A MORPHOLOGICAL STUDY OF THE ALGAL SYMBIONTS OF *CLADONIA RANGIFERINA* (L.) WEB. AND *PARMELIA CAPERATA* (L.) ACH.[1]

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Abstract: The cultural morphology of the algal symbionts of *Cladonia rangiferina* and *Parmelia caperata*, which were collected from various environmental habitats in different parts of the world, was studied. According to the criteria of vegetative cell characteristics and colony features, it was found that the algae isolated from different specimens of the same species of lichen collected from different localities were different. This finding indicated that, at the species level, lichen fungi were not specific toward their algal symbionts.

INTRODUCTION

The specificity of the algal symbiont to the lichen fungus has long been of interest and concern. Chodat (1913) first studied the specificity of lichen algae. He and Mlle. Korniloff isolated the algal symbionts of *Cladonia furcata* and *Cl. pyxidata* and found that, although the algae were morphologically similar, physiological differences, i. e, in growth rates and color, were apparent. Chodat described these algae as different varieties. He considered these isolated algae as being of different physiological strains and felt that his findings showed that lichens were specific to their algal symbiont. He also isolated different algae from the same species of lichen collected in different localities. Warén (1918–19) isolated the algae of *Xanthoria parietina* that he collected from different localities in Finland and did not find any differences. However, a Dutch specimen of *X. parietina* was found to have a species of *Trebouxia* different from the one isolated from a Finnish specimen of *X. parietina*. Warén showed that the intensity of the green color of the colony, when grown on the same medium, was different in algae obtained from different lichens. From this finding, Warén suggested that color could be used as an identifying and taxonomic character of an algal species or strain. He concluded that each lichen species had its own specific *Trebouxia* species, but with some exceptions. Jaag (1929) isolated and distinguished four different types of *Trebouxia* from *Parmelia caperata* collected from different localities and substrates. He classified these as four distinct algal species according to the shape of their colonies when grown on nutritional medium. Ahmadjian (1959b) isolated the algal symbiont of *Parmelia caperata* and named it *Trebouxia gelatinosa*.

The purpose of this investigation was to study the cultural morphology of the algal symbionts (phycobionts) of two lichens, namely, *Cladonia rangiferina* (L.) Web. and *Parmelia caperata* (L.) Ach., which were collected from a variety of environmental habitats in different parts of the world. An attempt was made to determine

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whether the algae isolated from different specimens of one species of lichen were of a uniform morphological type. That is, does each morphologically identical lichen association always have one specific type of algal partner or can a mycobiont form the same lichen with different algae.

The main criteria which were used in this study to determine differences among the isolated cultural phycobionts were the shape of the vegetative cells, the type of chromatophore, and the manner of cell division. Other criteria at the cell level included the production and number of aplanospores, shape and flagellar characteristics of zoospores, size of vegetative cells, and the presence or absence of an extracellular gelatinous sheath. The shape and color of colonies produced by the algal isolates on organic nutrient agar were compared as well as their relative growth rates under light and dark conditions.

MATERIALS AND METHODS

Two species of lichens, namely, *Cladonia rangiferina*(1) and *Parmelia caperata*(2) were used in this investigation. Successful algal isolations were made from 34 specimens of *C. rangiferina* and 32 specimens of *P. caperata*. The number of countries from which the specimens were collected were 13 and 6, respectively.

The isolation of lichen algae and the methods and media used in this investigation have been discussed in an earlier paper by Ahmadjian (1959b). When lichen specimens were received, the algae were isolated as soon as possible by means of a micropipette. The isolated single algal cells were inoculated into *Trebouxia*-agar slants in cotton-stoppered 15×150 mm. pyrex test tubes. The cultures were incubated at 19±1°C. under an alternate 12 hours light, 12 hours dark cycle at an illumination of 50–100 foot candles. Light was supplied by G. E. Standard, cool white fluorescent bulbs. After development of the colonies, a portion of the algal colony was inoculated into liquid media which were used to study the morphological differences of the cells. Two series of liquid media (Bristol's solution and *Trebouxia*-liquid medium) were inoculated with the same organism and incubated under the same conditions. Liquid cultures were made in cotton-stoppered, 125 ml. Erlenmeyer flasks which contained 30 ml of medium and grown under continuous light at 60–80 foot candles. A reciprocal shaker was used for the liquid cultures. A portion of each algal colony also was incubated onto solid medium in 250 ml. Erlenmeyer flasks with 90 ml. of *Trebouxia*-agar. One set was kept in complete darkness and the other at a light intensity of 60–80 foot candles. These were used for a comparative study of colonies grown under light and dark conditions. All of these cultures also were incubated at 19±1°C.

A camera lucida was used for the drawing of the different division stages of the cells. India Ink was used as a negative stain for determining the presence of a cellular gelatinous sheath.

RESULTS

All of the isolates were *Trebouxia*. Those from *Cladonia rangiferina* belonged to Group I and those from *Parmelia caperata* belonged to Group II. Ahmadjian

(1) All specimens of *Cl. ragiferina* were determined by Dr. T. Ahti, Univ. of Helsinki, Finland.
(2) All specimens of *P. caperata* were determined by Dr. V. Ahmadjian, Clark Univ., Worcester, Mass., U. S. A.

(1959b) divided the genus *Trebouxia* into two groups. In Group I, the chromatophore is deeply indented into narrow, irregular processes which extend to the cell wall and the vegetative cells are usually ellipsoidal. Group II has a smooth-margined chromatophore which is generally located at a distance from the cell wall and the vegetative cells are spherical. Within each group there were differences between the isolates in terms of cell size, presence or absence of a gelatinous sheath, and shape and color of colony on agar. The *Cladonia* isolates fell into five groups (See key to the phycobionts of *Cladonia rangiferina* (L.) Web.) and the *Parmelia* isolates into seven groups (See key to the phycobionts of *Parmelia caperata*). The number of successful clones isolated from each thallus ranged from 1 to 23. Cultural characteristics of the different groups of *Trebouxia* are listed in Tables I and II.

Key to the phycobionts of *Cladonia rangiferina* (L.) Web.

(Based on cultural characteristics)

1. Vegetative cells spherical 2
1. Vegetative cells mostly ellipsoidal, or ovate 3
2. Presence of a gelatinous sheath; zoospores are 11.0 μ long, 5.0 μ wide and their flagella are 11.0 to 12.0 μ long; there is a loss of color when the colony is grown in direct light; the colony has a vermiform surface; no internal air spaces ..... Group 2
2. No gelatinous sheath could be demonstrated under any of the cultural conditions used; zoospores are 7.0 μ long, 2.5 μ wide and their flagella are 8.5 to 9.0 μ long; no loss of color when the colony is grown in direct light; the colony has a granular surface; with many internal air spaces ..... Group 1
3. Presence of a gelatinous sheath ..... Group 5
3. No gelatinous sheath could be demonstrated under any of the cultural conditions 4
4. Vegetative cells reached maximum diameter of 25.22×35.83 μ; zoospores are 9.5 μ long, 2.5 μ wide and their flagella are 10.0 μ long ..... Group 3
4. Vegetative cell reached maximum diameter of 20.37×27.16 μ; zoospores are 7.0 μ long, 2.5 μ wide and their flagella are 8.5 to 9.0 μ long ..... Group 4

Key to the phycobionts of *Parmelia caperata* (L.) Ach.

(Based on cultural characteristics)

1. Vegetative cells generally tend to remain together ..... Group 1
1. Vegetative cells generally free-living 2
2. Presence of binucleate vegetative cells 3
2. No binucleate vegetative cells could be found 4
3. Presence of a gelatinous sheath under all cultural conditions; flattened zoospores produced ..... Group 2
3. Presence of a gelatinous sheath under some cultural conditions; rounded zoospores produced ..... Group 3
4. Loss of the color of the colony when grown in direct light; flattened zoospores produced ..... Group 4
4. No loss of the color of the colony when grown in direct light; rounded zoospores produced 5
5. Presence of gelatinous sheath under some cultural conditions 6
5. No gelatinous sheath could be demonstrated under any cultural conditions ..... Group 5
6. The number of aplanospores generally 8–32 ..... Group 6
6. The number of aplanospores more than 32 ..... Group 7

Table I. Cultural characteristics of the 5 groups of *Trebouxia* (Group I) from *Cladonia rangiferina*

Group of phycobiont, Locality & Specimen number	Shape of vegetative cell	Size of vegetative cell (micon)	No. of aplanospore	Type & size of zoospore (micron)	Gelatinous sheath	Color of colony	Type of colony	Other traits
Group 1 (Fig. 1) Thailand (#28); E. Princeton, Mass. (#41); Georgia (#46); Austria (#55, 56, 57, 58, 59, 60); Wisconsin (#62, 63); Vermont (#69); Mt. Fuji, Japan (#77); S. Scotland (#95); Bulgaria (#109); Hungary (#125).	spherical	19.49 to 24.25	more than 32, some have only 8-32	flat 7.0×2.5	−	green to dark green	convex; rough granular surface, with many internal air spaces	no loss of color in direct light
Group 2 Queen Charlotte Is. (#70); Finland (#96); Holden, Mass. (#110).	spherical	19.34 to 25.22	more than 32	flat 11.0×5.0	+	green to dark green	convex; vermiform surface	loss of color in direct light
Group 3 Queen Charlotte Is. (#71, 72, 73); Scotland (#85); Finland (#113).	mostly ellipsoidal, often ovate	16.49×26.19 to 25.22×35.83	more than 32	flat 9.5×2.5	−	dark green	convex; granular surface	
Group 4 Taiwan, China (#9); Canada (#31); Holland (#37); Finland (#111); Canada B.C. (#112); Czeckoslovakia (#120, 123).	mostly ellipsoidal, some spherical or ovate	13.58×17.46 to 20.30×27.16	more than 32	flat 7.0×2.5	−	same as group 1 & 2	convex; rough surface, with many internal air spaces	
Group 5 Scotland (#86); New Hampshire (#131).	mostly ellipsoidal	16.49×21.34 to 18.43×24.25	more than 32	flat 7.0×2.5	+		convex; vermiform surface	

Table II. Cultural characteristics of the 7 groups of *Trebouxia* (Group II) from *Parmelia caperata*

Group of phycobiont, Locality & Specimen number	Shape of vegetative cell	Size of vegetative cell (micron)	No. of aplanospore	Type & size of zoospore (micron)	Gelatinous sheath	Color of colony	Type of colony	Others traits
Group 1 (Fig. 2) E. Princeton, Mass. (#17-2); Peru (#34).	spherical	11.64 to 28.14 & 10.19 to 24.74	8, 16, & 32	flat 8.7×3.8	+	yellow in dark, green in light	convex; granular & vermiform surface; many internal air space	several cell groupings in mother cell wall
Group 2 Japan (#30); Tenn. (#45); Woods Hole, Mass. (#48); Bedford, Mass. (#53-2, 54-2); Germany (#82-5); Dennisport, Mass. (#102).	spherical	11.64 to 24.25	more than 32	flat 8.0×3.0	+	dark green	homogeneous vermiform surface	Thickening of the cell wall in older vegetative cells, binucleate vegetative cell
Group 3 (Fig. 3) Concord, Mass. (#10); Upton, Mass. (#14); N. Carolina (#43); Georgia (#44).	spherical	14.5 to 25.2		round, 5 μ in diameter	+	dark green	ridged surface in light, vermiform surface in dark	a few binucleate cells, thickening of the cel wall
Group 4 Petersham, Mass. (#6-1, 6-2, 6-3); Kingston, Canada (#32-1, 32-3).	spherical	12.1 to 21.5; max. to 31.05	32 or more	flat or round 9.0×3.0	present only in Bristol's solution	dark green	convex; knobby appearance	progressive loss of color in direct light
Gronp 5 Holden, Mass. (#1-2, 3-1, 3-2); Kingston, Canda (#32-2).	spherical	9.7 to 21.7; max to 34.93	8.16 or more than 32	round, 5 μ in diameter	—	grass green	convex, ridged & knobby surface	no loss of color in direct light
Group 6 Wisconsin (#62-1); France(#66); New Hampshire (#74-1, 74-2); Michigan (#75-1).	spherical	10.67 to 19.40	8, 16, or 32	round, 5 μ in diameter	—	green to dark green	vermiform, granular, & knobby surface	thickening fo the cell wall in older vegetative cell
Group 7 Holden, Mass. (#1-1, 2-4); Rutland, Mass.(#4); Bedford, Mass. (#52-1, 52-2).	spherical	11.6 to 22.1	more than 32	round, 5 μ in diameter	—	yellow green to dark green	ridged, vermiform surface	

DISCUSSION

From the results obatined, it is interesting to note that the phycobionts which were isolated from morphologically identical specimens of *Parmelia caperata* collected from different localities and substrates could be divided into seven groups (see key to the phycobionts of *Parmeila caperata*). The vegetative cells of the phycobionts of most specimens of *Parmelia caperata* generally did not remain attached to each other to form clumps of cells. However, the phycobionts of specimens from East Princeton, Massachusetts, U. S. A. and Peru (#17–2, 34) had vegetative cells which, in a very characteristic manner, did remain together in small groups. Also, the presence of binucleate vegetative cells was noted in some phycobionts and not in others, Ahmadjian (1959b) used these two cultural characteristics to delimit species in the taxonomy of *Trebouxia*. It should appear, therefore, that there are at least two distinct species of *Trebouxia* among these isolated phycobionts.

Ahmadjian (1959b) also reported that the most characteristic trait of the phycobiont which he isolated from a *Parmelia caperata* specimen was a cellular gelatinous sheath, which was demonstrated under all of his tested cultural conditions. Large balls of aplanospores and numerous zoospores were also found in his cultures and the alga formed a knobby-looking colony on agar-glucose-peptone medium. However, in our study, we found that although some of the algae isolated from specimens of *Parmelia caperata* collected from the same locality as Ahmadjian's, i. e., Bedford, Massachusetts, U. S. A. (i. e. #53–2, 54–2) were similar to Ahmadjian's original isolates, others were quite different. The differences were as follows: In some isolates, a gelatinous sheath was present only around cells grown in Bristol's solution; the zoospores were round in shape; the colonies were flat, dark green, and had a ridged surface when grown in the light; in the dark, the colonies were flat, dark green, with a vermiform surface (#52–1, 52–2). Another instance where the specimens of *Parmelia caperata* collected from the same locality had different cultural characteristics was found in the isolates from three Canadian specimens (#32–1, 32–2, 32–3). The colonies of phycobionts from two specimens of Canada (#32–1, 32–3) were dark green, with a convex shape and a knobby appearance, both in the light and in the dark, and there was a progressive loss of color when the cultures were grown in direct light. However, the colony from another specimen of Canada (#32–2) was grass green, had a rough granular surface, both in the light and dark grown cultures, and there was no loss of color when the colonies were grown in direct light. Phycobionts isolated from 5 specimens of *Parmelia caperata* (#1–1, 1–2, 2–4, 3–1, 3–2) from the same locality of Holden, Massachusetts, U. S. A. also were different. The differences were that two strains (#1–1 and 2–4) of phycobionts had a gelatinous sheath only around cells grown in Bristol's solution but in the other three strains (#1–2, 3–1 and 3–2) no gelatinous sheath could be demonstrated under any of the cultural conditions. The colonies of #1–2 and 3–1 were flat and had a ridged surface both in the light and dark grown cultures and the colonies of the other strain (#3–2) had a ridged surface when grown in the light but a knobby surface when grown in the dark. The color of the colonies of those phycobionts (#1–2, 3–1 and 3–2) without a gelatinous sheath was grass green. With regard to the colonies (#1–1 and 2–4) of algae of those phycobionts with a gelatinous sheath, one (#1–1) was flat, yellow-green, and had a ridged surface when grown in the light. When grown in the dark, it formed a colony which was flat, dark green, and had a vermiform surface. The colony of the other strain of phycobiont (#2–4) with a gelatinous sheath was flat,

grass green, and had a ridged surface when grown in the light; in the dark, the colony was convex-shaped, dark green and homogeneous. The appearance of the algal colonies in culture also varied among isolates from lichen specimen collected from different localities. Jaag (1929) described 4 distinct algal strains of *Trebouxia* isolated from *Parmelia caperata* which were collected from 4 localities. The 4 isolates had spherical cells that differed slightly in size but were unlike in the types of colonies they produced on agar; one formed lobes at the surface of its colony; another had no marginal lobes; another colony was of an irregular form; another colony was smooth and flattened.

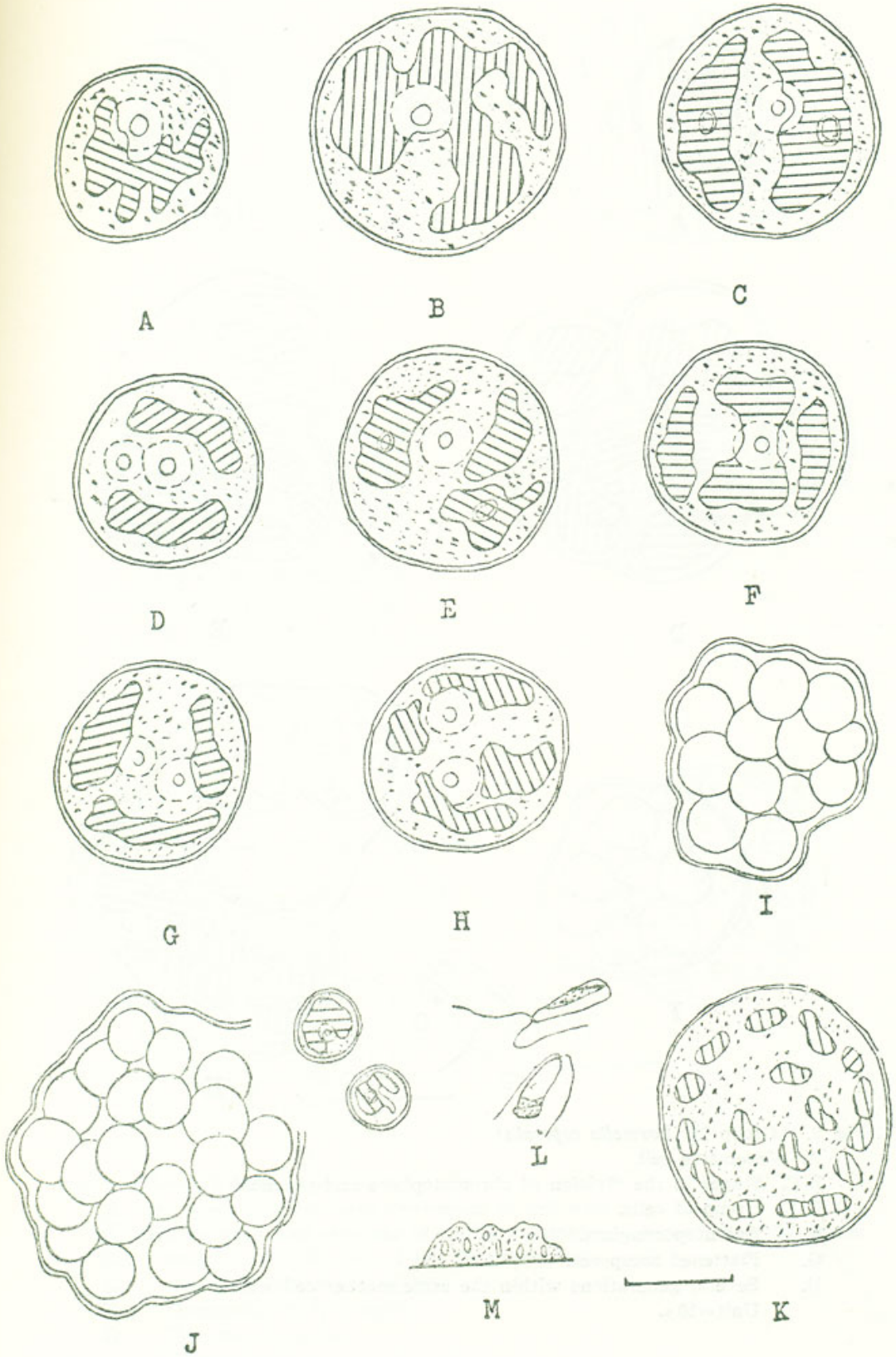
Ahmadjian (1960) used colony characteristics as an aid in differentiating isolates of the lichen alga *Trebouxia*. Bold and Parker (1961) used colony characteristics and changes in color as tools in distinguishing the species of the related unicellular alga *Chlorococcum*. From our cultural study it appeared that although individual cellular characteristics were the same, the algae differed in the intensity of the color and shape of their colonies and these traits depended on the area from which the lichens were collected and the illumination under which they were cultured. The shape and color of a colony are useful traits in distinguishing between species or strains of phycobionts. The problem of identifying species of phycobionts, however, is beyond the scope of this study.

The phycobionts isolated from specimens of *Cladonia rangiferina* which were collected from different localities and substrates could be divided into five groups (See key to the phycobionts of *Cl. rangiferina*). The algae obtained from different specimens of the same subspecies of *Cl. rangiferina* collected from the same locality showed differences in vegetative cells and the shape and color of their colonies. For example, the phycobionts isolated from the four specimens of Canada (#70, 71, 72, 73), had the following differences: The vegetative cells of #70 were spherical and cells of #71, 72, 73 were ellipsoidal or ovate. The color of their colonies was dark green and they had a vermiform (#70) and granular surface (#71, 72, 73) both in the light and dark. One strain from a Taiwan specimen (#9) showed differences from the strain of another specimen from Taiwan that was studied in 1970 (Yang-Wang, 1970.). Different subspecies of *Cl. rangiferina* collected from different lecalities had phycobionts with identical cultural characteristics and phycobionts isolated from the same subspecies of *Cl. rangiferina* which were collected from different localities had different cultural characteristics. According to Chodat (1913), there is a specificity of algae not only in lichen genera but also in lichen species. It was demonstrated in our study, however, that some of the algae isolated from different specimens of the same lichen species showed differences in their cultural morphology. On the other hand, all clones raised from the same lichen specimens were identical. A cellular gelatinous sheath was noted only around the algal cells of those strains

Explanation of figures:

Fig. 1. (Group 1) (*Cladonia rangiferina*)

A-B. Vegetative cell.
C-H. Stages in the division of the chromatophore.
I. Aplanosporangium with aplanospores.
J. Ruptured aplanosporangium, releasing aplanospores.
K. Developing zoosporangium.
L. Zoospores.
M. Section through colony grown on *Trebouxia* agar showing several air spaces. Unit = 10 μ.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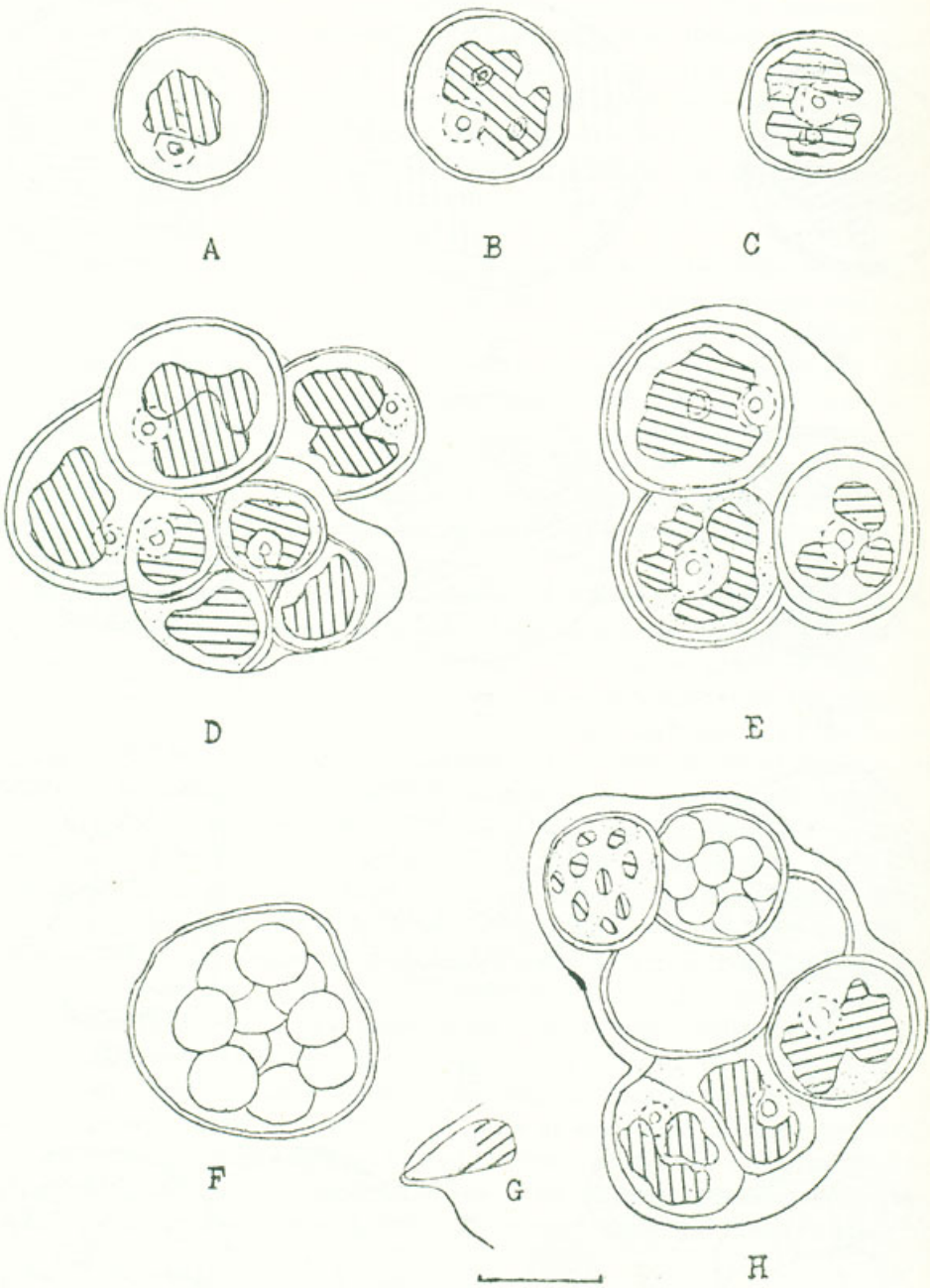

Fig. 2. (Group 1) (*Parmelia caperata*)
A. Vegetative cell.
B-C. Stages in the division of chromatophore and pyrenoid.
D-E. Group of cells.
F. Aplanosporangium.
G. Flattened zoospores.
H. Several generations within the same mother cell wall.
Unit = 10 μ.

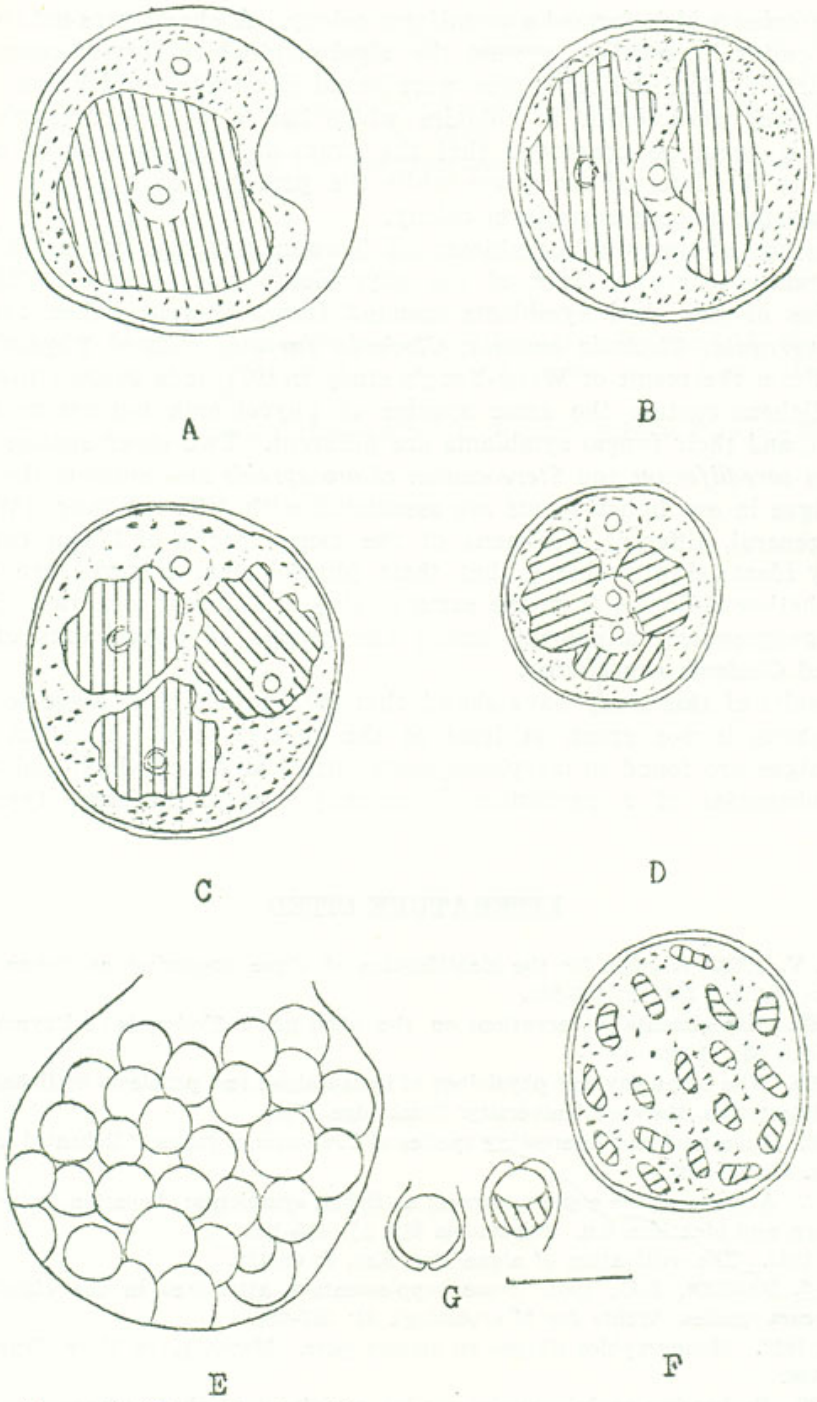

Fig. 3. (Group 3) (*Parmelia caperata*)
A. Old vegetative cell with thickening of cell wall.
B. Cell showing first cleavage of the chromatophore; nucleus near the center.
C-D. Stages in the division of the chromatophore.
E. Ruptured aplanosporangium.
F. Developing zoosporangium.
G. Rounded zoospores.
Unit = 10 μ.

of *Cl. rangiferina* which formed a vermiform colony. A sheath was not noted, under any of the cultural conditions, around the algal cells which formed colonies with a granular surface. Internal air spaces were found in colonies which had a granular surface but they were absent in colonies which had a vermiform surface. From this study, it could be concluded that the granulated appearance of a colony is attributable to the internal air spaces while the presence of a cellular gelatinous sheath causes the smooth vermiform colony.

In the study of the algal symbionts of Taiwan fruticose lichens (Wang-Yang, 1970), differences in growth rate of the phycobionts was observed. That is, the same species of the algal symbionts isolated from different lichen species (i.e. *Cladonia aggregata, Cladonia cornuta, Cladonia furcata*) showed physiological differences. From the result of Wang-Yang's study in 1971, it is evident that different species of lichens contain the same species of phycobionts but not necssarily the same strain, and their fungal symbionts are different. Two other species of lichens *Stereocaulon sorediiferum* and *Stereocaulon chlorocaproids* also support the view that different algae in one lichen genus are assoclated with different fungi (Wang-Yang, 1971). In general, different specimens of the same species of lichen contain morphologically identical phycobionts but their physiological features are sometimes different whether collected from the same locality or different localities. There was no logical geographical alignment among the groups of phycobionts of *Parmelia caperata* and *Cladonia rangiferina.*

The results of this study have shown that the specificity of algae to particular types of lichens is not great, at least at the species level. Different species or strains of algae are found in morphologically identical lichen thalli and conversely different subspecies of a particular lichen may contain the same type of algal symbiont.

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