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A REMARKABLE FOSSIL SELAGINELLA WITH PRESERVED FEMALE GAMETOPHYTES

BY

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OF ALL PLANTS known to the botanist, *Selaginella* is one of the most familiar. It is regarded as the classical example of a heterosporous plant. *Selaginella* has been recognized in the fossil record as far back in geological time as the lowest Cretaceous, but several earlier species, based upon fructifications, are similar enough to the living genus to be referred to *Selaginellites*. This form-genus is known from rocks of Carboniferous, Permian, and Mesozoic age.

Recently there was found in a newly acquired collection of fossil plants, received by the Botanical Museum of Harvard University from the environs of Mazon Creek, Illinois, a small strobilus bearing four large spores in each sporangium. The striking resemblance of this strobilus to strobili found in living species of *Selaginella* was especially interesting, because the perfect state of fossilization suggested the probability that cellular detail might be preserved. Following a study of the specimen by a combination of the maceration and peel methods, it was discovered that the fossil contained numerous early female gametophytes which exhibited remarkably well-preserved nuclei and nucleoli. So numerous were these

EXPLANATION OF THE ILLUSTRATION

SELAGINELLA AMESIANA *Darrah*. The figure at the top shows a large megaspore with its equatorial flange. Note the thickness of the spore wall. One hundred times natural size. Nitrocellulose peel from the holotype. Preparation number 1.

The figure at the bottom shows one megaspore pulled from a tetrad. The spore shows a dense vesicle, a large vacuole, and a thick spore wall. One hundred times natural size. Nitrocellulose peel from the holotype. Preparation number 2.

gametophytes and so fine their state of preservation, that it has been possible to compare the life-history of this fossil form, stage by stage, with the life-history of living species of *Selaginella.*

The specimen is referred without hesitation to the existing genus, *Selaginella.* It extends the paleontological history of the group to the upper Carboniferous. Its age, translated into years, according to the estimates of the geologist, is approximately 225 million years.

None of the other five or six known Paleozoic gametophytes exhibits cellular contents, so this discovery may, without over-estimation of the quality of the material, be regarded as one of the most marvellous petrifactions thus far recognized by paleobotanists. A study of the untouched reproductions in this paper should prove this claim.

Description of the specimen.

The single specimen known is a typical nodule from Mazon Creek, Illinois, which was collected by Frederick O. Thompson. The only plant fragment in the nodule, in addition to the strobilus, is a small unidentifiable fragment of stem. The strobilus is 27 mm. long, 3.5 mm. wide at its greatest width, and showed 34 sporangia, each containing four spores. The sporangia vary from 1.6 to 2.2 mm. in width. The strobilus is slightly curved, but it is not possible to determine whether the curvature was formed in life or during the process of petrifaction.

Methods employed.

The specimen contains megaspores which are visible to the naked eye (without ornamentation the spores attain a diameter of 0.6 mm.). These spores, which are brown in color, are infiltrated with calcium carbonate and iron carbonate (siderite). Minute crystals of pyrite (iron sulphide) and galenite (lead sulphide) can be observed under magnification of six to ten times. I first interpreted the brown color of the spores to be due to siderite, but upon the application of dilute hydrochloric acid (10%) they lost their muddy appearance and became "resinous." The brown color was due to a coalified residue.

Three spores were isolated from a sporangium by maceration with 25% hydrochloric acid. These were washed with distilled water, and subsequently bathed in absolute alcohol and permitted to dry on a glass slide. The air-dried spores were placed between two glass cover slips, and gently heated over the flame of an alcohol lamp. They volatilized quickly and left a negligible amount of white ash upon the slide.

From these observations it is believed that such mineral substances as were present — if the crystals were formed congenerically with the petrifaction—may have acted as preservatives against bacterial decay. It is also probable that the volatile, but resistant, resinous substances in the megaspores may have permitted almost perfect fossilization.

The fossil was smoothed slightly by scraping off the rough elevations on the strobilus by means of a dull scalpel. Serial nitrocellulose peels (1) were prepared at intervals as close as possible; i.e. 0.1 mm. for the first ten peels, and approximately 0.15 mm. for the next twenty-two peels. Eight peels were made from the counterpart at intervals of about 0.15 mm. The peels were mounted in "damar" in xylol.

Minute anatomy of the spores.

The average dimensions based upon ten spores cut longitudinally in a median plane are:

Total diameter including equatorial flange	750 micra
Diameter excluding equatorial flange	620 micra
Length of spore	420 micra

The wall of the spore is ornamented by a coarse reticulation. The wall is thick (20 to 25 micra) and is colored by a dense bituminous substance. The apex of the spore bears a large tetrad scar. Coal technologists have referred isolated spores of this type to the form-group *Perisporozonales*, and the closest resemblance is to be observed in Spore Type IX (2) or *Triletes circumtextus* Zerndt (3). The megaspores of the strobilus from Mazon Creek are of the type found in *Bothrodendron mundum* (4), but this species is believed to have been arborescent.

Comparison with Fossil Forms.

The genus *Selaginellites* was founded in 1906 by Zeiller(5) for herbaceous fossil lycopods believed to be heterosporous. Brongniart in 1822 (6) defined the form-genus *Lycopodites* to include the slender, dichotomously branched shoots which resembled the living *Lycopodium* or *Selaginella.* The name *Selaginites* has been given to various foliage types which appear to have been anisophyllous, but none of the so-called *Selaginites* are known from reproductive parts.

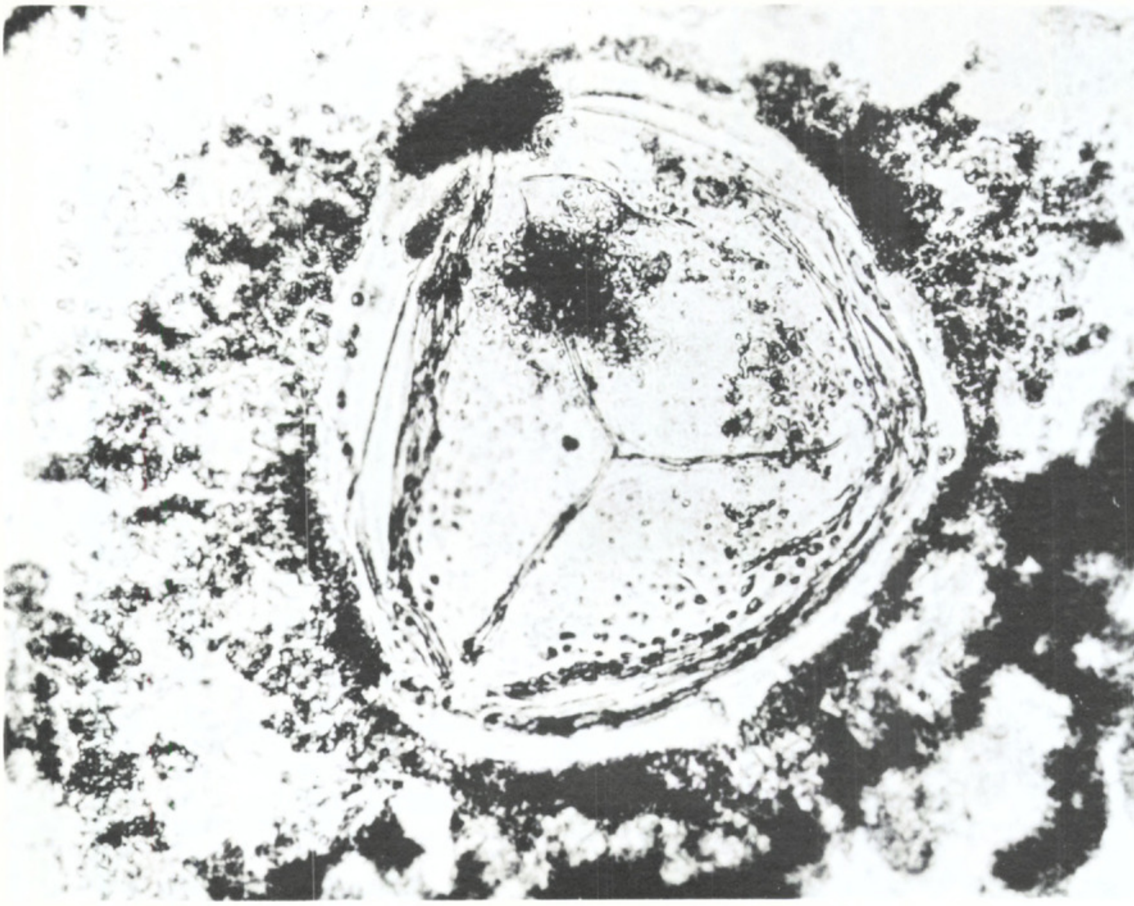
Lesquereux (7) has described three species of *Lycopodites (Selaginites)* from Morris and Mazon Creek, Illinois: *Lycopodites cavifolius*, *Lycopodites pendulus*, and *Lycopodites Meekii.* All of these are known from only foliage impressions.

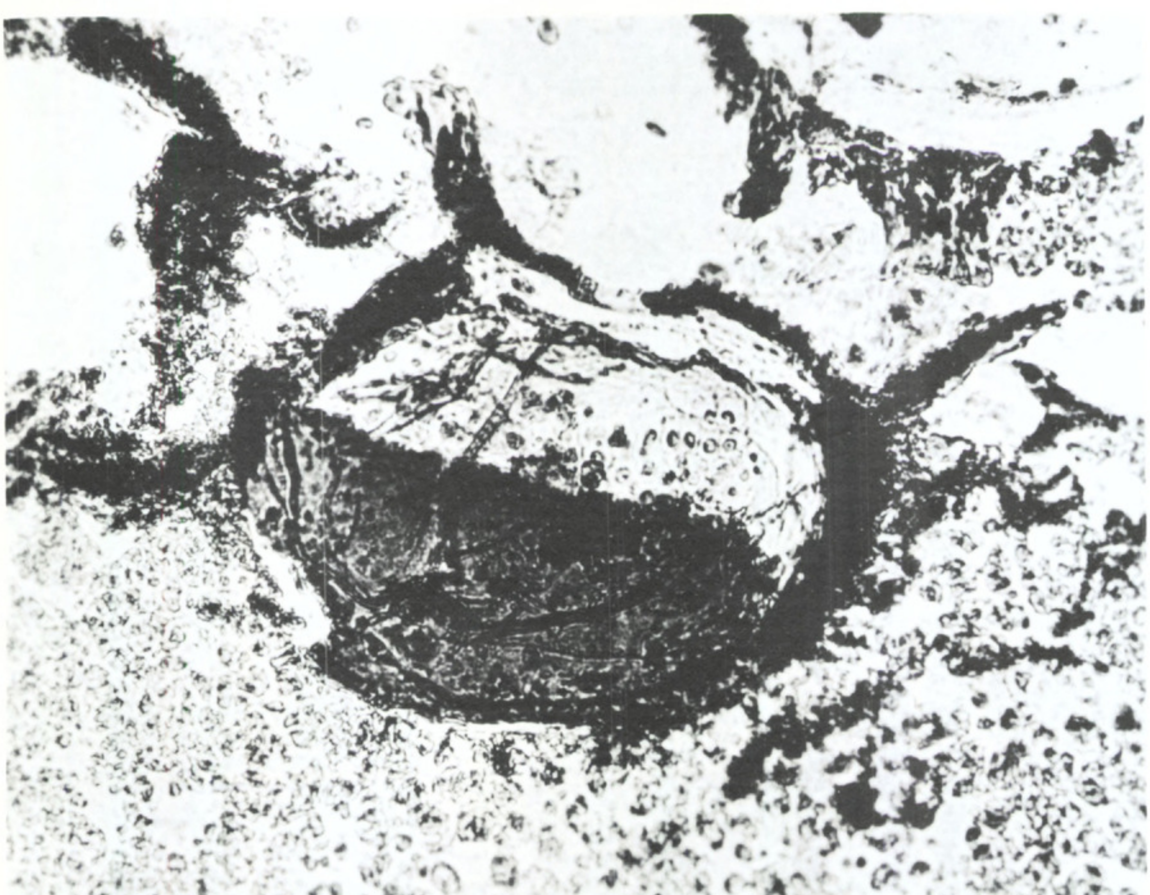
The various species of *Selaginellites* are much more completely known. The genotype is *Selaginellites Suessi* Zeiller (loc. cit.). The strobili were of comparatively great size, attaining a length of fifteen centimeters and a diameter of eight to ten millimeters. The sporophylls were very numerous, those near the summit of the strobilus bearing microsporangia filled with many microspores, and those in the lower part of the strobilus bearing megasporangia each with sixteen to twenty-four megaspores.

EXPLANATION OF THE ILLUSTRATION

Selaginella Amesiana *Darrah.* The figure at the top shows a megaspore with a tetrad scar. Note the three spore membranes, the mesospore and endospore have shrunken away from the exospore. One hundred times natural size. Nitrocellulose peel from holotype. Preparation number 2.

The figure at the bottom shows a megaspore and its enclosed megagametophyte. Note in the upper corners, portions of two sister spores. The gametophyte is composed of cells with large nuclei and moderately large nucleoli. Detail of cell-contents is beyond the depth of focus. One hundred times natural size. Nitrocellulose peel from the holotype. Preparation number 2.





Both kinds of spores were provided with an equatorial flange. The foliage was dimorphic. The megaspores resembled those of the existing *Selaginella caulescens*. They measured 500 to 650 micra in diameter. The microspores, in contrast, attained a diameter of only 40 to 60 micra.

Halle (8) in 1907, transferred *Lycopodites primaevus* Goldenberg and *L. elongatus* Goldenberg (9) to *Selaginellites*. These two species apparently bore only megaspores in the preserved sporangia. Those of *Selaginellites primaevus* attained a diameter of 400 to 500 micra, and those of *Selaginellites elongatus* had a diameter of 450 micra.

Curiously enough Zeiller compared *Selaginellites Suessi* with a third species of Goldenberg (10) *Lycopodites macrophyllus*. Spores have not been observed in this form, but the sporangia are preserved. They are not grouped into strobili, but are borne in the axils of leaves.

Seward (11) in 1913 described a lower Cretaceous (Wealden) *Selaginella* under the name *Selaginella Dawsoni*. This species was heterosporous. The microspores, which were still in tetrads, were finely tuberculate and measured 40 micra in diameter. The megaspore number could not be ascertained, but their size and ornamentation was observed. Their exines were irregularly reticulate and the spore diameter exceeded 300 micra.

The strobilus of the specimen from the Carboniferous of Illinois is distinct from all of the previously described fossil forms in possessing the following features: only four megaspores in each sporangium, the unusually large diameter of 750 micra—including the equatorial flange formed by the expanded arcuate ridges of the spore exine, and by having the gametophyte preserved. The spores are in general similar to the previously described species in having reticulated exines, equatorial flanges and large size. There are no close resemblances between this new species and those hitherto known.

It is noteworthy that the small number of megaspores per sporangium is a characteristic shared among fossil lycopsids only by *Bothrodendron mundum* and *Selaginellites primaevus*. This is not the less significant, because the relationships of the new form are with the existing *Selaginella*—in its most restricted sense.

Description of the Gametophyte.

More than fifty megaspores show nucleated cellular masses in varying degrees of complexity and organization. The material thus permits a detailed description.

The earliest stage in development is to be observed in preparation number 2. A tetrad of spores is still in conjunction, but the protoplasmic contents are crowded in the apex of each spore into a "vesicle" which has a dense (presumably nuclear) region near its own apex. This stage is shown in the lower figure of the first plate.

The succeeding events are best preserved in preparations 2, 13, 16, and 19, and are well preserved in preparations 1, 3, 4, 6, 14, 20, and 26. The vesicle appears to have become much larger and nuclei are present, at first without cell walls. The spore shown at the top of plate 2 shows many ovate nuclei and the several spore membranes which are not clear on the illustration because they are beyond the depth of focus. The larger gametophytes show increasing numbers of nuclei which are enclosed by cell walls. In several spores (preparations 1, 3, 6), the early cell-plates are beautifully preserved.

The most advanced and best preserved gametophyte is that shown under three different magnifications: the first, within the megaspore on plate 2, and two others on plate 3. The figure on plate 2 also shows the peripheries of two sister spores which are, of course, within the megasporangium. The gametophyte fills almost the entire spore, and all of the nuclei near the apex are enclosed in cell walls. Most of the nuclei show nucleoli and nuclear contents in varying degrees of preservation.

Comparison with the existing Selaginella.

Lyon (12) has fully described the development of the gametophytes of *Selaginella rupestris* and *Selaginella apus*. According to Miss Lyon the initial steps in the development of the female gametophyte are the rapid expansion of the protoplasmic vesicle and the repeated division of the nucleus. A thick envelope surrounds the vesicle, but this envelope becomes proportionately thin as the surface of the vesicle increases. There soon develops a large vacuole.

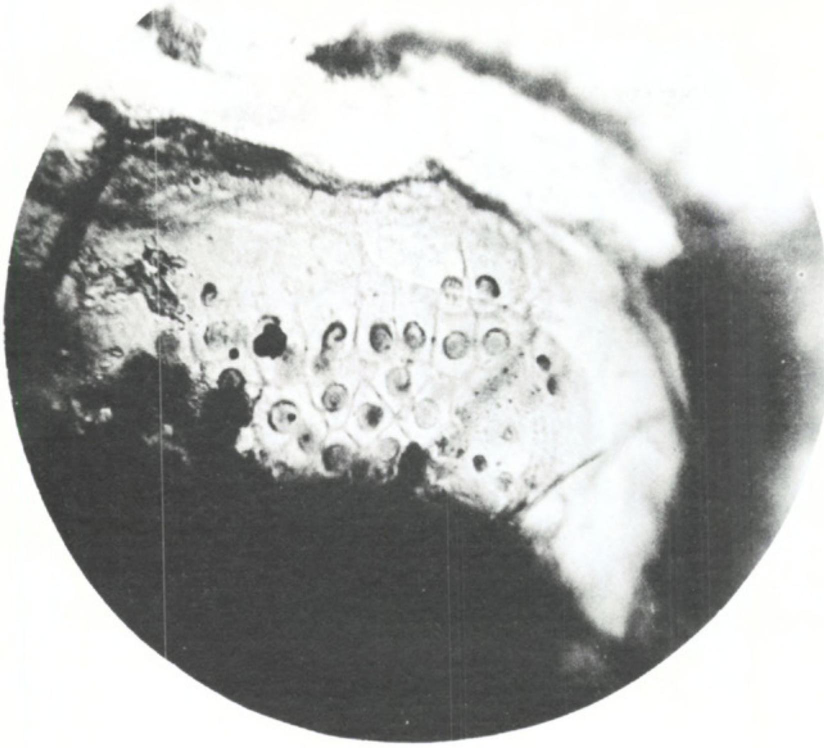
At this stage (Lyons, Plate VI, figure 46) the female gametophyte consists of the exospore, the mesospore, the endospore, the protoplasmic vesicle—which now consists of a thin layer of protoplasm in which there are imbedded numerous flattened ovate nuclei—and the vacuole. After the spore membranes have completed their growth, the nuclei undergo their final division—these divisions being marked by the formation of cell-plates and walls.

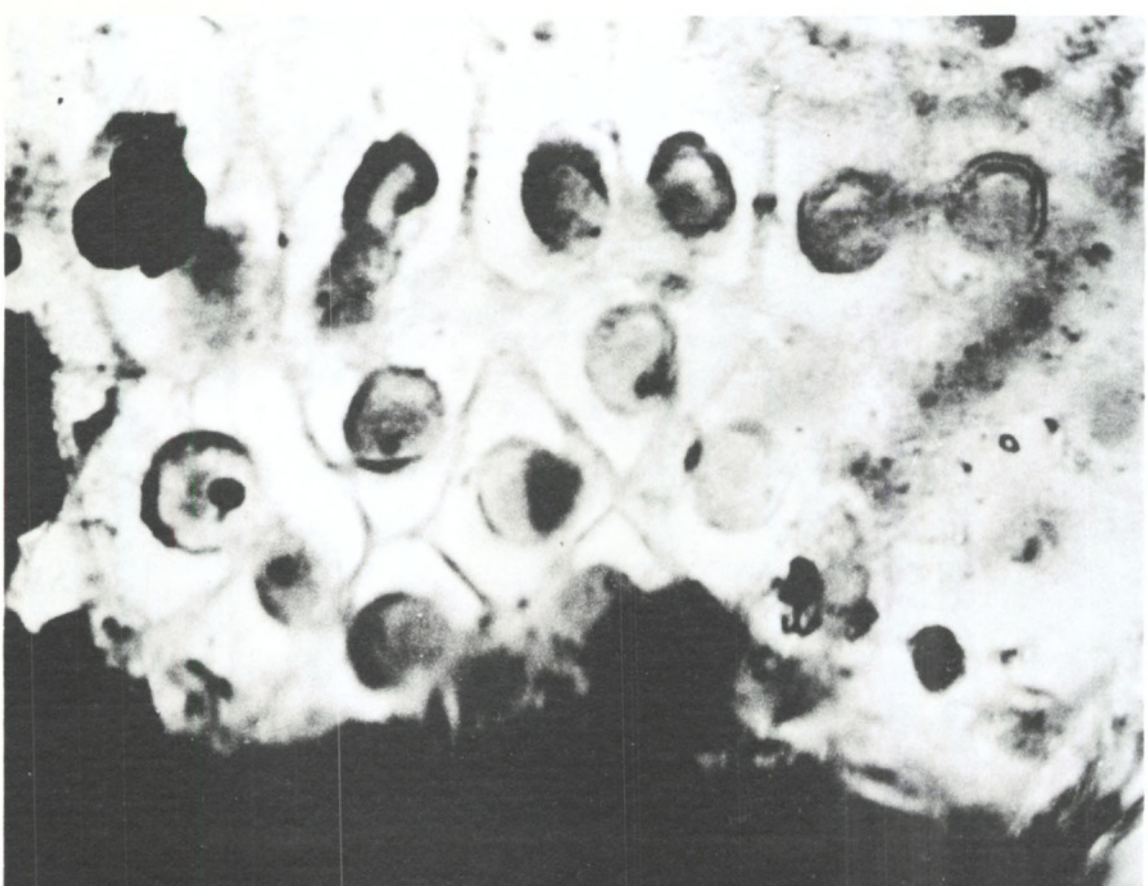
In the living species of *Selaginella* the megaspore usually germinates *in situ*. The nucleus undergoes repeated divisions, first producing a number of free nuclei that are subsequently surrounded by cell walls. This development occurs at the apical or scar end of the spore, and at the opposite end there forms a large vacuole. The multicellular female gametophyte is completely enclosed within the megaspore wall. As the gametophyte continues to enlarge, the spore is gradually forced open at the tetrad-scar and the gametophytic body protrudes to some extent. Following, or simultaneously with, this

EXPLANATION OF THE ILLUSTRATION

SELAGINELLA AMESIANA *Darrah.* The figure at the top shows the megagametophyte figured at the bottom of the second plate, magnified two hundred and twenty-five times. The figure shows cell walls, nuclei, and nucleoli. Nitrocellulose peel.

The figure at the bottom shows the same gametophyte magnified one thousand times. In the lower right is shown a moderately well preserved mitotic figure.





protrusion, archegonia are formed on the prothallus.

All of the gametophytic stages preserved in the fossil strobilus from Illinois are intermediate between the earliest nuclear divisions of the megaspore and the opening of the spore wall. No protruding gametophytes have been observed in more than 1500 sections representing more than 200 megaspores. No archegonia have been observed, presumably because they would have been borne upon the protruding portions of the gametophytes.

Description of the species.

Selaginella Amesiana *Darrah sp. nov.* 7 figures.

The strobilus is composed of spirally disposed megasporangia each bearing four megaspores. The megaspores were ornamented by a reticulate exospore and by a well developed equatorial flange. The female gametophyte was constructed of angular and somewhat elongate parenchymatous cells with nuclei and nucleoli preserved. Its development was endosporal.

The strobilus (as much as is preserved) measures 27 mm. in length and 3.5 mm. at the maximum width. The megaspores have a diameter of 750 micra including the equatorial flange, and a height of 450 micra.

Foliage, ligule, microspores, microsporangia, and archegonia are not known.

I have the pleasure of naming this species for Professor Oakes Ames, Director of the Botanical Museum of Harvard University, for his continued interest in paleobotany and for his enthusiastic support and encouragement of the activities of our Paleobotanical Laboratory.

Discussion of Relationships.

The megagametophyte of *Selaginella Amesiana* may be compared with the prothalli of *Bothrodendron mundum* and *Lepidodendron Veltheimianus.*

McLean (13) in 1912, described the prothallus of *Bothrodendron mundum* from a thin section of a coal-ball. The prothallus, which lies almost entirely outside of the spore, is composed of angular, elongated, parenchymatous tissue. The specimen shows at least three archegonia—represented by egg-cavities. McLean interpreted the prothallus as follows: ". . . it may be said that this specimen represents a stage in the reduction of the primitive free-living Lycopod gametophyte towards the condition obtaining in the "seed" of *Lepidocarpon*. The prothallus was not produced until after the megaspore had been shed. It developed outside of the spore, but remained attached to the spore-wall at its base, and in form resembled the prothalli of modern heterosporous ferns." (p. 318)

Gordon (14) in 1910 described the prothallus of *Lepidodendron Veltheimianus* from two thin sections, one showing a single archegonium and a small amount of adjacent tissue, the other showing an unripe (i.e. an unopened) spore completely filled with parenchymatous tissue which resembled to a considerable degree the gametophyte of *Selaginella*. Thus, in at least this species of *Lepidodendron*, the gametophytic development was endosporal and the archegonia developed at the scar in the spore wall, apparently not on a protrusion of the prothallus.

The prothallus of *Selaginella Amesiana* was endosporal and the evidence points to the fact that the archegonia were developed on a protruded part of the prothallus, that is to say, since no archegonia have been found in the prothalli of unopened spores, and some spores show early stages in the opening of the scar slit, presumably the archegonial stage was later than any of those stages which were preserved.

The only feature which militates against the reference of *Selaginella Amesiana* to the genus *Selaginella* is the absence of microsporangia in the strobilus. However, the deficiency in this case is of little importance because the gametophyte within the megaspore demonstrates clearly the female prothallus which, by all of the concepts of comparative morphology, implies the existence of the male—either on a part of the strobilus which was not preserved or upon a separate strobilus. *Selaginella Amesiana* is undoubtedly heterosporous, and is the earliest known species of *Selaginella.*

Conclusions.

The new species described in this paper as *Selaginella Amesiana* Darrah is a noteworthy addition to our knowledge of Carboniferous plants, not so much because it extends the geological history of the genus into the Paleozoic era, but rather because this specimen has the female gametophyte preserved with almost unbelievable detail. Nuclei, nucleoli, cell-plates and at least two recognizable mitotic figures are preserved.

It is so improbable that this example of preservation is unique, that one is fascinated by the possibilities suggested by its discovery. The occurrence of such delicate and elusive protoplasmic structures in a fossil of the carbonized compression type, reveals how inadequate are our conceptions of the process of petrifaction.

Three decades have passed since microtechnique was introduced into paleobotany by Nathorst, who utilized strong chemical reagents to macerate carbonized compressions. In more recent years Hamshaw Thomas, Walton, Lang and Harris have used improved methods in this type of work. Halle has developed a paraffin method for serial sectioning macerated carbonizations, and in our laboratory there has been developed a serial section method by the use of nitrocellulose films. The combined

EXPLANATION OF THE ILLUSTRATION

Selaginella Amesiana *Darrah.* Photograph of a nitrocellulose peel of the strobilus showing the arrangements of the megaspores and the megasporangia. The numerous spores exhibit various structures: the reticulated spore wall, the tetrad-scar, the equatorial flange, and a few spores filled with gametophytic tissue. Eight times natural size. Preparation number 3. The type specimen is number 30645 Paleobotanical Collection of the Botanical Museum.

result of these technical methods is that many thousands of specimens formerly considered to be worthless, are now available for investigation.

Thus the subjection of carbonizations, particularly those carbonized compressions which Hamshaw Thomas has called mummifications, to methods of serial section has marked the beginning of a new period of paleobotanical research.

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