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Lichen Mycobionts Transplanted into the Natural Habitat

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Abstract

Mycobionts of the four lichen species *Anaptychia ciliaris*, *Physcia tenella*, *Physconia distorta* and *Xanthoria parietina*, which were grown for more than one year under axenic conditions, were then attached to twigs of *Quercus robur*. The mycelia were subsequently exposed to natural conditions for one year. The observations show, that the isolated lichen mycobionts remain viable over a very long period of time and under various conditions. The transplanted mycelia served as substrate for lichen diaspores and free-living algae. In addition symbiotic interactions occurred between the isolated lichen fungi and their photoautotrophic partner. The mycobiont of *Physcia tenella* in contact with free-living algae showed a transition from mycelial growth to the symbiotic state and free-living young lobules of this lichen colonized the transplanted mycobiont and became an integral part of it. Intrahyphal hyphae took part in this relichenization process.

Keywords: Isolated lichen mycobionts, morphogenesis, relichenization, species specific recognition

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1. Introduction

Many lichens produce ascospores for sexual reproduction. After germination the outgrowing hyphae depend on the presence of suitable algae for the formation of a lichen thallus. If the germinating spores do not lichenize then the mycobiont must survive until contact is made. It is not known if mycobionts in an unlichenized state occur free-living in nature (Lawrey, 1984; Deacon, 1997) but there has been no systematic investigation of the complete mycoflora of the natural vegetation at any site (Carroll, 1997). Survival should be possible because Ott (1987a) described the growth of the mycobiont of *Xanthoria parietina* in saprotrophic contact with green algae that are not suitable for lichenization. Saprotrophic behaviour of the mycobiont also occurs in *Ochrolechia frigida*, as recently shown by Gassmann and Ott (2000). There is evidence that unlichenized mycobionts occur on the bark of trees or in the soil as part of the mycoflora (Honegger, 1996), but are morphologically undistinguishable from other filamentous fungi. Only modern methods of molecular biology will permit detailed examination of this problem in the future.

To investigate the ability of lichen mycobionts to survive in an aposymbiotic state in the natural environment, the isolated mycobionts of four lichen species cultured under axenic conditions for one year were transplanted onto twigs of *Quercus robur*. They were exposed to the conditions of the natural environment for one year. This experiment offers an opportunity to examine the response of lichen fungi to the environment and to other organisms that are found in the transplant habitat.

Although the artificial synthesis of lichens is challenging (Ahmadjian, 1993), at present it is not possible to routinely produce a lichen thallus from the isolated bionts in the laboratory (Galun, 1988). In artificial cultures contact of bionts often is only achieved between photobionts and germinating spores and not between photobionts and old mycelia. There are still gaps in the understanding of relichenisation processes and experiments with isolated mycobionts in the natural environment may provide new insights. This study reveals information about the potential of the isolated mycobionts to re-establish the symbiotic state even after a prolonged period of growth without its photoautotrophic partner. Some biotrophic parasites (e.g. rust fungi), which are difficult to culture, loose their potential to infect plants after growing in an axenic state (Maclean, 1982). This implies that they have changed irreversibly to a saprotrophic form and it was necessary to examine if this also happens to lichen fungi.

2. Materials and Methods

Lichen species

Xanthoria parietina (L.) Th.Fr., *Anaptychia ciliaris* (L.) Koerb. and *Physconia distorta* (With.) Laundon were collected in Mullsjö, Västergötland, Sweden. *Physcia tenella* (Scop.) DC. was collected in Schouwen Duiveland, The Netherlands.

Methods

Spores of the freshly collected lichens *Anaptychia ciliaris*, *Physcia tenella*, *Physconia distorta* and *Xanthoria parietina* were cultivated either on MF-Millipore membrane filter discs (ester of cellulose acetate and nitrate) or on Durapore membrane filter discs (polyvinyldendifluorid) with pore sizes of 0.45 µm (Millipore, Eschborn, Germany). The filters were placed over malt yeast extract agar medium (MY) (Ahmadjian, 1993) or mineral agar medium (BG110) (Rippka et al., 1979) with the addition of ribitol (1.5%) or glucose (1.5%).

The cultures were stored at room temperature and in a natural day-night cycle for one year without renewing the media. After this period the polyspore mycelia were cut out with the filter and transferred to twigs of *Quercus robur* in Düsseldorf, Germany. The samples were attached to the branches with silicon glue and exposed to natural conditions for one year.

Sections (25 µm) of the mycelia were made with a cryomicrotome, stained with lactoglycerin cotton blue (which dyes the protoplasts) and examined using a light microscope. For investigations with the scanning electron microscope (SEM) (Stereoscan 200, Cambridge) the mycelia were air dried and sputter-coated with gold.

3. Results

Description of the habitat

On the twigs of the oak abundant natural growth of *Physcia tenella*, *Parmelia sulcata*, *Parmelia elegantula* occurred and a few small specimens of *Hypogymnia physodes* and *Evernia prunastri* were found. In addition, the bark was closely overgrown by filamentous cyanobacteria, lumps of free-living green algae and lichen primordia (Fig. 1).

Very young stages of *P. sulcata* and *P. tenella* can be identified by their morphology (Ott, 1987b), which is important for the experiment. Primordia of

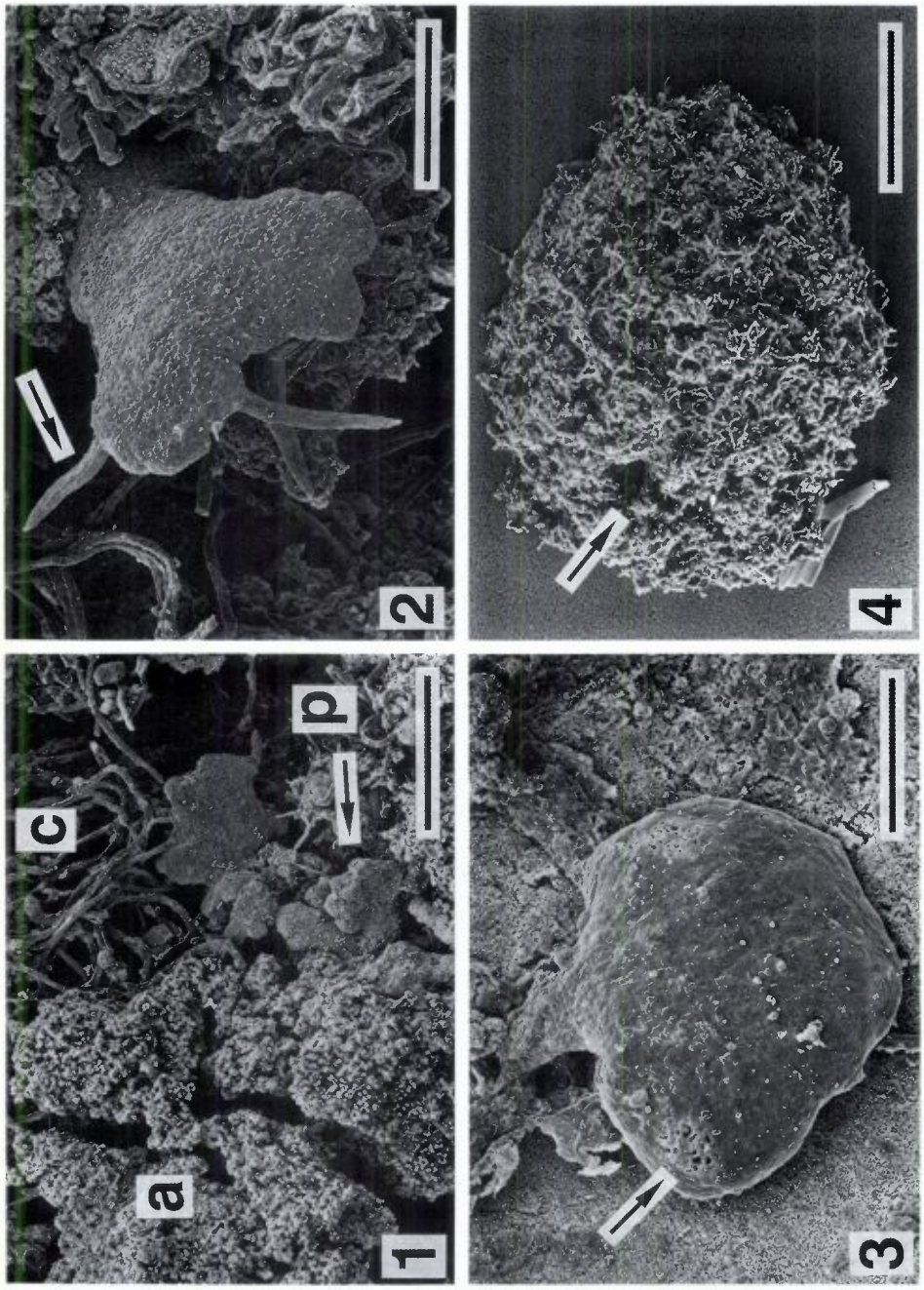


Figure 1. Bark of *Quercus robur* with filamentous cyanobacteria (c), free living algae (a) and lichen primordia (p). Scale bar = 300 µm.

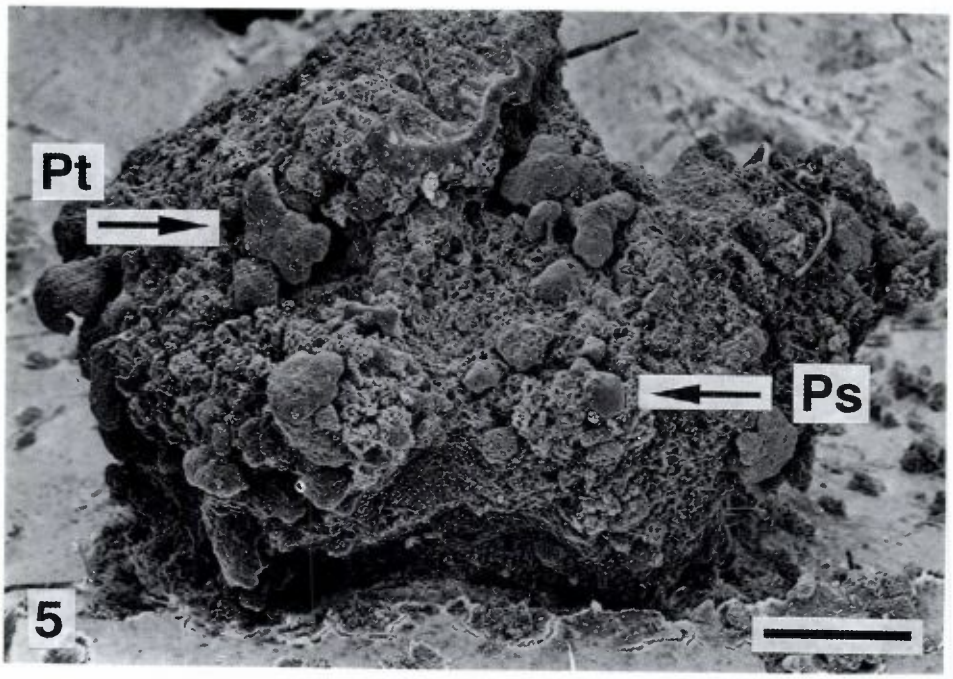


Figure 5. Mycobiont of *Physcia tenella* after a cultivation period of one year in the field. The mycobiont is colonized by lichen primordia and young lichen thalli of *Physcia tenella* (Pt) and *Parmelia sulcata* (Ps). Scale bar = 320 µm.

P. tenella are characterized by an uneven surface and by the development of rhizines at a very early stage (Fig. 2). Primordia of *P. sulcata* have a smooth surface with characteristic fissures, which develop into soralia (Fig. 3). Both lichens produce soredia as vegetative diaspores.

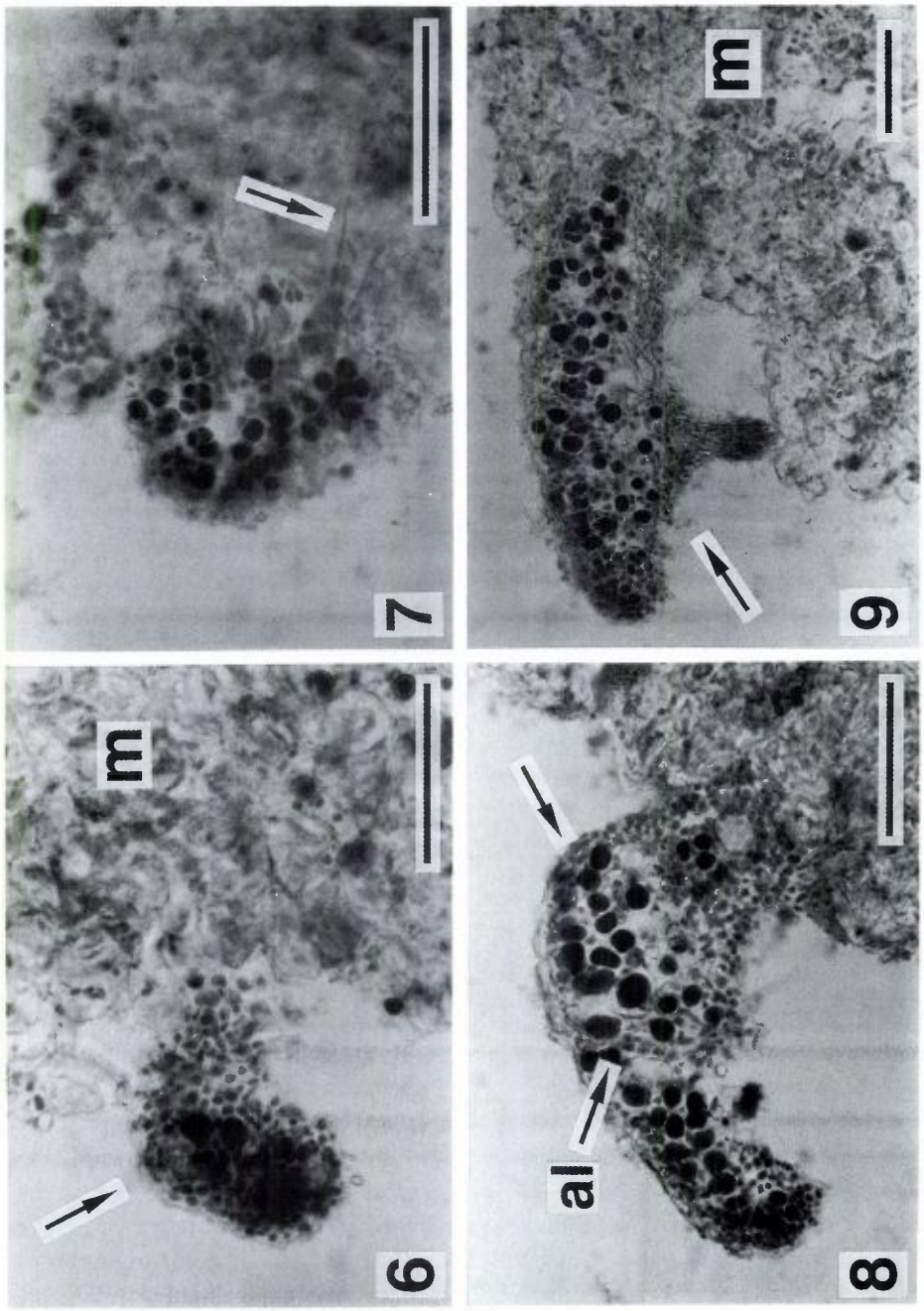
Lichen vegetation on the branches increased during the year of observation.

Figures 2–4 on opposite page:

Figure 2. Primordium of *Physcia tenella* with an uneven surface and rhizines (arrow). Scale bar = 190 µm.

Figure 3. Primordium of *Parmelia sulcata* characterized by a smooth surface with fissures (arrow). Scale bar = 130 µm.

Figure 4. Mycobiont of *Physcia tenella* grown for one year in axenic culture. A loosely structured mycelium with cavities (arrow) is formed. Scale bar = 250 µm.



Figs. 6–9. Colonization of the mycobiont of *Physcia tenella* by soredia of the same species. Scale bars = 50 µm.

Development of the mycobionts

All transplanted mycelia did not show marked growth or differentiation during the cultivation period of one year in the field, but they were still present and not degenerated. The mycobiont of *P. tenella* gave the most interesting results and this paper concentrates on it. Before exposure to the natural environment the one-year-old mycobiont of *P. tenella* had formed a loosely structured mycelium in the axenic culture; this is typical for the species (Fig. 4). After transplantation on the *Quercus robur* twigs, the mycobiont became covered by numerous lichen primordia (Fig. 5). Most can be identified as *P. tenella*, characterized by their rough cortex and by their rhizines. But also small *P. sulcata* lobules with their smooth surface became established on the exposed mycobiont.

Cross sections of the transplanted mycelia show short-celled hyphae, which are stained intensive blue with cotton blue, a characteristic feature for growing lichen tissue. The mycobiont kept its loose structure and algae and soredia from the vegetation covering the branches of the tree became loosely attached to its surface. In some areas lichen primordia were observed. The primordia and the new lobes may either be derived from relichenization of the exposed mycobiont or may be the result of colonization of the mycobiont by external soredia from the surrounding vegetation. A detailed analysis revealed several distinct developmental processes:

Colonization of the mycobiont by soredia of the same species

When a soredium of the same species comes into contact with the mycelium (Fig. 6), outgrowing hyphae of the soredium penetrate the cultured mycobiont, while the algal cells inside the developing primordium proceed to divide (Fig. 7). The differentiation of a cortex and an algal layer initiates the development of a thalline scale (Fig. 8) and the formation of a rhizine shows that the young thallus is *P. tenella* (Fig. 9). It is important to emphasize that the tissue of the soredium and the transplanted mycobiont merge completely and no border remains.

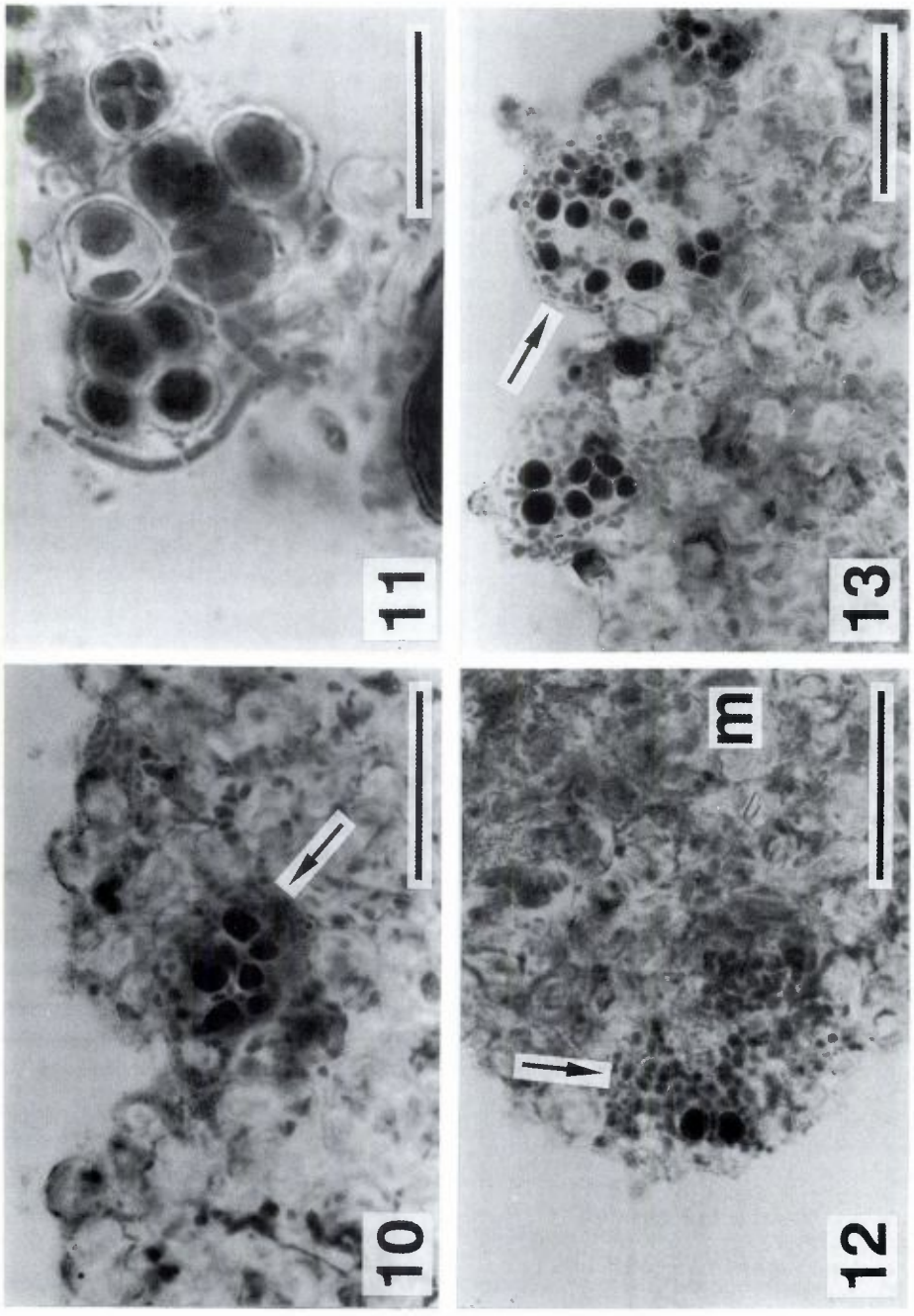
Figures 6–9 on opposite page:

Figure 6. A soredium (arrow) gets into contact with the mycobiont (m).

Figure 7. Hyphae of the soredium penetrate the mycobiont (arrow).

Figure 8. The primordium has formed a cortex (arrow) and an algal layer (al).

Figure 9. Young *Physcia tenella* thallus (arrow) with a rhizine. The lichen and the mycobiont (m) form a unified entity.



Figs. 10–11. Interaction of the mycobiont of *Physcia tenella* with free living algae.

Interactions of the mycobiont with free living algae

The branches of the tree were covered with a layer of green algae, quite likely including *Trebouxia impressa*, the photobiont of *P. tenella*, which is set free from decaying old thalli. The algae may also have come into contact with transplanted mycobionts of *P. tenella*. When algal cells penetrate the loosely structured hyphal network, this triggers growth of the hyphae inside the mycelium (Fig. 10). At the margin of the mycelia lumps of algae that are not similar to lichenized soredia are invaded by growing hyphae from the transplanted mycelium. Branches of the hyphae grow towards the algal cells (Fig. 11).

The specific photosynthetic partner of *P. tenella*, the green algae *T. impressa*, appears to initiate symbiotic growth responses. Branching hyphae of the mycobiont incorporate the algae into the mycelium by the formation of a thin fungal layer, while the algae divide (Figs. 12 and 13). A small primordium develops (Fig. 14) and differentiation of cortex and algal layer leads to the formation of a *P. tenella* thallus, which can be identified even without rhizines (Fig. 15). The developing lichen primordium and the transplanted mycobiont form a unified entity right from the beginning of morphogenesis, which differs from the secondary fusion of the mycobiont with the corresponding soredia.

Mycobionts as a substrate for unrelated lichen diaspores

The transplanted mycobiont of *P. tenella* was also colonized by *P. sulcata* (Fig. 5). The small lobule of *P. sulcata* can be seen anchored to the mycobiont by only a few outgrowing hyphae (Fig. 16). The tissue of the lichen and the transplanted mycobiont do not fuse. Obviously, the interactions between the soredia of *P. tenella* and the transplanted mycobiont of this species are much more intimate. Only intraspecific fusion appears to be possible. Colonization of the mycobionts of *Physconia distorta* and *Anaptychia ciliaris* (these mycobionts were also transplanted) by *P. tenella* supports this observation.

Figures 10–13 on opposite page:

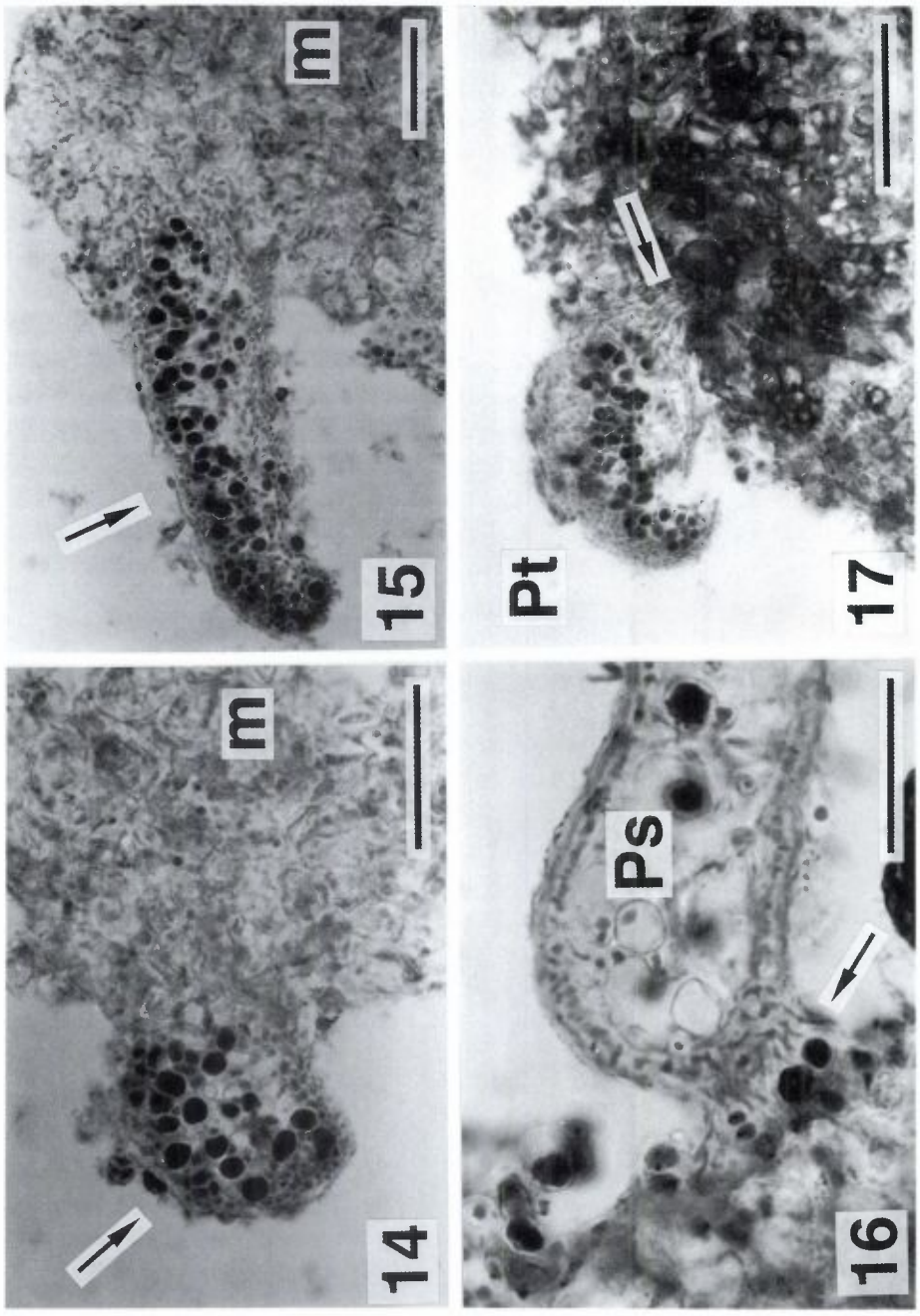
Figure 10. Algal cells that penetrate the loosely structured network of hyphae trigger a growth response of the mycobiont (arrow). Scale bar = 40 µm.

Figure 11. Lumps of free living algae at the margin of a mycelium are invaded by hyphae from the transplanted mycobiont. Scale bar = 15 µm.

Figs. 12–13. Development of a young lobule of *Physcia tenella* from incorporated algae. Scale bars = 50 µm.

Figure 12. Branching hyphae of the mycobiont (m) incorporate algae into the mycelium (arrow).

Figure 13. A thin fungal layer is formed (arrow). The algal cells proceed to divide.



Figs. 14–15. Development of a young lobule of *Physcia tenella* from incorporated algae. Scale bars = 50 µm.

Young lobes of *P. tenella* growing on the mycelium of *P. distorta* are anchored only by a few hyphae (Fig. 17). The small thallus of *P. tenella* that grows beneath the *A. ciliaris* mycobiont uses the niche between the mycobiont and the surface of the filter for colonization, again without forming intimate contact (Fig. 18).

Interactions of other mycobionts

Isolated mycobionts of *A. ciliaris*, *P. distorta* and *X. parietina* were also transplanted. These mycelia are possible substrates for outgrowing diaspores of other lichens but symbiotic interactions, which were observed in the mycobiont of *P. tenella*, did not occur. The mycelia remained vital but relichenization was not observed, probably because there were no free-living thalli in the vicinity to release the specific photobiont of these species.

4. Discussion

This experiment with isolated lichen mycobionts transplanted into the field demonstrates that mycelia from axenic cultures remain viable in a natural habitat for a long time. Although no marked radial growth was observed, the examined specimens from several species did not degenerate. As outgrowing hyphae could be observed making contact with free-living algae the non-lichenized transplants are still alive. It cannot be proven whether the mycobiont is dormant without this stimulus, but it seems likely that for survival the formation of intrahyphal hyphae (Fig. 19) is essential. Ahmadjian (1980, 1982) briefly described this growth behaviour in isolated mycobionts and mentioned that it results in the compact form of aposymbiotic mycelia cultured on organic media. Tips of hyphae grow in and out of other hyphae and ultra structural investigations reveal that these hyphae represent the growing part of a compact mycelium even after a very long cultivation period (unpublished data). The ability of the mycobiont to penetrate its own

Figures 14–17 on opposite page:

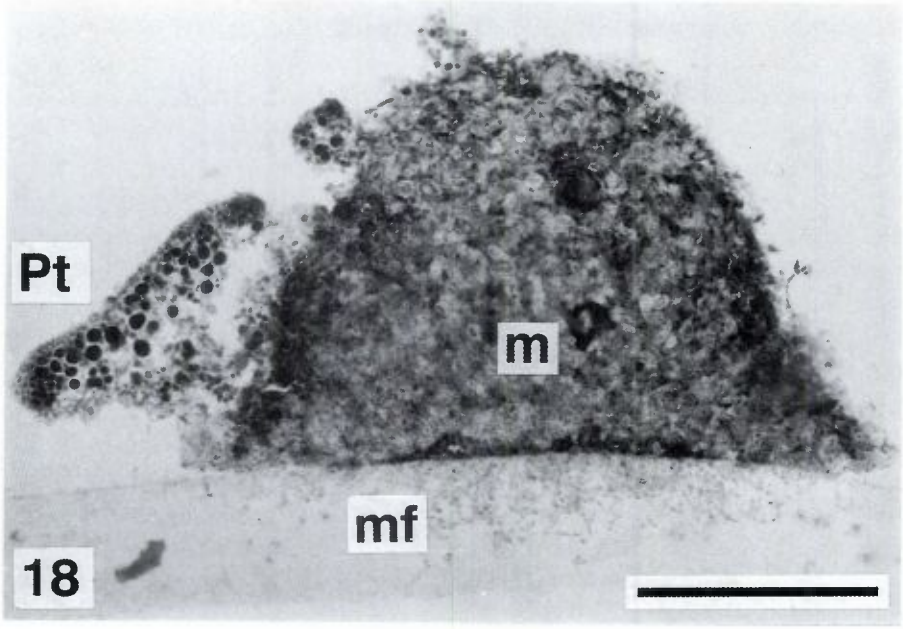
Figure 14. A small primordium (arrow) evolves from the mycobiont (m).

Figure 15. A small scale of *Physcia tenella* (arrow), differentiated into cortex and algal layer, forms a unity with the mycobiont (m).

Fig. 16–17. Mycobionts as substrate for unrelated lichen primordia.

Figure 16. Lobule of *Parmelia sulcata* (Ps) on the mycobiont of *Physcia tenella*. The lichen is anchored to the mycobiont by only a few outgrowing hyphae (arrow). Scale bar = 40 µm.

Figure 17. Lobule of *Physcia tenella* (Pt) growing on the mycobiont of *Physconia distorta*. Some anchoring hyphae penetrate the mycobiont (arrow). Scale bar = 80 µm.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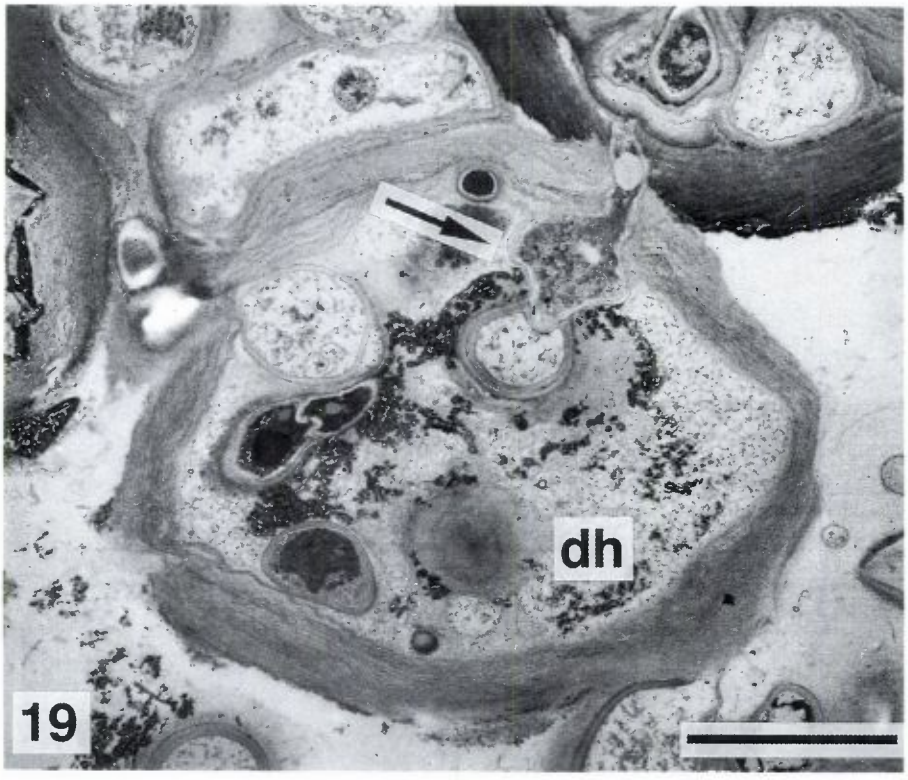
m
mf
18


dh
19

hyphae enhances exploitation of available nutrients, which may account for the longevity of mycelia in the natural environment. A second possibility for getting nutrients might be the ability of isolated mycobionts to utilize cellulosic substrate as a carbon source (Ahmadjian, 1977).

Lichenization is feasible for old transplanted mycelia even after a year of axenic growth in the laboratory. The mycobiont of *Physcia tenella* showed transition from mycelial growth to the symbiotic state through interactions with its photoautotrophic partner, which led to the development of young lobules. Earlier studies with lichen mycobionts have shown that young growing hyphae are more suitable for relichenisation than old mycelia (Ahmadjian, 1993). In most experiments reported up to now compact fungal colonies, which are characteristic for most lichen mycobionts, are regularly homogenized in the laboratory to produce small fragments as "starter units" (Armaleo, 1991). In the present experiment, mycelia have not been reduced to small pieces, but mycelia were transplanted into the field intact. It is likely that the young growing tips of internal hyphae can respond to free-living algae and initiate lichenization. Similar processes have been described by Ahmadjian (1982) for the mycobiont of *Cladonia cristatella*: After removal from liquid culture, thin-walled aerial hyphae were formed and these were able to bind algal cells.

Symbiotic interactions with free-living algae were not observed in transplanted mycobionts of *A. ciliaris*, *P. distorta* and *X. parietina*. It seems likely that these lichens need species-specific photobionts, which were absent from the habitat. There is no local source of photobionts from decaying thalli, as the lichens *A. ciliaris* and *P. distorta* do not occur in the region around Düsseldorf. *X. parietina* is found in this region on trees and on rocks but it has just re-colonized the area in recent years and is not yet found on twigs of *Quercus robur*, which were used for the experiment. Microclimatic conditions or other environmental parameters, such as the pH of the bark, may inhibit the development of *X. parietina* on this tree. Additional transplant studies are now in progress, to investigate the response of the mycobionts of *A. ciliaris*, *P. distorta* and *X. parietina* in a habitat that contains the corresponding lichens.

Figures 18–19 on opposite page:

Figure 18. Mycobionts as substrate for unrelated lichen primordia. Lobule of *Physcia tenella* (Pt) colonizing the niche between the mycobiont of *Anaptychia ciliaris* (m) and the membrane filter (mf). Scale bar = 400 µm.

Figure 19. Mycobiont of *Physcia tenella* with intrahyphal hyphae (arrow) penetrating an adjacent hyphae. Note the penetration tube. Degenerated hyphae (dh). Scale bar = 0.5 µm.
(With courtesy of Dr. Robert Bauer, University of Tübingen, Germany.)

Interaction between precultured mycobionts and free-living lichens or vegetative diaspores of these free-living species is complex. The soredia of *P. tenella* germinate on the transplanted mycobiont of this species and develop into new lobules. This is not an obvious response. In the soralia of lichens germination of soredia is usually inhibited. Here a growth restricting control prevents the further development of the diaspores as long as the symbiotic equilibrium is intact. Only in degenerated thalli do soredia differentiate into new lobules inside the soralia as an adaptation that secures the habitat for the species (Jahns and Ott, 1990). Although interaction between the soredium and the transplanted mycelium is very close, the isolated mycobiont obviously has no inhibiting effect on the soredia. Either the mycobiont looses these properties during the cultivation period or only an intact lichen thallus containing both bionts can influence the soredia.

The soredia of *P. tenella* on the transplanted mycobiont of this species grow directly into thalline scales without previous degeneration. This differs from observations by Jahns (1984, 1993) on cultures with vegetative diaspores. In these experiments the lichen propagules degenerated into undifferentiated tissue before developing new scales. Under favourable conditions in the natural habitat this degeneration prior to organized growth appears to be absent and direct growth was observed in the soredia of *Pseudevernia norwegica* (Hilmo and Ott, pers. comm.). In our experiment either microclimatic conditions in the natural habitat were sufficiently favourable for direct growth or the influence of the overgrown mycobiont initiated this positive reaction.

All transplanted mycobionts are colonised by the soredia of the lichen species found on the tree. Where a mycobiont is in contact with an unrelated soredium the popagules use the mycelia merely as substrate. The formation of anchoring hyphae is evident in some cases and may provide the young primordium with additional supply of water. It is not known if nutrients are exchanged, but transport of organic substances is possible (Crittenden, 1991). The outgrowing soredium, the developing lobe and the transplanted mycobiont always remain separate units. Intimate contact is evident only if the transplanted mycobiont and the soredium belong to the same species. It has been stated before that the cultured mycobiont of *P. tenella* is colonized by soredia from a free living thallus of the same species and it is significant that the soredium becomes an integral part of the mycobiont. After a certain developmental stage the young lobules do not differ from those originating from lichenization. This implies that the tissue of the soredium from the external source and the hyphae of the transplanted mycelium merge completely. Obviously, only intraspecific contact permits such fusion and this requires species-specific recognition. Many details of this process remain to be discovered; investigations using genetic markers suggest a promising new avenue

for better understanding of the mode of interaction in the fused organisms. Future studies will focus on this approach.

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