going their final major regression back to the ocean basins (*18*). Productivity in the epeiric seas can only be guessed at, but a reasonable assumption is that it approximated the present day *P/A* value for the continental shelf of 0.16. At the time of the terminal Cretaceous regression, angiosperm vegetation covered wide areas and the tropical regions were more latitudinally widespread than they are today. Thus, during the regression, replacement of the epeiric seas (*P/A* value, 0.16) by tropical and temperate vegetation (average *P/A* value, 0.68) should have resulted in an increase in the total world primary productivity; any reduction of phytoplankton productivity would have been more than offset by the expansion of terrestrial plants. Because of the expanded Late Cretaceous tropical regions, the total productivity then could easily have exceeded the modern value.

During an interval of relatively sudden late Mesozoic or Cenozoic phytoplankton failure, as, for example, the catastrophic late Maestrichtian reduction in diversity and abundance of the coccolithophorids, a minor, geologically short-term interval of reduced productivity could have occurred. However, because of the great productivity and wide distribution of Cretaceous terrestrial vegetation and because other phytoplankton species would have quickly occupied the vacated niche, such a failure would probably have been reflected as a minor inflection on the world productivity-time scale. The failure, and the slight increase in atmospheric CO_2 and decline in O_2, unless they disrupted the atmosphere/ocean CO_2 balance, would in themselves probably have been insufficient to generate worldwide terrestrial and marine faunal extinctions.

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Scanning Electron Microscope Study of Early Lichen Synthesis

Abstract. *In the early stages of cellular interaction between the symbionts of* Lecidea albocaerulescens, *the phycobiont produced an extracellular sheath which bound to it hyphae of the mycobiont. Such a sheath may be a means by which the symbionts recognize each other. Hyphae of the mycobiont formed flattened appressoria as they grew over the algal cells and in this way secured the autotrophic population necessary for the development of a lichen thallus.*

There have been many attempts to reconstitute lichens from their separate fungal and algal symbionts. Some of the attempts have been successful (*1*), but, in general, they have not contributed much to our understanding of the cellular events that occur during resynthesis. Our study, the first at the ultrastructural level of the early stages of lichen synthesis, provides new clues on the mechanism of interaction between the symbionts of lichens.

The symbionts of *Lecidea albocaerulescens* (Wulf.) Ach. (*2*) were isolated and maintained in separate cultures for about 1 year before they were recombined. A culture of the fungal component (mycobiont) was obtained from several spores that were discharged from a fruiting body onto an agar surface. The fungus was cultivated in malt extract-yeast extract medium. A culture of the algal partner (phycobiont) was obtained from a single cell that was isolated by the micropipette method (*3*). The alga was cultured in a tris-buffered inorganic medium (*4*) and identified as *Trebouxia glomerata* (Warén) Ahm. Colonies of the fungus that had grown in liquid medium were washed in sterile distilled water for 1 hour and then placed onto a purified agar substrate (Difco 0560-02) in plastic petri dishes (60 by 20 mm) together with cells of the alga taken from an agar slant. The mixed cultures were incubated at 18°C under 2140 lm/m² of illumination and with a daily cycle of 12 hours of light and 12 hours of darkness. Observations with a scanning electron microscope (SEM) were made about 4 months after incubation of the cultures.

Colonies of the resynthesized lichen were cut out of the agar and fixed for 30 minutes at room temperature in 2 percent glutaraldehyde in 0.1*M* phosphate buffer, *p*H 7.2. The colonies were rinsed briefly in the phosphate buffer and then fixed for 30 minutes at room temperature in 1 percent OsO_4 in 0.1*M* phosphate buffer. The specimens were rapidly dehydrated through an ascending alcohol series, placed in a critical-point drying apparatus (model DCP-1, Denton Vacuum, Inc., Cherry Hill, N.J.) and dried by the method of Anderson (*5*). Specimens were cemented to specimen stubs, coated with carbon and then with 150 Å of gold-palladium, and examined at 10 kv in an SEM (JEOL model JSM 35U).

Lichenized interactions between mycobiont and phycobiont were observed with a light microscope 25 days after the beginning of the joint culture. Early contacts between the symbionts as well as a thallus-like structure were seen with an SEM in some areas of our mixed cultures (Fig. 1). The algal cells associated with fungal hyphae were dark green whereas those algae free from the fungus were yellow and filled with large droplets. Thus, the influence of the fungus on the phycobiont appears in the earliest stages of synthesis. Hill and Ahmadjian (*6*) reported that the fungus influenced the metabolism of the phycobiont even in a mixed culture where there was no visible physical contact between the symbionts.

One of the most evident features of lichens at the ultrastructural level is an extracellular, fibrillar material that surrounds the symbionts. Such a substance, presumably a polysaccharide (*7, 8*), binds hyphae of the cortex and medulla and provides a structural integrity to the lichen thallus. It has been assumed that this material is mostly of fungal origin since it is evident mainly in the cortex and medulla which are thallus layers that consist of fungal hyphae.

Our study revealed that in the mixed culture the algal symbiont produced an extracellular substance that bound together its cells with fungal hyphae (Fig. 2). In separate culture, the alga also formed a gelatinous sheath around its cells. A sheath may be present around cells of all species of *Trebouxia* in culture even though it is not visible with India ink preparation under a light microscope. For example, Jacobs and Ahmadjian (*9*), using freeze-etching and carbon replication methods, reported that the cells of *Trebouxia erici* Ahm. in culture had a thin rim of a lead-staining, fibrillar substance outside of their walls. This substance appeared to be identical to the one we found around the algal cells of our mixed cultures. Such a sheath was not seen by Jacobs and Ahmadjian around algal cells examined directly from the lichen thallus. The fungus does not appear to be involved in the formation of extracellular substance during the early stages of synthesis, although in separate culture a thin sheath was produced around the fungal hyphae. New growth of the fungus, as seen with an SEM as it grew out of the algal matrix, was free of any extracellular substance (Fig. 3). Thus, any binding substance produced by the fungus appears to be a later development of the symbiosis and may occur when the association begins to differentiate into specific thallus layers.

The fungal hyphae formed appressoria when they contacted the algal cells. An appressorium is a flat, distinctive structure that attaches to an algal cell (Fig. 4). Jacobs and Ahmadjian (*8*) showed that hyphal cells in contact with algae had thinner walls than the cells of unattached hyphae. We could not see with an SEM if there were haustorial penetrations. With a light microscope, however, we observed that many of the algal cells were penetrated by peglike haustoria. About 40 percent of the cells were infected by haustoria, the same percentage as that found in the natural lichen. A single hyphal filament bound several algal cells and formed an appressorium on each cell as it grew over and made contact with the cell surface (Fig. 5). An appressorium did not necessarily terminate a hyphal filament; rather, the hypha could resume its normal growth and then form new appressoria on other algal cells (Fig. 5). The advantage of a fungal hypha binding several algal cells rather than one is that a fungus can quickly secure many algal cells that will form the autotrophic population necessary for the formation of a lichen thallus. Algal cells enveloped by several hyphae (Fig. 6) were rare in our cultures. Undoubtedly, a secondary envelopment of the algal cells by fungal hyphae occurs, but in our cultures these older algal cells were obscured by the gelatinous matrix and overlying hyphae. An SEM micrograph by Lawrey (*10*) of a section of the thallus of *Cladonia cristatella* Tuck. showed a cell of *Trebouxia erici* Ahm. that was bound by only one appressorium.

The envelopment of an algal cell by hyphae is not complete; that is, there are still surface areas of the algal cell that are free of fungus. This means that substances such as water and minerals may be absorbed directly by the algal cells rather than being transferred through the fungal hyphae. In his SEM study of thallus surfaces Hale (*11*) found that some lichens had numerous pores that lead into the algal layer. These pores could be a means of entry for water and other substances.

Lichenization in our mixed cultures occurred only in regions of new growth of the fungus from the original colonies that were inoculated into the agar. Such growth was mycelial and consisted of elongated hyphae that radiated out from the main colony. Lichen fungi generally are cultured on media that are rich in organic supplements (*12*). On such media

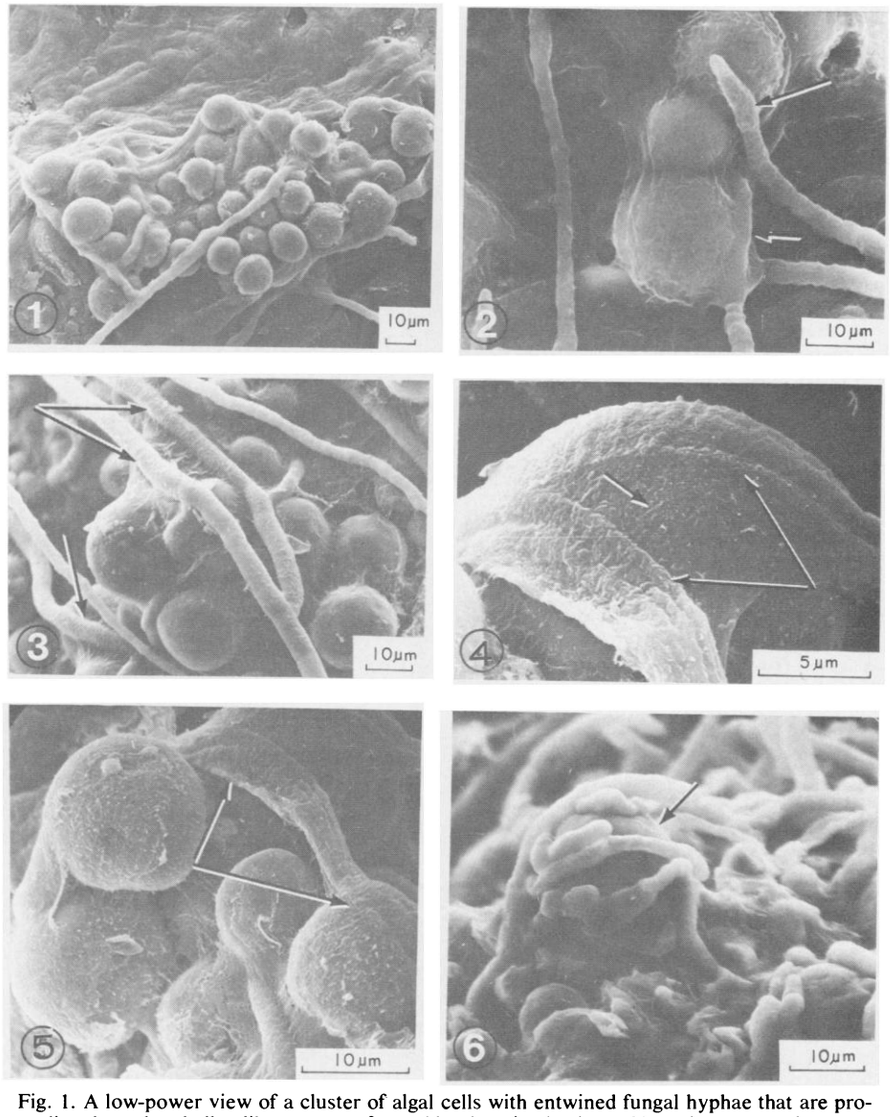

Fig. 1. A low-power view of a cluster of algal cells with entwined fungal hyphae that are protruding through a thallus-like structure formed by the mixed culture. Note the contacts between fungal hyphae and algal cells. Fig. 2. Several algal cells enveloped by a thin veil of extracellular substance which binds the tip of one hypha (short arrow). Another hyphal tip (long arrow) appears to be forming an appressorium. Fig. 3. A low-power view of fungal hyphae that have made contact with numerous algal cells. Only those fungal hyphae in contact with algal cells are covered by extracellular substance (arrows). Fig. 4. A high-power view of one algal cell on which are two appressoria (long arrows). Note the thin veil of extracellular substance coating the appressoria (short arrow) and the flat nature of the appressoria. Fig. 5. One fungal hypha that has formed appressoria on two algal cells (arrows). Fig. 6. An algal cell (arrow) enveloped by several hyphae, a rather rare finding.

the fungi grow well, but their growth is of a colonial form; that is, the colonies are made up of tightly compacted hyphae. The fungus assumes a more openly mycelial state on a nutrient-poor medium.

Ahmadjian (*1*) recognizes several stages of lichen resynthesis. The first stage is the contact between the symbionts, either through appressoria or by envelopment of algal cells by fungal hyphae. The second stage is the formation by the fungus of a tissuelike mat of cells called a pseudoparenchyma. This tissue binds the algal cells into a compact group and is the forerunner of thallus differentiation into cortical, algal, and medullary layers. We did not observe pseudoparenchyma tissue in our cultures, but some of the fungal hyphae consisted of a series of short cells which suggested the beginning of such a tissue formation.

Since the algal sheath binds the fungal hyphae during the early stages of association of the symbionts, it is conceivable that such a sheath is a means by which the symbionts recognize each other. However, there is no evidence at present to support such a possibility. Recently, it has been shown that lectins, which are plant proteins, may be a way by which symbiotic bacteria are recognized and accepted by their plant hosts (*13*). Although we know that some specificity exists between lichen symbionts, that is, one cannot randomly mix different symbionts and achieve lichen formation, we still are not clear about the limits of specificity. We do not know how many different types of algal symbionts one lichen fungus can associate with and vice versa. Moreover, we are not sure of the stimulus that causes the fungus to find and make contact with the alga. There is no evidence to support a chemotropic response. Rather, the limited findings indicate that the initial response of the fungus is thigmotropic; that is, it responds to a specific surface shape. In our study, we have seen a fungal hypha form an appressorium around another hyphal cell, and in an earlier study (*7*) we observed with transmission electron microscopy the presence of intrahyphal hyphae. The algal sheath, if it does play a role in symbiont recognition, may be a secondary factor that binds the appropriate fungus and stimulates it to begin thallus differentiation.

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A Harvester Ant Venom: Chemistry and Pharmacology

Abstract. *The mammalian toxicity of the potently algogenic venom of the ant* Pogonomyrmex badius *is greater than that reported for any other insect venom. This enzyme-rich venom contains high concentrations of phospholipase A_2 and B, hyaluronidase, acid phosphatase, lipase, and esterases. This hemolytic secretion from the poison gland produces unusual symptoms in mammals and appears to have been evolved as a deterrent for vertebrate predators.*

Stings by harvester ants of the genus *Pogonomyrmex* have long been recognized for their painfulness and for the highly unusual symptoms that follow envenomation (*1*). In addition to the typical symptoms—pain, redness, burning and itching, and slight swelling—which ensue after stings by most ants, bees, or wasps, the symptoms resulting from envenomation by *P. badius* include localized piloerection and sweating plus long-lasting pain and tenderness of the nearest lymph nodes. We report our analysis of some toxicological, pharmacological, and biochemical properties of this venom.

Fresh venom from worker ants of *P. badius* was used in all experiments. In the ant, the venom is stored in a spherical reservoir (about 0.5 mm in diameter) located near the tip of the abdomen (Fig. 1). To obtain pure venom, reservoirs were removed, rinsed twice with distilled water to remove hemolymph, fat body, or other contaminants, and ruptured in a droplet of distilled water. The empty reservoirs were discarded.

The toxicity of the venom to mice was 0.42 mg/kg [LD_{50} (lethal dose for 50 percent of the animals), administered intraperitoneally] with 95 percent confidence intervals of 0.31 to 0.58 mg/kg (*2*). This is not only the highest toxicity reported from an insect venom, but also is roughly five times higher than the toxicity (LD_{50}, administered intravenously to mice) reported for the venom of the Oriental hornet and eight to ten times higher than reported for honeybee venom (LD_{50}, administered intravenously to mice) (*3*, *4*). These two venoms are the most toxic venoms reported from insects outside the genus *Pogonomyrmex*.

Venom from *P. badius* is not especially toxic to insects, nor does it produce permanent paralysis of envenomated insects. The toxicity (LD_{50}) of the venom injected into the hemocoel of larvae of the flesh fly, *Sarcophaga bullata*, or wax moth, *Galleria mellonella*, falls in the range of 50 to 100 mg/kg (*5*). Thus, the toxicity of harvester ant venom appears to be different from that of the venoms of paralyzing wasps, which are usually not lethal to the injected animal, but which may produce total permanent paralysis when as little venom as 10 to 100 μg/kg are injected (*6*).

A powerful, direct hemolytic factor is present in *P. badius* venom. As little venom as 4 μg/ml induces more than 95 percent hemolysis of a 5 percent suspension of washed mouse blood (*7*). The direct hemolytic activity is not reduced by incubation of the venom with a 100-fold excess of heparin. In this respect, *P. badius* venom is different from the venoms of the honeybee or the Oriental hornet, both of which contain direct hemolysins that are inhibited by heparin (*4*, *8*). Heparin inhibits the hemolytic activities of these two as well as other venoms through formation of acid-base complexes with venom peptides (*9*). These complexes form white precipitation zones when 100 μg of honeybee venom and 10 μg of heparin are allowed to diffuse toward each other on Ouchterlony diffusion plates (*10*, *11*). No zones of precipitation are discernible when 500 μg of *P. badius* venom are diffused toward either 10 or 100 μg of heparin. Even 100

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