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Genetic variation within and among populations of the threatened lichen *Lobaria pulmonaria* in Switzerland and implications for its conservation

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Abstract

The foliose epiphytic lichen *Lobaria pulmonaria* has suffered a significant decline in European lowlands during the last decades and therefore is considered as endangered throughout Europe. An assessment of the genetic variability is necessary to formulate biologically sound conservation recommendations for this species. We investigated the genetic diversity of the fungal symbiont of *L. pulmonaria* using 143 specimens sampled from six populations (two small, one medium, three large) in the lowland, the Jura Mountains, the pre-Alps and the Alps of Switzerland. Among all nuclear and mitochondrial regions sequenced for this study, variability was found only in the internal transcribed spacer (ITS I), with three polymorphic sites, and in the nuclear ribosomal large subunit (nrLSU), with four polymorphic sites. The variable sites in the nrLSU are all located within a putative spliceosomal intron. We sequenced these two regions for 81 specimens and detected six genotypes. Two genotypes were common, two were found only in the more diverse populations and two were found only in one population each. There was no correlation between population size and genetic diversity. The highest genetic diversity was found in populations where the fungal symbiont is reproducing sexually. Populations with low genetic diversity included only the two same common genotypes. Our study provides evidence suggesting that *L. pulmonaria* is self-incompatible and heterothallic. Based on our results we give populations with sexually reproducing individuals a higher rank in terms of conservation priority than strictly asexual populations. The remaining lowland populations are so small, that one single catastrophic event such as a windthrow might destroy the entire population. Hence we suggest augmenting such populations in size and genetic diversity using small thallus fragments or vegetative diaspores collected in other populations. As we did not detect any locally adapted genotypes, these transplants can be taken from any other genetically diverse population in Switzerland.

Keywords: conservation, genetic variation, lichens, nuclear and mitochondrial DNA sequences, sexual reproduction

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Introduction

The assessment of genetic variation and its conservation is widely used in preserving threatened organisms particularly in species with small and isolated populations (Daniels *et al*. 1996; James & Ashburner 1997). This procedure is recognized as an important conservation measure by many authors (Falk & Holsinger 1991; Loeschcke *et al*. 1994; Petit *et al*. 1998) although some of the existing empirical data are conflicting (see Ellstrand & Elam 1993). In general, a higher genetic variation is assumed to improve the fitness of a species and enhance the probability of

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population survival (Mitton & Grant 1984; Wildt *et al*. 1987; Hedrick & Miller 1992).

The epiphytic lichen *Lobaria pulmonaria* (L.) Hoffm. is distributed over parts of Europe, Asia, North America and Africa (Yoshimura 1971). In Switzerland it grows mainly on deciduous trees such as beech (*Fagus sylvatica*) and sycamore maple (*Acer pseudoplatanus*). Vegetative diaspores (isidioid soredia) include both symbionts and are formed on the upper cortex of the thallus. Occasionally, apothecia, the fruiting bodies of the fungal symbiont (producing ascospores), are formed along the ridges and margins of the thallus. The photobiont is a green algae and rarely a third symbiont, a cyanobacteria is found in cephallodia on the upper cortex. The thallus is loosely attached on the substratum and spreads widely, often exceeding 20 cm in diameter. This species, similar to many other lichens, is sensitive to acidic air pollution (Sigal & Johnston 1986) and ozone (Scheidegger & Schroeter 1995). Growth experiments using soredia, thallus fragments (Scheidegger 1995; Scheidegger *et al*. 1995) and ascospores (B. Frey, personal communication) show that *L. pulmonaria* is a species with generation cycles of up to 25 years. Furthermore, Scheidegger (1995) has shown that *L. pulmonaria* is limited in its capability to establish new thalli on neighbouring trees.

The foliose lichen *L. pulmonaria* has suffered a considerable decline in central and northern Europe since the last century (Hallingbäck & Martinsson 1987) and is considered to be critically endangered particularly in the lowland regions (Türk & Wittmann 1986; Clerc *et al*. 1992; Wirth *et al*. 1996). The sharp decline in number and size of populations in lower elevations of Switzerland is most probably due to habitat destruction, changes in forest management (such as extensive plantation of conifers) and air pollution, coupled with poor dispersal mechanisms and long generation time of this species. The remaining small lowland populations consist of only a few thalli and are widely separated from each other and from the larger populations in the Alps and the Jura mountains. Only two lowland populations are known to have more than five thalli. Such small populations are presumed to be more sensitive to stochastic environmental changes (Shaffer 1981; Lande 1988) and more vulnerable to extinction due to loss of genetic variation (Huenneke 1991; Lynch *et al*. 1995).

Studies of variation within lichen species have been conducted using enzyme polymorphism (Fahselt 1986; Hageman & Fahselt 1990; Nevo *et al*. 1997) and restriction-site patterns of the nuclear ribosomal small subunit RNA gene (nrSSU) resulting from the presence/absence of group-I introns in the fungal component of the lichen genus *Cladonia* (DePriest & Been 1992; DePriest 1993; Beard & DePriest 1996). In these studies, considerable within-population variation was detected. In the *Cladonia chlorophaea* complex, which probably includes several taxa, 13 genotypes were found within one mat (DePriest 1994). On the other hand, in *Cladina subtenuis* no variation in the nrSSU was found within populations but some was detected among populations (Beard & DePriest 1996).

In our study we investigated the genetic diversity of the fungal symbiont of *L. pulmonaria* in 81 samples from six populations in the lowland, the Jura mountains, the northern pre-Alps and the Alps in Switzerland. We present, for the first time, an extensive study on the genetic variation of a lichen species using DNA sequences. We investigated several mitochondrial and nuclear genes for variability using PCR and automated sequencing. We examined the influence of population size, history, isolation and the mode of reproduction (sexual or asexual) on the genetic variability of the populations. These results provide information essential for biologically sound recommendations for appropriate conservation measures and management priorities.

Materials and methods

Lichen material and preparation

A total of 143 *Lobaria pulmonaria* thallus fragments were collected from six locations in Switzerland north of the Alps (Fig. 1). In the two small (less than 50 thalli) and isolated lowland populations of Vordemwald (coordinates 634.0/237.4) and Forst Bern (coordinates 589.1/196.7), we collected 25 fragments out of approximately 40 known thalli and six fragments out of six known thalli, respectively. In the two large populations of Muotatal (coordinates 705.8/204.5) and Lauenen (coordinates 591.9/137.8) in the eastern and western Alps, 30 fragments out of 600 known thalli and 32 fragments out of 400 known thalli were collected, respectively. In Marchairuz (coordinates 509.0/156.2), a large population in the Jura mountains of 600 known thalli, and in Toggenburg (coordinates 737.5/227.2), a medium population in the eastern pre-Alps of around 150 known thalli, 35 and 15 fragments were collected, respectively. The fragments were about 5–10 cm^2 in size. In general we collected one sample per tree, but for two trees in Vordemwald four thalli were sampled per tree. Trees were chosen in order to cover the total distribution of the population. No preferences were made concerning tree species, size or exposure. To avoid any damage to populations, the thallus size had to be larger than at least twice the size of the collected fragment. To get haploid fungal material only, fragments were taken from vegetative parts of the thallus where no apothecia were visible. Fragments were stored air dried and prepared within a few days in the laboratory. For each thallus that was sampled we documented the presence or absence of apothecia and the type of vegetative diaspore (soredia, isidioid soredia).

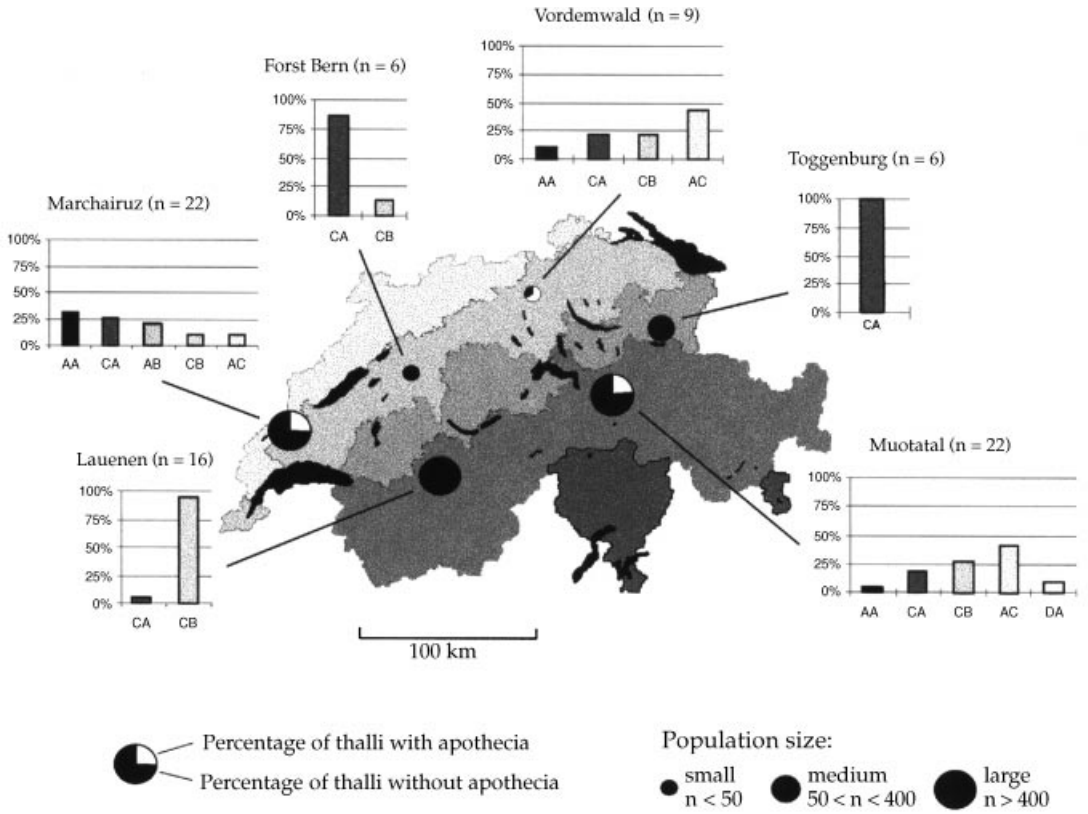

Fig. 1 Geographic location and genotype frequencies of the six populations investigated. Six genotypes were found: AA, AB, AC, CA, CB and DA. The five regions on the map are (from top left to bottom right, in light grey to dark grey): Jura mountains, lowland, northern pre-Alps, Alps, southern pre-Alps. Lakes in black. n, number of thallus fragments sequenced.

DNA amplification and sequencing

The upper cortex, photobiont layer and lower cortex of the lichen thallus were removed manually from the fungal medullary layer using tweezers and a scalpel. The fungal material was cleaned and any abnormality (in colour or structure) was excised. From these purely fungal samples (10–20 mg in dry weight) DNA was extracted using a column method described in Zoller *et al*. (1999).

PCR was performed in a 50-μL reaction volume (34.5 μL of H_2O, 5 μL of 10× PCR buffer, 2 μL of dNTPs [1 mM], 2 μL of $MgCl_2$ [50 mM], 2 μL of each primer [10 μM], 2.5 U *Taq*, 2 μL of DNA extract) in a thermal cycler (PTC-100, MJ Research) using a standard cycling protocol: denaturation at 94 °C for 3 min; 30 cycles of 94 °C for 1 min, annealing temperature (see Table 1) for 1 min, extension at 72 °C for 1 min; final extension of 7 min. We tested several mitochondrial and nuclear genes for variability (see Table 1 and Fig. 2).

Sequencing of both strands of each PCR fragment was performed using an automated sequencer (Perkin-Elmer ABI 377). Sequence data were assembled and aligned using Sequencher 3.0 (Gene Codes Corporation 1995). The alignments were optimized by eye.

From the 143 collected fragments, 81 were sequenced (Fig. 1). To decide when we had sequenced enough samples from a specific population, we used the following procedure. Sequences from the population were randomly sampled with replacement. The sample size ranged from one thallus to 22 thalli. For each sample size the number of genotypes per number of samples was counted. By doing this several times, we obtained a mean number of genotypes for each sample size and were able to compute the most probable genotype saturation curve for each population. We stopped sequencing more samples from a population when its curve reached the plateau level as shown in Fig. 3. Sequences of *L. pulmonaria* nrDNA obtained during our study are deposited in GenBank.

Statistical analyses

Genetic variability among and within populations were estimated using the K_{ST} statistic developed by Hudson *et al*. (1992) which is similar to the statistics y_{ST} (Nei 1982) and N_{ST} (Lynch & Crease 1990). Statistical significance of

Table 1 Nuclear and mitochondrial genes as well as noncoding regions tested to detect genetic variability in the lichen species *Lobaria pulmonaria* in Switzerland. See also Fig. 2

Gene/region	Polymorphic sites	Annealing temp. (°C)	Symetric PCR amplification	Sequencing primers	References
nr LSU	4	50	LR3R–LR5, LR0R–LR7, LR17R–LR10, LR17R–LR12	LR0R, LR3R, LR10R LR17R, LR3, LR5, LR7 LR12, LR16, LR21	Vilgalys & Hester (1990); Moncalvo *et al*. (1993); Rehner & Samuels (1994); Vilgalys & Sun (1994)
nr IGS	No consistent amplification	52	LR13R–NS1R, LR12R–NS1R		Vilgalys & Sun (1994)
nr SSU	1	50	NS17–NS24,	NS2, NS3, NS4, NS17, NS22, NS24, SR7R, SR11R	White *et al*. (1990); Gargas & Taylor (1992); DePriest 1994
ITS	3	50	ITS1–ITS4	ITS1, ITS4	White *et al*. (1990)
mtr LSU	None	58	ML1–ML6, ML1–ML4	ML1, ML3, ML4, ML6	White *et al*. (1990)
beta-tubulin	None	58	Bt1a–Bt1b	Bt1a, Bt1b	Glass & Donaldson (1995)
histone 3	None	58	H3–1a–H3–1b	H3–1a, H3–1b	Glass & Donaldson (1995)
mtr SSU	None	52	mSSU1–mSSU3R	mSSU1, mSSU2 mSSU2R, mSSU3R	Zoller *et al*. (1999)

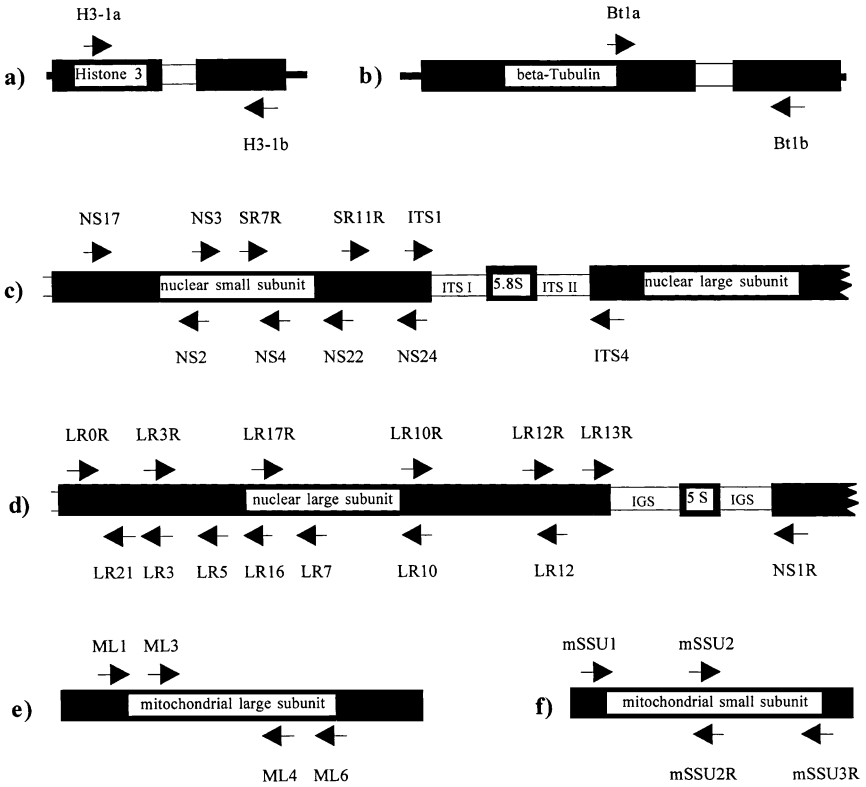

Fig. 2 Noncoding and coding DNA regions we sequenced to detect inter- and intrapopulation genetic variability in the lichen-forming fungal species *Lobaria pulmonaria*. See also Table 1. (a) Histone 3 (five specimens sequenced); (b) beta-Tubulin (five specimens sequenced); (c) nuclear small subunit (six specimens sequenced) and ITS (81 specimens sequenced); (d) nuclear large subunit (81 specimens sequenced) and IGS (seven specimens sequenced); (e) mitochondrial large subunit (six specimens sequenced); (f) mitochondrial small subunit (six specimens sequenced). The test specimens were collected in different populations. Dark boxes denote exons and the open, smaller boxes denote noncoding regions (ITS I, ITS II and IGS). Arrows denote the positions of the primers.

the values were estimated using a permutation test suggested by Hudson *et al*. (1992) and implemented using an in-house program written by S. Zoller. Correlations were tested using the software package DataDesk 5.0 (Data Description Inc.). Sexual and asexual populations were compared using the nonparametric Mann–Whitney rank test.

A phenogram was constructed from the K_{ST} matrix using the program FITCH in PHYLIP version 3.57 (Felsenstein 1995).

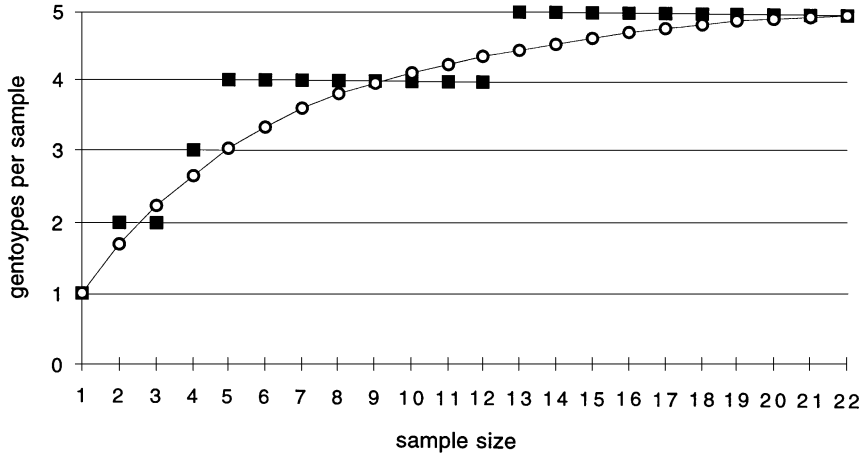

Fig. 3 Calculated saturation curve (open circles) and observed occurrence of new genotypes in the sequencing process (black squares) for the Marchairuz population. See text for explanations.

Table 2 Polymorphic sites in the nuclear ribosomal ITS I and LSU for 81 specimens of *Lobaria pulmonaria* distributed in six populations. ITS I positions relative to ITS I sequence of *Ramalina fastigiata* (GenBank Accession no. U84582). LSU positions relative to the beginning of the insertion which is exclusive to *Lobaria* (see Fig. 4). Gaps are represented by —

	Nucleotide position						
	ITS I			LSU			
Genotype	37	Indel at position 128		19	33	48	55
AA	C	T	G	C	C	T	T
AB	C	T	G	T	T	—	—
AC	C	T	G	C	C	—	—
CA	T	—	—	C	C	T	T
CB	T	—	—	T	T	—	—
DA	T	T	G	C	C	T	T

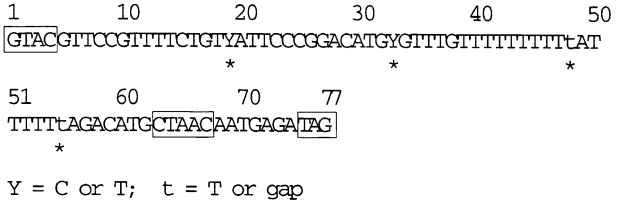

Fig. 4 Sequence of the nrLSU small insertion which is exclusive to *Lobaria*. Four variable nucleotide positions including two indels have been found within this insertion. Relative to *Saccharomyces cerevisiae* nrLSU sequence (GenBank Accession no. J01355) this insertion is located after position 921. Sequences in open rectangles are typical of spliceosomal introns (see text).

Results

Variability appropriate for our study was found in the ITS I and the nrLSU (Table 1, Fig. 2). All other nuclear and mitochondrial DNA regions that we tested (Fig. 2) did not show any variability with the exception of the nrSSU with one polymorphic site. DNA amplification of the ITS I resulted in a fragment of 550 bp. Three variable nucleotide positions were found within the ITS I: one polymorphic site at position 37 and two contiguous gaps representing one indel at position 128 (Table 2). Amplification and sequencing of the nrLSU with LR3R and LR5 revealed a 400 bp fragment with four variable nucleotide positions including two indels. Comparisons with nrLSU sequences of several other lichen species (data not shown) and a BLAST search show that these variable positions are located in a small insertion 75–77 bp long that is exclusive to the genus *Lobaria*. The location of this insertion relative to the nrLSU sequence of *Saccharomyces cerevisiae* (GenBank Accession no. J01355) is after position 921. The sequence of the insertion and the locations of the variable nucleotide positions are presented in Fig. 4.

Together, these seven variable positions characterize six different genotypes (Table 2). Only in one individual did one position of the sequencing chromatogram show two overlaying peaks which could be due to different copies of the multicopy nuclear ribosomal DNA. This sequence was excluded from all analyses.

Two genotypes (CB and CA) are common and appear in most populations (Fig. 1). Genotypes AA and AC are found only in the more diverse populations Marchairuz, Vordemwald and Muotatal. Genotypes AB and DA are rare and were found only in one population each (Marchairuz and Muotatal, respectively). Genotype AC is slightly more abundant in eastern populations.

In the 22 sequenced samples of the Marchairuz population we detected five genotypes. Seven samples are genotype AA, six are CA, five are AB, whereas CB and AC each appear twice. Marchairuz has the most homogeneous arrangement of genotype frequencies of all populations.

Table 3 Genetic variation among and within populations of the lichen-forming species *Lobaria pulmonaria* in Switzerland

Population	K^{log}_i	K_{ST} values V	L	FB	Tg	Mu
Vordemwald (V)	0.65	—				
Lauenen (L)	0.16	0.36*	—			
Forst Bern (FB)	0.32	0.16	0.56*	—		
Toggenburg (Tg)	0.00	0.30*	0.80*	0.00	—	
Muotatal (Mu)	0.64	0.03	0.26*	0.16	0.26*	—
Marchairuz (M)	0.62	0.01	0.36*	0.11	0.18	0.05

*Populations genetically different at $P < 0.05$ (Permutation test, following Hudson *et al*. 1992).

We consider it to be the largest of all the populations we investigated with approximately 600–800 individual thalli. The 22 sequenced samples from the Muotatal population also include five genotypes. More than 40% of the thalli sequenced are AC (nine samples) and more than 25% are CB (six samples). Genotype CA appears four times, DA twice and AA once. We estimate that this population consists of up to 600 thalli. Vordemwald consists of four genotypes. In the nine sequenced samples for this population AA appears once, CA and CB each were found twice, and AC was found four times. Vordemwald is a small population with less than 50 thalli. In the Lauenen and Forst Bern populations, we detected only two genotypes CA and CB. In Lauenen, which is a large population with approximately 400 thalli, 15 of the 16 thalli sequenced are genotype CB and one is CA. In Forst Bern, the smallest population investigated with only six thalli, CB appears once and CA five times. In the medium population of Toggenburg, with approximately 150 thalli, we found only the genotype CA (six samples). The additional thalli sampled from two trees in Vordemwald (four samples/tree) belong to the genotypes AC and CB. Each genotype was restricted to one of the two trees. The two large populations in Marchairuz and Muotatal as well as the small one in Vordemwald form a group showing high nucleotide diversity with K^{log}_i values above 0.6 (Table 3). A second group consists of the large population in Lauenen, the medium population in Toggenburg and the small population in Forst Bern. This group is characterized by low nucleotide diversity (K^{log}_i below 0.35). In populations of the first group, i.e. with high genotypic diversity, apothecia (which are sexual reproductive structures producing meiospores) are present in up to 72% of the thalli (Fig. 1). No apothecia were found in the three populations of the second group, i.e. with low genotypic diversity. Therefore, populations with some level of sexual reproduction are significantly more variable than populations without any apothecia (Fig. 5). Significant levels of genetic differentiation were also detected among Lauenen and all other populations, between Vordemwald and Toggenburg and between Muotatal and Toggenburg (Table 3). No correlation was found between geographical distances and genetic distances nor between genetic diversity and population size. All genotypes except AB were found to produce apothecia. No genotype was found to be specializing on one specific tree species and no correlation was found between the number of host tree species in a population and the level of genetic variation.

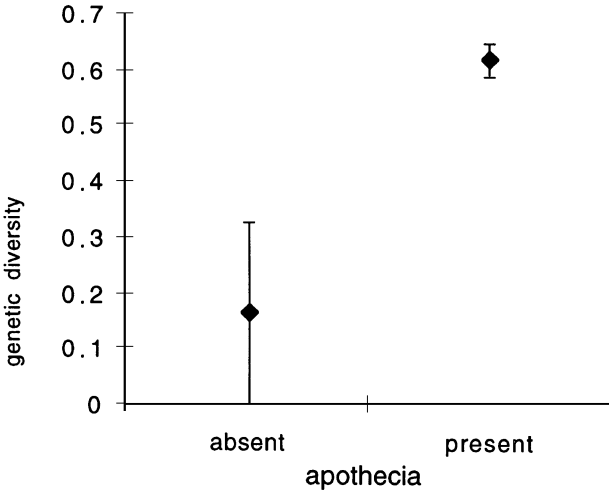

Fig. 5 Correlation between sexual reproduction and within-population diversity. Populations with apothecia (fruiting bodies of the fungal component of the lichen symbiosis producing meiospores) are significantly more diverse than strictly asexual populations (Mann–Whitney rank test, $P < 0.01$).

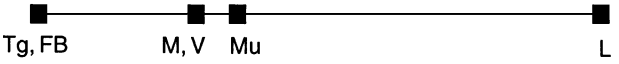

Fig. 6 Unrooted FITCH phenogram (PHYLIP version 3.57) constructed from K_{ST} estimates of genetic differentiation between six populations of the lichen species *Lobaria pulmonaria* in Switzerland. Tg, Toggenburg; FB, Forst Bern; M, Marchairuz; V, Vordemwald; Mu, Muotatal; L, Lauenen.

A FITCH phenogram constructed from the K_{ST} matrix illustrates the relatedness of populations in terms of genotypic diversity (Fig. 6). The phenogram depicts the strong similarity between populations having fruiting bodies (Vordemwald, Muotatal, Marchairuz).

Discussion

Amount and distribution of genetic variation

Genetic variation was found only in the ITS I and in the

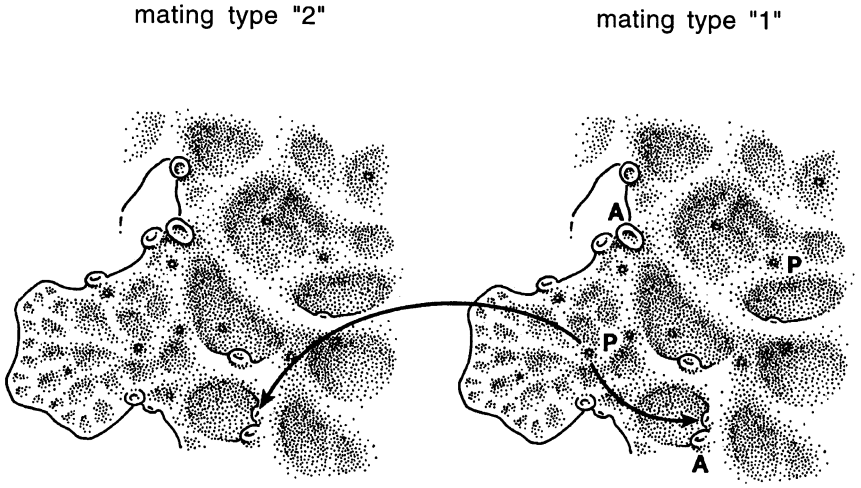

Fig. 7 Two *Lobaria pulmonaria* thalli showing pycnidia (P) which are forming conidiospores with an expected function as spermatia. Plasmogamy, leading to dikaryotic hyphae, is expected to occur in early developmental stages of apothecial primordia (A). Heterothallic species are self-incompatible as indicated by the long arrow going from mating type '1' to mating type '2'. Homothallic species are self-compatible as shown by the short arrow.

nrLSU. Remarkably, no variation was found in the mitochondrial (mtr) SSU and the mtr LSU, despite the fact that in several fungal species high levels of nucleotide substitutions and length mutations were detected in these regions (Hibbett & Donoghue 1995; Bruns *et al*. 1998; Gonzales & Labarere 1998). Each copy of the multicopy rDNA repeats is very similar to the other copies within individuals and the few differences are mostly length variations within the intergenic spacer region (Stambrook 1978; Arnheim *et al*. 1982). The most important processes leading to this phenomenon of homogenization are unequal crossing over (Smith 1976) and gene conversion (Nagylaki & Petes 1982). It is expected that this concerted evolution (Arnheim *et al*. 1980) produces a low intraspecific variation in the rRNA genes compared with single-copy genes (Hillis & Dixon 1991). Surprisingly, in *Lobaria pulmonaria* genetic variation was found only in the rRNA genes but not in the investigated single-copy genes.

By sequencing the ITS I and parts of the nrLSU from 81 *L. pulmonaria* thalli we detected six different fungal genotypes. The four variable nucleotide positions detected in the nrLSU were part of a small insertion known only from the genus *Lobaria*. This insertion shows several characteristics typical of spliceosomal introns, such as the consensus GTAC with C/TAG donor and acceptor sequences at the 5′ and 3′ termini, respectively, and a putative branch-point sequence (CTAAC) similar to that in higher plants (CTA/GAC/T, Sinibaldi & Mettler 1992) (D. Bhattacharya, personal communication). Small insertions of this type have been reported from the nrSSU in other lichen-forming fungi by Stenroos & DePriest (1998) and Grube *et al*. (1996), but were misinterpreted as degenerated group-I introns. Consequently, the splicing site was often placed at a wrong position as they were not aware of the correct splicing mechanism of spliceosomal introns. This intron seems to be stably inherited and has also been found in a specimen of *Lobaria quercizans* (J. Miadlikowska and F. Lutzoni, unpublished) and in specimens of *L. pulmonaria* from Corsica (Mediterranean Sea, France) and from the Canary Islands (Eastern Atlantic Ocean, Spain).

No correlation between population size and genetic variation was found, nor was it found between genetic distance and geographical distance between populations. The distribution of genotypes in Switzerland shows no geographical pattern except that genotype AC is slightly more abundant in eastern populations. In populations where the fungus is reproducing sexually (apothecia present), the genetic variation was much higher than in populations reproducing strictly asexually, regardless of the population size.

The genetic variation of the algal symbiont was not investigated. We focused on the fungus, the species to which the name of the lichen refers and the species we intend to preserve. Most lichen-forming fungi are ecologically obligate and only the symbiotic phenotype is found in nature (Honegger 1996). Light-microscopy studies show that many lichen species share the same algae with a low host specificity throughout a wide distribution area. Several of these algae have only very few sequence polymorphism in the nrDNA repeat (F. Lutzoni, unpublished). Therefore, a study on the genetic variability of the algae might reveal only a low level of sequence polymorphism.

Mating system

Conidiospores, which are formed in pycnidia (Fig. 7) are expected to act as spermatia. Plasmogamy between a pycnidiospore and a trichogyne leads to the development of an apothecium. Heterothallic species are self-incompatible; thus spermatia must reach a thallus of

different genotype to initiate apothecial development (Fig. 7). Homothallic species are self-compatible. Remarkably, populations with low genetic variation (Lauenen, Forst Bern, Toggenburg) involved genotypes CA and CB exclusively. These populations produce no apothecia, but in the more diverse populations genotypes CA and CB did produce apothecia. These results suggest self-incompatibility and therefore that *L. pulmonaria* might be heterothallic. If this assumption is true, we would expect the absence of apothecia in unigenotypic populations. This also means that populations with two genotypes are less likely to be compatible, and have apothecia, than populations with four and five genotypes. Such mating type systems are well known in many other ascomycetes (Esser 1992) and might exist in lichens as well.

Historical distribution and demography

L. pulmonaria is an epiphytic species that grows mainly on *Acer pseudoplatanus*, *Fagus sylvatica* and other deciduous trees in Switzerland. Therefore, its migration most probably followed the migration of these tree species during their recolonization of western Europe after the last Pleistocene glaciation period (about 8000 years ago) (see Huntley & Birks 1983; Demesure *et al*. 1996). *L. pulmonaria* is now distributed over large portions of Europe, but considered critically endangered, particularly in the lowland regions (Türk & Wittmann 1986; Clerc *et al*. 1992; Wirth *et al*. 1996). Outdoor growth experiments of isidioid soredia and thallus fragments (Scheidegger 1995) as well as ascospore growth in the laboratory (B. Frey, personal communication) show that *L. pulmonaria* has a very low growth rate in the first years after the colonization of a new phorophyte. Scheidegger *et al*. (1998) demonstrated that *L. pulmonaria* has a very limited dispersal capability and probably has a generation cycle of up to 25 years. The fact that some populations consist of one or two genotypes only confirms limited diaspore dispersal and gene flow. Despite this propagation constraint, genotypes are rather evenly distributed over Switzerland (Fig. 1), including small populations such as Vordemwald. The populations investigated are all located in rather large forests. Consequently, the size of the forest patch had no influence on both the population size and the genetic variation of the *L. pulmonaria*.

The large population in Lauenen shows very low genetic variability. This forest has been used for centuries for wood production by selective cutting. Generally, the pressure from forest management is lower than in lowland forests and natural catastrophes that could have destroyed the whole population or big parts of it are not reported from this site. In addition, the lichen vegetation is very rich and includes several red list species which are rare elsewhere. The site is geographically isolated and located at the end of an alpine valley surrounded by high mountains. For oceanic lichen species, the best access for colonization of this valley is from the north, along the bottom of the valley. A narrow, continuous, more than 15 km-long corridor of broad-leaved deciduous forest is present there and connects potential *L. pulmonaria* habitats of the Alpine region with the potential habitats of the pre-Alpine region. As a result, opportunities to colonize this valley were probably limited in the past, and immigration of new sexual or vegetative diaspores (e.g. ascospores or soredia) and hence gene flow is limited. Such situations can lead to founder effects and a reduction in local genetic variability (Hedrick & Miller 1992; Lynch 1996). We therefore assume the low genetic diversity to be an outcome of a founder effect.

In contrast with the large but genotypically poor population of Lauenen, the small lowland population of Vordemwald has the highest within-population variation of all populations analysed in this study. Vordemwald is an old forest where fertile *L. pulmonaria* thalli have been collected since at least 1881 and 1877 by Siegfried and Fischer-Siegwart, respectively (Scheidegger 1995). There are no reports of large clear cuts at this site in the forest, although logging by selective cutting has been carried out regularly. However, modern forest management practices favour shorter cutting cycles and rather dense, dark deciduous or coniferous stands. In addition, *L. pulmonaria* is sensitive to acid pollutants (Sigal & Johnston 1986) and ozone (Scheidegger & Schroeter 1995). Moreover, in recent decades, air quality decreased and was especially poor in the lowland regions of Switzerland where most of the large cities and industrial facilities are located. Such pollutants might be a further cause of the population decline in Vordemwald. All of these harsh environmental stresses on this *L. pulmonaria* population did not lead to a drastic reduction of genetic diversity. We assume that the existing thalli in Vordemwald represent remnants of a formerly larger and genetically diverse population. Somehow, despite the slow and continuous decline of this population, it was able to maintain a high genetic diversity.

Conservation

Sexual reproduction between individuals from different populations adapted to rather different local environmental conditions (i.e. different adaptive peaks) should be avoided as this can disrupt locally optimal gene combinations (Leberg 1993) and produce less fit offsprings (Templeton 1986). Our study of *L. pulmonaria* shows no geographical pattern of the genotypes resolved by rDNA types and consequently we had to reject the hypothesis of locally adapted ecotypes. This is despite the fact that two genotypes (AB and DA) were found only in one location, Marchairuz and Muotatal, respectively.

Genetic variability and population size are considered important factors for the survival of a population or species, particularly in a changing environment (Shaffer 1981). Larger populations with higher genetic variability are expected to have a higher probability of survival and a higher evolutionary potential (Avise 1994). Based on this assumption and because we have shown in this study that populations with apothecia have a higher genetic diversity, we give populations of *L. pulmonaria* in Switzerland where sexual reproduction is known a higher rank in terms of conservation priority than strictly asexual populations of this species.

The lowland populations, particularly the population in Forst Bern, are so small that one single catastrophic event (e.g. windthrow) might destroy the entire population. Augmentation in size and viability of such small populations with thallus fragments or vegetative diaspores from other populations seem to be the only possibility for their preservation. The lack of local adaptation, as identified with these markers, suggests that transplants may be taken successfully from any other genetically diverse population in Switzerland. By transplanting specimens from the more diverse populations we could probably increase the level of sexual reproduction as more, different mating types will be present. Hence, the amount of ascospores produced in such a threatened population would increase. As these diaspores are easily dispersed, the probability of founding new thalli on other trees increases.

For the first transplantations we will use thalli from the nearest geographical vicinity or coming from sites with similar forest vegetation. Transplantations of *L. pulmonaria* have already been shown to be very successful and an effective way to increase the number of individuals and subpopulations within a threatened population (Scheidegger 1995). For example, to augment the size and genotypic diversity of the very small lowland population in Forst Bern, we suggest transplanting soredia or very small thallus fragments from the more diverse lowland population in Vordemwald. This will increase the probability of preserving genotypes adapted to the full range of ecological variation. Although Vordemwald is a small population, this practice would not threaten its survival.

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