triradii (Fig. 2B). These patterns contain features resembling those of the digital ridges of humans.

The development of epidermal ridges in embryonic life has been described (*7*, *8*). The process seems to begin with the formation of epidermal folds at around 4 months of gestation, perhaps at sites determined by nerves and blood vessels (*8*). While it has been suggested that the formation of ridges probably results from increased local proliferation in the underlying folds, this does not explain why the ridges should be curved into such ordered patterns as whorls. It has been

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