Myco-photobiontal selection in a Mediterranean cryptogam community with *Fulgensia fulgida*

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Received: *2 August 2001*
Accepted: *19 September 2001*

Summary

• To examine the mode of dispersal and photobiont selectivity of *Fulgensia fulgida* we investigated the photobionts associated with the lichens of a community related to the *Toninio–Psoretum decipientis* association.

• Photobionts and mycobionts were analysed using morphological (light microscopy) and molecular (internal transcribed spacer (ITS) and partial large subunit (LSU) nuclear ribosomal DNA sequencing) techniques.

• The thalli of the mycobiont of *F. fulgida*, which were identical in their ITS and partial LSU-sequence, contained two strains of *Trebouxia asymmetrica*, one of them also present in a thallus of *Toninia sedifolia*. Other co-occurring lichens, namely *Squamarina lentigera*, *Catapyrenium michelii* and *Collema cristatum*, were shown to have *Asterochloris irregularis*, *Myrmecia* and *Nostoc* as photobionts.

• It is suggested that *F. fulgida* is selective in its photobiont choice, that dispersal of the lichen has occurred by way of ascospores and subsequent independent relichenization events and that *F. fulgida* and *T. sedifolia* share the same photobiont pool. The results also suggest possible mechanisms of relichenization and strategies for future research on the selectivity of lichen bionts.

Key words: lichens, mycobiont, photobiont, selectivity, specificity, relichenization, classification of specificity/selectivity, Lecanorales, Teloschistaceae.

Introduction

Lichens are prime examples of symbiotic associations. They were even referred to in the original definition of a symbiosis (De Bary, 1879), but the associations are still poorly understood. In the majority of cases (about 97%) it is unclear which species of algal organisms are involved. However, to answer questions concerning selectivity, coevolution and the mode of relichenization, knowledge of the identity of both partners is a prerequisite.

In sexually reproducing lichens, where fungal components propagate via ascospores, relichenization is suspected to be a necessary event in the life cycle of the system. For the establishment of a functioning, mutualistic symbiosis, germinating hyphae of the mycobionts need to meet and associate with compatible free-living or already lichenized photobiontal cells (Beck *et al.*, 1998). But the likelihood of potential mycobionts meeting a compatible, aposymbiotic alga has been considered to be rather low. However, the chance of contact is considerably increased if a potential mycobiont is capable of forming a temporary aggregate with a less compatible biont and remains in that state until the association with a compatible photobiont is established and thallus formation initiated (Ott, 1987). In this way, a lichen mycobiont can survive and spread on the substrate for an extended period of time.

Where such interim photobionts are not present and relichenization events rare, a high mycobiontal spore input is necessary to assure the establishment of a lichen population. Such high input may be provided by parental thalli in the surrounding environment. In populations with a closely neighboured, genetically homogenous source of spores, the newly developing mycobionts can be expected to be genetically homogenous as well. Should the neighbouring source of spores be already genetically inhomogeneous or recombination between genetically different chromosomes from different germ cells has occurred during the process of meiosis in

the parental asci, the offspring are expected to be genetically heterogeneous.

Genetic uniformity of the algal photobiont depends on the photobiont-referring selectivity of the mycobiont as well as on the availability of suitable photobiontal species at a particular stand. In the case of low selectivity and accessibility, more than one algal clone is expected to occur in lichen populations, even those comprising genetically uniform mycobionts. Such heterogeneity of the photobiont may be considered to reflect multiple relichenization events actually having taken place in the population. In studies on lichen populations, morphological, chemical, isoenzymatic and molecular traits of the fungal component have been used for the identification of the lichen individuals (Fahselt, 1996). However, the genetic traits and their variation of both the fungal and the algal components need to be examined to understand the dynamics of the whole systems.

In the present study, the ITS and partial LSU nrDNA polymorphism of fungal and the ITS nrDNA polymorphism of the algal bionts of a whole population of a sexually reproducing lichen species was examined. This was exemplified by a population of *Fulgensia fulgida* (Nyl.) Szatala (Teloschistaceae) in Southern France, growing in a stand which was covered by a lichen community related to the *Toninio–Psoretum decipientis* Stodiek association. *F. fulgida* is a frequently occurring species in the Mediterranean but nearly absent North of the Alps. It grows on calcareous ground in open grassland, usually directly on soil or over mosses. By contrast to other species of the genus, *F. fulgida* occasionally covers rock surfaces as well. The species is well characterised by its growth form, pyriform and clavate ascospores and the presence of depsidones in addition to anthraquinones.

Materials and Methods

Lichen samples

All investigated lichen thalli were taken from a stand of a *Toninio-Psoretum decipientis*-related community. The population selected was a rather close assemblage of thalli in a sociologically uniform stand of lichens, about 30 dm^2. It was collected in France, Alpes Maritimes, 4 km northeast of Gourdon, on a 45° inclined, west-exposed boulder, which is elevated about 30 cm above the ground. Among the *Fulgensia* thalli present, five were selected and analysed with respect to their myco- and photobionts (photobiont clones M-99.021 to M-99.025). In addition, the photobiont of co-occurring thalli of *Toninia sedifolia* (Scop.) Timdal (B-207) and *Squamarina lentigera* (Weber) Poelt (M-99.109) were analysed in more detail. All material was collected by D. Triebel and G. Rambold (no. 6254 a–g) and has been deposited in the lichen herbarium of the Botanische Staatssammlung München (M).

Photobionts

Photobionts were isolated using a single-cell-manipulator as described in Beck & Koop (2001) and grown as described in Friedl (1989a). Culture strains of the isolated photobionts are maintained at the Lehrstuhl für Pflanzensystematik, University of Bayreuth. The photobionts were examined both in the lichenized and cultured states by standard light microscopic techniques. For identification, the isolated strains were compared with cultures of all known species of *Trebouxia* (Gärtner, 1985; Ettl & Gärtner, 1995), obtained from culture collections (SAG, Schlösser, 1994, UTEX, Starr & Zeikus, 1993).

DNA extraction, PCR and sequencing

Photobionts For the DNA sequence analyses one photobiont clone of each lichen thallus was selected from the isolated lichen photobionts (Table 1). This was considered to be sufficient as all clones from one strain were found to be morphologically identical. Additionally four more clones, obtained from the thallus with the differing photobiont clone, were sequenced. DNA was extracted from log phase cultures using a Qiagen DNeasy Plant Mini Kit, following the standard extraction protocol as supplied by the manufacturer. ITS-regions were amplified using PCR with primers and under conditions as described by Friedl (1996). The ITS-region of the photobiont of *Toninia sedifolia* was amplified from DNA of two whole areoles using the photobiont specific

Table 1 Photobionts analysed from samples of collection GR 6254, fragments a–f

Clone no.	Lichen species	Fragment	Photobiont species	Number of clones analysed by LM	Sequenced clone	Type of sequence
M-99.021	*Fulgensia fulgida*	a	*Trebouxia asymmetrica*	13	B2	a
M-99.022	*Fulgensia fulgida*	a	*Trebouxia asymmetrica*	14	B4	a
M-99.023	*Fulgensia fulgida*	b	*Trebouxia asymmetrica*	13	A3, B2, B4, C2, C3	b
M-99.024	*Fulgensia fulgida*	c	*Trebouxia asymmetrica*	15	B3	a
M-99.025	*Fulgensia fulgida*	f	*Trebouxia asymmetrica*	13	A5	a
M-99.109	*Squamarina lentigera*	d	*Asterochloris irregularis*	10		
B207	*Toninia sedifolia*	b	*Trebouxia asymmetrica*	–	sequenced directly	a

Table 2 GenBank accession no. of ITS nrDNA sequence and references of Trebouxia strains used for the phylogenetic analyses

Trebouxia species	Strain number	source of photobionts	Accession no.
T. arboricola	SAG 219–1a	Free-living?	Z68705
	M-96.025C1	*Xanthoria parietina*	AJ007387
T. asymmetrica	SAG 48.88	*Diploschistes diacapsis*	AJ249565
	M-99.023C2	*Fulgensia fulgida*	AF344175
	M-99.024B3	*Fulgensia fulgida*	AF344176
	B207	*Toninia sedifolia*	AF344177
T. gelatinosa	UTEX 905	*Flavoparmelia caperata*	Z68698
	G-86.108B2	*Flavoparmelia caperata*	Z68697
T. gigantea	UTEX 2231	*Caloplaca cerina*	AJ249577
T. impressa	M-96.027D1	*Physcia adscendens*	AJ007383
T. incrustata	UTEX 784	*Lecanora dispersa*	AJ293795
T. jamesii	M-97.017A2	*Lecidea silacea*	AF128270
T. usneae	G-87.019A1	*Parmotrema tinctorum*	Z68702

primer AL1500bf (Helms *et al.*, 2001) instead of NS7m. The PCR products were cleaned with Qiaquick (Qiagen GmbH, Hilden, Germany) spin columns, following instructions in the manual and sequenced directly over both strands using a Licor X4 automated sequencer. Sequencing primers were 1800F (Friedl, 1996) and ITS 4 (White *et al.*, 1990).

Data analysis The sequences obtained were manually aligned with available *Trebouxia* ITS-sequences (Table 2), representing the major lineages within the genus *Trebouxia* as reported by Beck *et al.* (1998) and Helms *et al.* (2001), using the program GeneDoc (http://www.psc.edu/biomed/genedoc/). After regions of ambiguous alignment and the 5.8S nrDNA had been excluded (positions 44–80, 152–185, 300–452 (5.8S nrDNA), 478–495 and 579–592; positions are given in respect to the sequence of *T. arboricola* SAG 219–1a, GenBank acc. no. Z68705), the alignment with 453 aligned nucleotides was subjected to maximum parsimony analyses using the program-package PAUP*4.0b4a (Swofford, 2000). From the 453 positions used, 277 were constant and 122 variable positions were parsimony informative. The analyses were done using a branch and bound search and the resulting phylogram was midpoint-rooted as no suitable outgroup for *Trebouxia* species was available. Stability of monophyletic groups was tested using the bootstrap method (Felsenstein, 1985) with 500 replications.

Mycobionts Total DNA of the mycobionts was extracted using Qiagen DNeasy Plant Mini Kit, following the standard extraction protocol with minor modifications (elution buffer AE was replaced with sterile water, in two elution steps). The undiluted mycobiont DNA extraction was amplified using PCR and Perkin-Elmer Amplitaq Gold polymerase. After an initial denaturation step of 95°C for 10 min, the PCR ran for five cycles (95°C for 1 min, 53°C for 30 s, 72°C for 2 min) and 35 cycles (95°C for 1 min, 50°C for 20 s, 72°C for 2 min) with a final extension step of 72°C for 10 min. The primers nu-SSU-1203–5′ ('nongreen algal'; Gargas & DePriest, 1996), ITS1F ('fungal-specific'; Gardes & Bruns, 1993), ITS4 (White *et al.*, 1990), S155–5′ (Döring *et al.*, 2000) LR3, LR5 (http://www.botany.duke.edu/fungi/mycolab/primers.htm), and the recently developed LSU155–5′ ('mycobiont-specific'; Döring *et al.*, 2000) were used as PCR primers. The PCR products were purified with QIAquick PCR Purification Kit (Qiagen) and eluted with sterile water. Both strands were sequenced using the PCR primers and primer ITS3 (White *et al.*, 1990). The cycle sequencing reaction ran for 28 cycles (95°C for 30 s, 50°C for 15 s, 60°C for 4 min). Sequences were obtained by a Perkin-Elmer ABI 377 automatic sequencer, using the Big Dye Terminator Reaction Kit (Perkin-Elmer Inc., Wellesley, MA, USA). Fragments were assembled with the Sequencher 3.0 package (Gene Code Corporation Inc., Ann Arbor, MI, USA).

Sequence alignment Overlapping PCR products generated sequences covering the 3′ part of the SSU, the ITS-regions (the spacers ITS1 and ITS2 and the enclosed highly conserved 5.8S rRNA gene) and the 5′ part of the LSU. The sequences were aligned with MegAlign in the LaserGene 1.61 package (DNASTAR Inc., Madison, WI, USA), using the default (gap penalty 10, gap length penalty 10) and manually optimised settings. PCR priming sites were excluded.

Results

The lichen community investigated shares only one species, *Toninia sedifolia*, with the original stand of the *Toninio-Psoretum decipientis* (= *Thalloedema-caeruleonigricans–Lecidea–decipiens* association, Stodiek, 1937), but is similar to the *Fulgensietum fulgentis* Gams 1938. The main differences were the absence of *Psora decipiens* (Hedwig) Hoffm. and *Squamarina cartilaginea* (Wirth) P. James and the replacement of *F. fulgens* (Sw.) Elenkin with *F. fulgida*. The *Fulgensietum fulgentis* has been considered a synonym to the first community by Wirth (1980). The species *F. fulgens* or *F. bracteata* (Hoffm.) Räsänen, often reported for this association, have not been found in the investigated stand. They are replaced by *F. fulgida*, which is much more common in the Mediterranean region. Very similar communities, which also comprise

Table 3 Species inventory of the *Toninio-Psoretum decipientis* and related lichen communities

	Stodiek (1937)	Gams (1938)	Llimona (1982)	Scholz (1995)	Present study
Catapyrenium michelii (A. Massal.) R. Sant.				+	+
Catapyrenium squamulosum (Ach.) O. Breuss				+	
Collema fuscovirens (With.) J. R. Laundon	Rare				
Collema cristatum (L.) Wigg.					+
Diploschistes ocellatus (Vill.) Norman			+		
Endocarpon pusillum Hedw.				+	
Fulgensia bracteata (Hoffm.) Räsänen				+	
Fulgensia fulgens (Sw.) Elenkin		+		+	
Fulgensia fulgida (Nyl.) Szatala			+		+
Psora decipiens (Ehrh.) Hoffm.	+	+	+	+	
Psora saviczii (Tomin) Follm. & Crespo				+	
Squamarina cartilaginea (With.) P. James in Hawksw.		+			
Squamarina lentigera (Weber) Poelt		+			+
Toninia sedifolia (Scop.) Timdal	+	+	+	+	+

F. fulgida and lack *F. fulgens*, were described from Spain by Llimona (1982), who assigned them to the *Toninion caeruleonigricantis* Hadac 1948. The most characteristic species of some described *Toninio-Psoretum decipientis*-related lichen sociological units and the presently studied community are listed in Table 3. Since the inventory of the investigated stand seems to correspond sufficiently with other reports of the *Toninio-Psoretum decipientis* (one character species is replaced by a congeneric species), the investigated community is considered as belonging to the *Toninio-Psoretum decipientis* in a wider sense. It may be possible to differentiate between different subgroups within this association as soon as an improved data background has been achieved.

Mycobionts

Within the population examined, all mycobiont sequences were identical, lacking any variation. Compared with two samples of *F. fulgida* from Greece (GenBank acc. nos AF278776, AF 278777) and two samples of the same species from Italy (GenBank acc. nos AF278774, AF 278775), which were genetically identical to each other, the obtained sequences of the population's mycobionts (GenBank acc. nos AY051357, AY051358, AY051359) showed only the following minor differences: one transition in the ITS1-region (C/T change), an additional nucleotide (A) in the ITS2-region, one transition in the 5′ part of the LSU (C/T change) and one transition in a spliceosomal intron of the LSU (C/T change). All sequences contained a spliceosomal intron starting at position 808 in the LSU with respect to the sequence of *Saccharomyces cerevisiae* (GenBank acc. no. J01355) (Kasalicky *et al.*, 2000).

Photobionts

All photobiont clones from the *Fulgensia fulgida* thalli were morphologically identical. The cells possessed a finely lobed chloroplast ('crenulate', as defined by Gärtner, 1985) and were often arranged in packages of autospores (mainly tetrads). This feature is characteristic of *T. gigantea* (Hildreth & Ahmadjian) Gärtner (1985) in the sense of Friedl (1989b). Very few cells, however, had cell wall thickenings at one side of the cell, a feature characteristic of *Trebouxia asymmetrica* Friedl & Gärtner, 1998, which is closely related to *T. gigantea* (Friedl, 1989b). To test the relationships of the *F. fulgida* photobiont the ITS nrDNA was sequenced.

The sequence analyses demonstrated that all the studied photobiont clones did indeed belong to *Trebouxia asymmetrica* and that they were genetically closely related (Fig. 1). The photobiontal isolates of the *Fulgensia* thalli examined appeared to be genetically homogenous (Type a sequences in Table 1) except the material derived from one particular thallus (Type b sequences in Table 1). Within that thallus the obtained photobiont strains were homogenous as well. The photobiont strains from four thalli (M-99.021B2, M-99.022B4, M-99.024B3 and M-99.025A5) were identical in their ITS and 5.8 S nrDNA sequence. The five photobiont strains analysed from the fifth thallus (M-99.023A3, B2, B4, C2 and C3) all differed from the strains of the other thalli in only one position, a C/T transition at the base corresponding to position 475 in the sequence of *Trebouxia arboricola* (SAG 219–1a, GenBank acc. no. Z68705). All these ITS-sequences of photobiont clones from *Fulgensia fulgida* were identical to the sequence of the type strain of *Trebouxia asymmetrica* (GenBank acc. no. AJ249565) except for three transitions and two deletions (transition C/T at positions 67 and 551, transition A/G at position 496, deletion of a T at positions 158 and 271/272; numbers are given with respect to the sequence of *T. arboricola* SAG 219–1a). The genetic differences between all other available *Trebouxia* ITS-sequences are much larger. None of the photobiont-strains from *Fulgensia fulgida* had a Group I-intron in position 1512 of the SSU nrDNA (with respect to the *Escherichia coli* rDNA), which

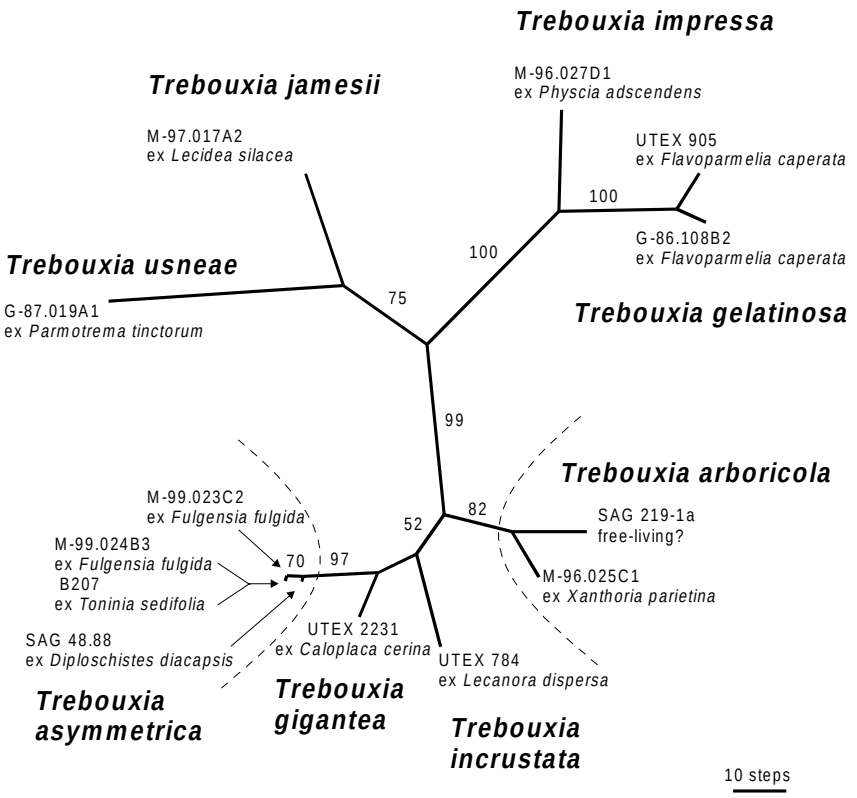


Fig. 1 Unrooted phylogeny of *Trebouxia* ITS nrDNA sequences. One tree has been obtained using the maximum parsimony method and a branch and bound search. Bootstrap values were independently calculated for 500 replicates using unweighted maximum parsimony methods. The scale indicates the distance due to 10 evolutionary steps.

has often been found in other *Trebouxia* strains, being genetically identical in this respect too.

The maximum parsimony analyses of *Trebouxia* strains resulted in a single, most parsimonious tree with a consistency index (CI) of 0.7655 (Fig. 1). The investigated photobiont clones from *Fulgensia fulgida* clearly belong to *Trebouxia asymmetrica*, because their monophyly with *T. asymmetrica* is well supported in bootstrap tests (97%). Therefore it is evident, that the ability to develop cell wall thickenings at one side of the cell is an important phylogenetic trait characteristic of this species, even if only a few cells exhibit this trait. The relationship of this species to other, morphologically quite similar, species mentioned earlier (namely *T. gigantea* and *T. incrustata* Ahmadjian ex Gärtner, 1985) was not well resolved (bootstrap values around or below 50%). It will be interesting to test whether the inclusion of further sequences from strains of these species will clarify or blur the boundaries within this species complex. The clones of *T. arboricola* formed a monophyletic clade, but with only 82% bootstrap support.

Apart from *Fulgensia fulgida*, the photobiont inventory of some co-occurring lichen species, such as those of *Toninia sedifolia* and *Squamarina lentigera*, was investigated as well. Within the community *Toninia sedifolia* was poorly represented, whereas *S. lentigera* had a rather high percentage of coverage. The ITS and 5.8 S nrDNA sequence of the photobiont of *Toninia sedifolia* proved to be identical to the sequence of those *Trebouxia asymmetrica* strains from the four *Fulgensia* thalli examined (Type a sequences in Table 1). Thus the early record of *T. potteri* for *Toninia caeruleonigricans* (Lightf.) Th.Fr. [= *T. sedifolia*] by Reháková (1968) could not be verified. The photobiont clones M-99.109, obtained from the thallus of *S. lentigera*, all belonged to the genus *Asterochloris*. They corresponded to *Asterochloris irregularis* (Hildreth & Ahmadjian) Friedl ined. because of the elliptical cells and the broadly lobed chloroplast. Further species occurring at the examined stand were *Collema cristatum* (L.) Wigg. and *Catapyrenium michelii* (A. Massal.) R. Sant. The algal partners of these were checked in squash preparations of the thallus and resulted to be *Nostoc* Vaucher and *Myrmecia* Printz, respectively. Additionally, traces of indeterminable sterile crustose lichens were present but not considered in this analysis. The dominant moss was identified as belonging to the genus *Grimmia* Hedw.

Discussion

This investigation demonstrates that the *Fulgensia fulgida*-population is highly selective in its photobiont choice, being associated with only one photobiont species, namely *T. asymmetrica*. As this photobiont is associated with other lichen mycobionts as well (e.g. *Toninia sedifolia*), the fungal–algal association is not a specific one at morpho-species level. *T. asymmetrica* is also known from very distantly related lichens as *Diploschistes* Norman (Graphidales,

Thelotremataceae; Friedl & Gärtner, 1988). However it might be possible that different strains within a morpho-species are confined to certain lichen groups. The genetic investigations showed that two different strains of *T. asymmetrica*, which were almost identical in their ITS-sequence, are acceptable algal partners for *F. fulgida* at this stand. Only one of these strains has been shown to form lichens with other mycobionts, namely *Toninia sedifolia*. If the other strain should not be found in other lichens, this association would be a specific one.

Alternatively the occurrence of only one *Trebouxia* species within this *F. fulgida*-population could be explained by the absence of other *Trebouxia* species in this stand rather than to the high photobiont selectivity of the mycobiont. We regard this to be unlikely as there is a generally high input of vegetative diaspores of lichens (Ahmadjian, 1988), most likely containing several species of *Trebouxia*. As the investigated stand was not protected from this input these diaspores should have been accessible for a possible relichenization. Moreover preliminary results suggest that *Fulgensia* is associated with *T. asymmetrica* in Italy as well (A. Beck, unpubl.).

Contrary to the photobionts, the mycobionts have been found to be genetically homogenous. The fact that genetic differences exist between mycobionts of *Fulgensia fulgida* from different localities, for example France and Italy (Kasalicky *et al.*, 2000), gives significance to the observed homogeneity within the population. Mycobiontal genetic variation within lichen populations has been observed already, for example by Zoller *et al.* (1999), who showed that the ITS-region – and specifically the ITS1-region – revealed genetic variation within and among different populations of *Lobaria pulmonaria* (L.) Hoffm. mycobionts. The fact that only one ITS-sequence has been found in apothecia of one and the same thallus could be explained by homothallism, which means a fertilisation of trichogynes with pycnospores (which are bacilliform and could serve as spermatia) from the same thallus or cytogamy. Pycnospores have already been interpreted as spermatia, for example by Poelt (1986), amongst others referring to observations made by Honegger (1984) on fusions of pycnospores and trichogynes in *Cladonia furcata* (Hudson) Schrader. Pycnidia and apothecia were observed on one and the same thallus.

The finding of the uniformity of ITS-sequences within the apothecia of the mycobionts and heterogeneity of the photobionts suggests that the lichen thalli of this stand derive from propagation by ascospore dispersal (which had identical ITS-sequences) and multiple independent relichenization events rather than by thallus fragmentation. Alternatively, independent relichenization events may have occurred already before the establishment of this population and lichenized propagules have played the major role as secondary dispersal units, despite the presence of apothecia. However, nothing is indicative of such a scenario, as *F. fulgida* appears to be a purely sexual species, showing sterile thalli only at the juvenile stage and never forming asexual, lichenized propagules. In other *Fulgensia* species, a special type of vegetative diaspore has been recognized and described by Poelt (1965). These flake-like structures have been called schizidia. They are parallel aligned to the thallus and develop from the upper thalline layers, consisting of the upper cortex and parts of the photobiontal and medullar layers, lacking a lower cortex. These fragile structures can easily split off the thallus and are therefore assumed to serve as lichenized dispersal units. Within the genus *Fulgensia*, they are known from *F. bracteata* ssp. *deformis* (Erichsen) Poelt, *F. fulgens*, *F. delphinensis* Poelt, *F. desertorum* (Tomin) Poelt, *F. poeltii* Llimona and *F. subbracteata* (Nyl.) Poelt (Poelt, 1965; Gilbert, 1978; Westberg & Kärnefelt, 1998), and are assumed to be of taxonomic relevance, for example by Poelt (1973). *F. fulgida* has no schizidia and the strictly monophyllous and placodioid thalli also lack other structures, which might facilitate thallus fragmentation. Its compact thallus is firmly fixed to the substrate. For these reasons, dispersal by ascospores seems the most likely distribution mechanism of this species.

The examination of this lichen community from southern France also suggests that *Toninia sedifolia* and *Fulgensia fulgida* share the same photobiont pool, a finding that might support the observations of Ott *et al.* (1995) on *Fulgensia bracteata*, which is supposed to take over the photobionts of *Toninia sedifolia* (= *T. caeruleonigricans*). However it has not yet been shown that *Toninia sedifolia* is really a potential photobiont source for germinating or aposymbiotic hyphae of *Fulgensia*. *Toninia sedifolia* at least seems to be a suitable substrate providing improved conditions for the thallus establishment of *Fulgensia*, and the results from this study demonstrate that the two lichens have photobionts with identical ITS-sequences. Co-existence of the two lichens in the same habitat and with a narrow ecological amplitude of the photobiont may have favoured photobiont-sharing between *Toninia* and *Fulgensia*. It can be estimated that additional lichen species participate in this pool, without being involved in close interactions. Cyanolichens, as *Collema cristatum* in the investigated stand, often occur together with *F. fulgida* and may serve as a substrate during the initial growth of the phycolichen by representing a suitable environment with improved water and nitrogen supply.

Perspectives of studies on myco-photobiont relationships in lichenized systems

For characterising symbiotic interactions Galun & Bubrik (1984) distinguished between the terms 'selectivity', meaning 'preferential interaction between organisms', and 'specificity', meaning 'cell–cell interactions with absolute exclusivity'. Smith & Douglas (1987) referred to specificity as the degree of taxonomic difference between partners with which an organism associates. Other authors treat the two terms as synonyms. The terminology describing preferential partner selection in symbiotic systems is therefore in need of

Table 4 Levels of selectivity in symbioses (with minor alterations from Smith & Douglas, 1987)

Levels of selectivity	Ranges of acceptable partners
Very high	Within the same strain
High	Within the same species
Moderate	Within the same genus
Low	Within the same order
Very low	Within groupings of higher taxonomic levels

clarification. In lichenological literature, 'selectivity' has hitherto been attributed only to the mycobiont of a lichenized system but has never been applied to the photobiontal partner. However, in a classification that has to cover the whole range of possible kinds of reciprocal interactions, it seems appropriate that this term should be addressed to each biont of a symbiotic system. We suggest using the term 'selectivity' for the characterisation of interactions between organisms viewed from the perspective of one biont only. Thus it characterises the range of possible partners that can be selected by this biont – its degree of selectivity. 'Specificity' should be used for the symbiotic association as a whole, therefore depending on the degree of selectivity of the partaking bionts. A suitable classification of selectivity and specificity may be best elaborated by considering the whole range of theoretically possible constellations. Five levels of selectivity can be distinguished, covering the whole range from very high – association with members of the same strain only – to very low – association with members of different orders or even classes (Table 4, Fig. 2). It is evident from this definition that the correct systematic placement of the bionts is a prerequisite for determining the degree of selectivity.

If a mycobiont forms a lichen with photobionts of different families, it exhibits a low degree of selectivity. An example of this kind of interaction is *Chaenotheca chrysocephala* (Turner ex Ach.) Th.Fr., which can form lichen thalli with either *Trebouxia* (ord.: Trebouxiales, class: Trebouxiophyceae) or *Stichococcus* (ord.: inc. sed., class: Trebouxiophyceae) (Tibell, 1980). As other mycobionts can form lichens with the same photobionts, the association is not exclusive. A mycobiont that is found lichenized with only one photobiont species is considered to exhibit a high selectivity. *Phlyctis argena* (Sprengel) Flotow is an example of this type of association, where the mycobiont was found to be always associated with *Dictyochloropsis splendida* Geitler (Tschermak-Woess, 1995). The corresponding photobiont establishes associations also with other mycobionts, for instance *Chaenotheca brunneola* (Ach.) Müll.Arg. (Tschermak-Woess, 1978), so the interaction as a whole is therefore an unspecific one.

If both (or all) partaking organisms exhibit a high degree of selectivity, the association is specific (Table 5). Actually it is impossible to give an example of a truly specific association, because the identity of the photobiont at the species level is

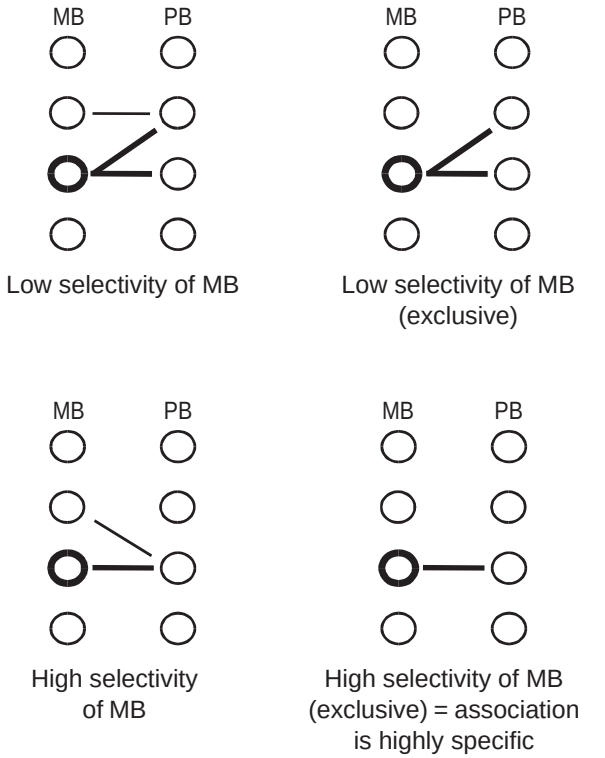
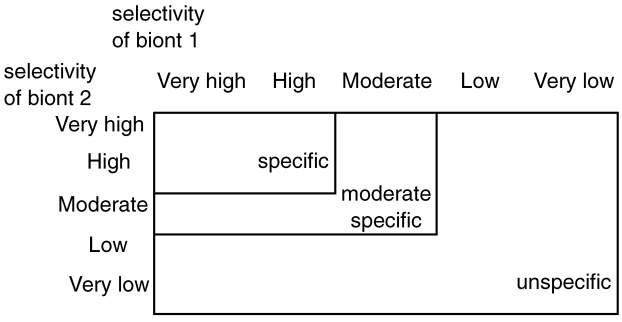


Fig. 2 Schematic illustration of the definition of selectivity, exemplified for lichen mycobionts.

Table 5 Definition of the different degrees of specificity in binary symbiotic associations



only known for about 3% of all described lichens (Honegger, 1996). For this reason it is premature to assign an exclusive selectivity to a photobiont. However, judging by the available data, most photobiont species seem to have a relatively low degree of selectivity, for instance *Trebouxia jamesii*, which is associated with several Lecanoralean fungi (Beck, 1999).

Tripartite associations, as present in cephalodiate lichens, have to be resolved in three binary ones and the pairs have to be estimated individually.

The study of the kind of selectivity in lichen associations will be an important task for the near future to clarify whether a high degree of mycobiontal and/or photobiontal selectivity can be found in certain lichen groups, which would be indicative that coevolutionary processes may have taken place

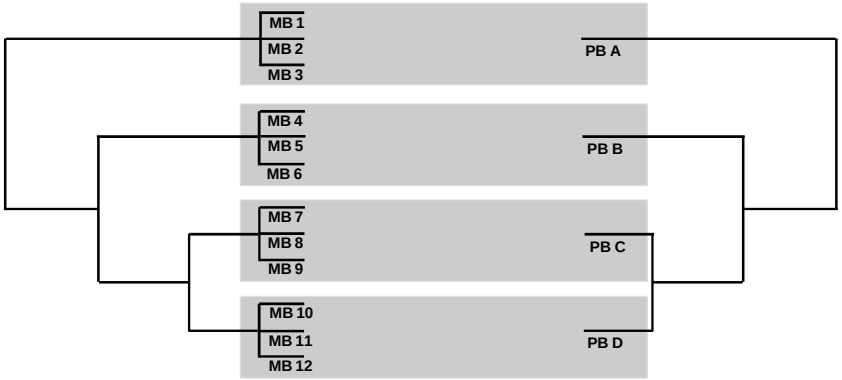


Fig. 3 Scheme for supposed coevolution between myco-(MB) and photobionts (PB) in a lichen associations leading to cospeciation.

there. These studies are also necessary to test whether the photobionts are potential indicators of phylogenetic relationships of the mycobionts (Rambold *et al.*, 1998).

Investigations of the selectivity of single mycobiont species from different localities and their algal counterparts can focus on various aspects: focusing on individual lichen thalli; on whole lichen populations; and on the phylogenetic relationships (of the mycobionts). The investigation of a single mycobiont species may be of interest and may be carried out at a local or global scale. On a local scale, it may reveal polymorphism in the mycobiontal populations. However, to test for the degree of selectivity of a mycobiont, the associated photobionts have to be investigated at a global scale, preferentially from the most diverse habitats. Based on morphology, studies of this kind have first been performed by Tschermak-Woess on *Phlyctis argena* by Tschermak-Woess (1995). Currently various working groups focus on such questions by applying molecular techniques. Recently Kroken & Taylor (2000) demonstrated selectivity of photobiont choice in the genus *Letharia*, always being associated with algae from the *Trebouxia jamesii* species complex. Analysing both, the myco- and photobionts of each thallus by molecular techniques they conclude, that cospeciation is not a common rule, but that there is evidence for selectivity of a given fungal species for an algal species. More such investigations will certainly provide better insights on the degree of photobiont-referring selectivity of lichen mycobionts.

Information on the environmental influence of selectivity is provided by studies of whole lichen communities. Different lichen species may share the photobionts when growing side by side, an observation that can only be addressed when all thalli of a lichen community are analysed (Beck, 1999). Studies of this kind are very important in order to make estimations on the relichenization events taking place in saturated communities, where established lichen thalli may act as a photobiont source for arriving mycobiontal spore material.

Population studies like the present one, combined with mycobiontal spore germination capability tests, will allow the investigation of the modes of relichenization in more detail. Furthermore, experiments on lichen resynthesis with the bionts of the original association and lichen resynthesis with one mycobiontal strain and with different algal strains and vice versa will provide further knowledge on the potential range of compatible partners.

The only way of proving specificity of the partner selection is to investigate different lichen species and to determine whether the partners are exclusively associated with each other (even when occurring in different lichen communities). This means there is a huge number of lichens to be investigated, which means that only a very limited number of thalli per species can be investigated. Possible results of such investigations include the verification of perfect coevolution – leading to cospeciation. Should such cospeciation have taken place, it could be visualized by congruent underlying myco- and photobiontal phylogenies (Fig. 3).

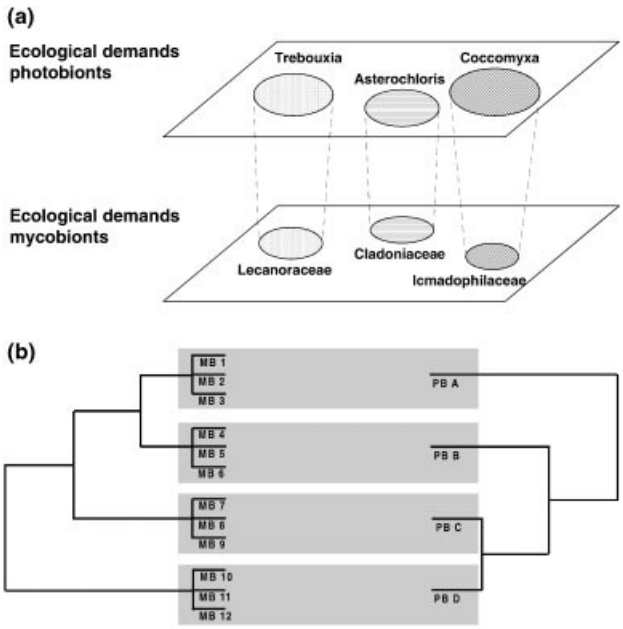


Fig. 4 (a) Partner selection in lichen symbioses could be due to similar ecological preferences of the corresponding bionts. Thus the phylogenies of the underlying myco- and photobionts do not need to be congruent (b).

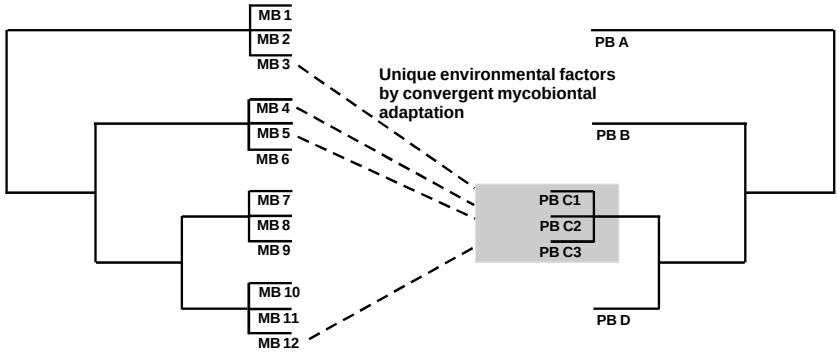


Fig. 5 Partner selection in lichen symbioses might be due to similar environmental factors for the photobiont provided by the mycobionts because of convergent mycobiontal evolution.

Up until now not enough data are available to estimate whether such scenarios exist for taxa associated in lichenized systems at all. In the case of trebouxiophyte photobiont *Coccomyxa* (Friedl, 1998) being found in families of various relationships, as in the Icmadophilaceae, Peltigeraceae, Clavariaceae (*Multiclavula*) and Tricholomataceae (*Omphalina*), it is evident that cospeciation did not occur as these unrelated mycobionts are associated with members of the same photobiont genus.

Existing patterns of selectivity in lichens may also be due to environmental factors. It seems possible that certain ancestral, as well as recent, myco-photobiontal-constellations were due to the similar ecological preferences of both bionts (Fig. 4a). In this case, the underlying phylogeny of the myco- and photobionts would not need to be congruent (Fig. 4b). It is an important task for upcoming studies to investigate such preferences in detail.

As phylogenetically related species might share similar environmental demands, the resulting pattern of photobiont distribution would look similar to one caused by perfect coevolution (but lack congruence of the underlying phylogenies).

As many lichen photobionts are found mainly within lichen thalli (e.g. *Trebouxia*, the most common lichen photobiont), mycobiontal factors are an important part of their environment. Therefore even distantly related taxa may share the same photobiont taxon through providing a suitable environment for these algae (Fig. 5). Perfect cospeciation between the partaking bionts would not be a precondition for such patterns, only a general ability to associate by lichenization. Recent results from investigations of the *Acarosporetum sinopicae* Hil. 1924 seem to support this hypothesis (Beck, 1999).

Acknowledgements

We thank C. Stadtkus (Botanische Staatssammlung München), for help with the sequencing work, and Gert Helms (University of Göttingen) for making available the sequence of an unpublished algal-specific PCR-primer. T.K. acknowledges Heidi Döring (Natural History Museum London, now University of Bayreuth) and Mats Wedin (Natural History Museum London, now University Umeå) for advice during molecular analysis of the *Fulgensia* mycobionts. We extend our thanks to an anonymous referee for his suggestions on an earlier version of this manuscript, A. Davies for improving the English text and to F. Schuhwerk for his advice in lichen sociological questions.

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