Spatial differences in genetic diversity within a population of the endangered forest lichen *Lobaria pulmonaria*

Diploma thesis of Isabelle Stähli
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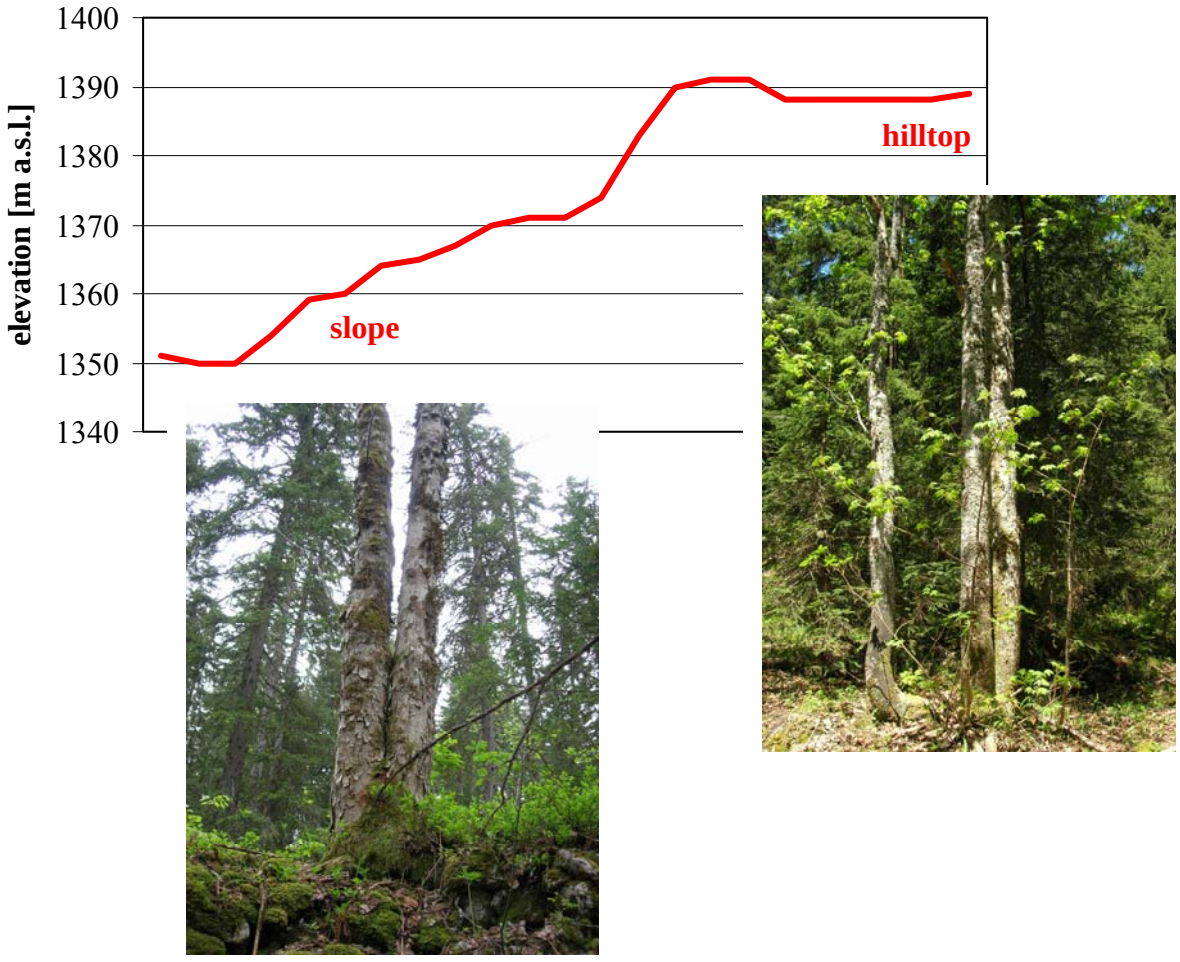

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Abstract

Summary in English

Lobaria pulmonaria is an epiphytic lichen that has been proposed to be an indicator for natural, continuous forests. It is sensitive to changes in the forest environment and habitat loss due to natural or human disturbance. However, a recent large-scale survey could not confirm the proposed negative association between the occurrence of *L. pulmonaria* and disturbance. Large and diverse populations were also found in disturbed areas.
A tree map of the present distribution of colonized and potential host trees of *L. pulmonaria* in the northern part of the Parc Jurassien Vaudois shows four areas where this lichen is very frequent. The lichen populations of an undisturbed and two disturbed areas of this tree map were already studied by another researcher. This diploma thesis aimed to investigate whether or not the fourth area of the tree map is undisturbed. The investigated lichen population was compared to the populations in the other three areas at genetic level. Comparisons of gene diversity revealed strong similarities between the study area and the undisturbed area. Therefore, the study area can be considered as undisturbed, too.

Summary in German

Die epiphytische Lungenflechte *Lobaria pulmonaria* wird als Indikatorart für naturnahe, über lange Zeiträume bestehende Wälder beschrieben. Sie reagiert empfindlich auf Störungen ihres Habitates durch Naturkatastrophen oder menschliche Eingriffe. Eine kürzlich gemachte Untersuchung zeigte jedoch, dass sich eine Störung des Habitates nicht unbedingt nur negativ auf das Vorkommen der Lungenflechte auswirkt. Grosse und diverse Populationen wurden auch auf gestörten Flächen gefunden.
Eine Verbreitungskarte der Lungenflechte zeigt, dass sich auf vier Flächen im nördlichen Teil des Parc Jurassien Vaudois dichte Populationen angesiedelt haben. Eine gestörte und zwei ungestörte Flächen dieser Verbreitungskarte wurden bereits in einer anderen Studie untersucht. Eines der Ziele der vorliegenden Diplomarbeit war herauszufinden, ob die vierte Fläche gestört oder ungestört ist. Dazu wurde die untersuchte Lungenflechten Population mit den drei weiteren Populationen der Verbreitungskarte auf molekularer Ebene verglichen. Es stellte sich heraus, dass die untersuchte Population bezüglich genetischer Diversität derjenigen Population im ungestörten Gebiet am ähnlichsten ist und deshalb selbst als ungestört betrachtet werden kann.

1 Introduction

The lungwort *Lobaria pulmonaria* (L., Hoffm. 1796) is a macrolichen with a worldwide distribution (appendix 1). Yoshimura (1971) mapped *L. pulmonaria* in cooler regions of the tropics and in temperate and boreal regions of the northern hemisphere. Hallingbäck and Martinsson (1987) reported a substantial decline of the occurrence in several European countries. In Switzerland, *L. pulmonaria* has almost disappeared from the Swiss Plateau. Populations where it grows on more than 5 trees are mainly known from the Jura Mountains and from the Pre-Alps (Roth *et al*., 1997; Zoller *et al*., 1999; Scheidegger *et al*., 2002; appendix 2). Cited in the Red List 2002 of threatened epiphytic and terricolous lichens of Switzerland, *L. pulmonaria* is one of 230 endangered epiphytic Swiss lichens (Scheidegger *et al*., 2002). It is our responsibility to conserve endangered species. Conservation measures for lichens can only be effective if parameters of life history, colonization and dispersal are known. However, there is still little known about capacity and effectiveness of lichen dispersal (Sillett *et al*., 2000; Walser *et al*., 2005).

Lobaria pulmonaria grows mainly on old deciduous trees in natural forests (Wirth, 1995). According to Schöller (1997), this lichen has mainly disappeared because of air pollution and habitat change or loss. Forestry management often includes logging that removes not only the host trees of epiphytic species but may also alter the microclimate in terms of light, temperature and water availability. A subsequent replacement of logged stands by monocultures worsens the situation even more. However, habitat change or loss may also occur due to natural disturbance events such as fire or wind fall.
It has been proposed that *L. pulmonaria* is an "old-forest indicator" which is especially sensitive to changes in the forest environment and whose presence may indicate continuous forests (Rose, 1976; Nilsson *et al*., 1995). Several studies attempt to find reasons for the assumed sensitivity of *L. pulmonaria* to habitat change. In the following, some reasons are given.
According to Snäll (2003), *L. pulmonaria* is a patch-tracking organism for which trees act as patches. The destruction of a patch by natural or human reasons causes the extinction of a local *L. pulmonaria* population. Therefore, protecting *L. pulmonaria* also includes the conservation of its host trees.
The re-colonization of lost patches may be difficult because *L. pulmonaria* is said to be dispersal limited. Its vegetative and sexual propagules lack morphological adaptations (Zoller *et al*., 1999; Walser *et al*., 2005) and therefore, dispersal over larger distances may be a problem. However, the lightweight sexual propagules are believed to be more important for long-distance dispersal than the heavier vegetative propagules (Bailey, 1976).
Lobaria pulmonaria has a long generation time of over two decades (Roth *et al*., 1997; Zoller *et al*., 1999). This means that propagules are only formed after a thallus has reached the age of up to 30 years. Thus, a scarce availability of propagules may complicate re-colonization of lost patches.

The study area was located in the Swiss Jura Mountains, where the landscape is dominated by a mix of woodland and wooded pastures. Recently, two studies about *L. pulmonaria* were conducted in this region. In their dissertations, Jesse Kalwij and Silke Werth both worked on the subject of "Pasture-woodland landscape dynamics", a NCCR Plant Survival project at the Swiss Federal Institute for Forest, Snow and Landscape Research, WSL (Kalwij, 2005; Werth, 2005).

Kalwij (2005) mapped the present distribution of colonized and potential host trees of *L. pulmonaria* (sycamore maple and beech) within 251 randomly placed one-hectare plots in the northern part of the Parc Jurassien Vaudois. His tree map showed four local "hot spots" where *L. pulmonaria* is very frequent (appendix 3). As it has been proposed that *L. pulmonaria* is an "old-forest indicator", one may assume that these hot spots were located in undisturbed forests. However, two populations grew in disturbed areas that were visually delineated by comparing orthophotos from 1933 to orthophotos from 1998 and applying known dendroecological methods (Bolli *et al*., in prep.). One stand-level disturbance was an area intensively logged between 1850 and 1900, where predominantly *Picea abies* were probably removed for charcoal production. The other was an area intensively logged in 1870, followed by wind throw and fire in 1871 replacing the stand (Kalwij *et al.*, in press). In the large-scale survey of Kalwij *et al*. (in press), this proposed negative association between the occurrence of *L. pulmonaria* and disturbance could not be confirmed.

Werth (2005) studied the genetic diversity of the *L. pulmonaria* populations in the disturbed areas West (charcoal) and North (logged & burnt) and the in the undisturbed area East of Kalwij *et al.* (in press), where only uneven-aged forestry had been applied for centuries (appendix 3).
Although the area West was largely affected by stand-level timber logging, genetic diversity of the *L. pulmonaria* population in the concerned plots was especially high. According to Werth *et al.* (subm.), there might be two possible explanations: an increase of available host trees such as sycamore maples, supported by temporally enhanced light conditions due to timber logging (Kalwij *et al.*, in press), or a selective logging of tree species such as Norway spruce and beech where sycamore maples were spared. In contrast, genetic diversity in the northern population, disturbed by a fire 130 years ago, was much lower. A possible reason for this low genetic diversity per plot may be the small number of dispersal propagules which acted as colonizers and reproduced mainly asexually after germination (Werth *et al.*, subm.). If this pattern of few colonization events, followed by a rapid clonal spread, can be shown at tree level, too, was not investigated.
Werth (2005) also analyzed the genetic differentiation between the three areas East, West and North and found very high levels of gene flow. The gene flow was assumed to be of historical origin reflecting a once connected landscape that contained one big *L. pulmonaria* population. Additionally, the occurrence of the same three gene pools was detected in each area. This result confirmed the idea that the areas East, West and North were connected in former times.

In this study, a *L. pulmonaria* population in the Parc Jurassien Vaudois was investigated where the tree map of Kalwij *et al*. (in press) revealed the fourth "hot spot" of this species (appendix 3). Within the study area, the *L. pulmonaria* population was spatially distributed on a slope and a hilltop. According to the tree map, there were less host trees with *L. pulmonaria* on the hilltop than on the slope, although slope and hilltop seemed to have an equal number of potential host trees (appendix 4). This study aimed to investigate whether or not the different number of colonized trees reflected differences between population genetic parameters, which would indicate that there are two different populations on slope and hilltop. If the study area

contains only one *L. pulmonaria* population, comparisons with the three areas of Werth *et al.* (subm.) would be legitimate.
The study area was located next to the areas North and East of Werth *et al.* (subm.) and is called "Northeast".

These considerations led to the following main questions:

A 1. Do slope and hilltop in the study area Northeast differ with regard to
- habitat parameters:
  - a density of potential host trees
  - b density of colonized trees
  - c light conditions
  - d age of colonized trees
  - e trunk width of colonized trees
- spatial aggregation of:
  - f all host trees
  - g colonized host trees
  - h uncolonized host trees
- *Lobaria pulmonaria* population parameters:
  - i density
  - j aggregation
  - k cover
- population genetic parameters:
  - l genotypes
  - m clonality
  - n colonization events
  - o diversity

B 1. Is the *Lobaria pulmonaria* population in the study area Northeast more similar to the population of the undisturbed area East than to the populations of the disturbed areas West (charcoal) and North (logged & burnt) of Werth *et al.* (subm.) with regard to
- population genetic parameters:
  - a genotypes
  - b diversity
- spatial genetic structures:
  - c clonal pattern
  - d total diversity
  - e recombination pattern

B 2. Was the *L. pulmonaria* population in the study area Northeast once also connected to the populations of the areas East, West and North?

C 1. Is there a high level of clonal propagation within trees?

2 Material and Methods

2.1 Species

In Switzerland, *L. pulmonaria* grows mainly on beech (*Fagus sylvatica*) and sycamore maple (*Acer pseudoplatanus*; Zoller *et al*., 1999), but the preference for sycamore maple is stronger (Kalwij *et al.*, in press). The availability of sycamore maples as potential host trees plays therefore a decisive role in the spatial distribution of *L. pulmonaria*.
Lobaria pulmonaria is able to form both sexual and vegetative dispersal propagules (Yoshimura, 1971; Scheidegger, 1995). The sexual propagules, ascospores, are produced in apothecia, the fruiting bodies of the fungal symbiont. *L. pulmonaria* is assumed to be heterothallic, i.e., *L. pulmonaria* is an outcrossing species. Thus, mating partners of different but compatible genotypes are required (Zoller *et al*., 1999).
In contrast to ascospores, vegetative propagules such as soredia, isidia, isidioid soredia or thallus fragments are symbiontic. Therefore, the ascomycete and the primary photobiont of the genus *Dictyochloropsis* are dispersed together. Aside from dispersal function, vegetative propagules may also have a regenerative function within a population when re-establishing a new thallus where the previous grew (Büdel *et al*., 1996). Compared to vegetative propagules, ascospores are formed more rarely (Denison, 2003). In Switzerland, fertile thalli were only found in luxuriant *L. pulmonaria* populations which consist of many large thalli (Scheidegger, 1995).

2.2 Study area

The study area was located in the northern part of the Parc Jurassien Vaudois, Canton Vaud, Switzerland. It was sited below the Col du Marchairuz at 46°33.5'N and 6°14'E in a region called "Grande Rolat". The spatial extent of the study area was about 1000 m x 500 m and included a slope, rising from 1340 m a.s.l. to the hilltop at 1390 m a.s.l.
The climate in the north-western Jura Mountains is cold and humid, leading to frequent rainfall. The mean annual temperature at Les Trois Chalets (1295 m a.s.l.) is 2.9°C and the annual precipitation at the Col du Marchairuz (1447 m a.s.l.) comes to 1631 mm/yr. Nearby the summit of La Dôle at an elevation of 1670 m a.s.l., the dominating winds are west or east winds that can be very violent: wind speed often exceeds 100 km/h (Vittoz, 1998).
The typical landscape at higher elevations of the Jura Mountains is a pasture woodland landscape (Combe, 1999), dominated by Norway spruce (*Picea abies*). These sparse forests also consist of light depending sycamore maples (*Acer pseudoplatanus*), beeches (*Fagus sylvatica*) and silver firs (*Abies alba*), but the latter are less abundant. The phytosociological description of the study area is mainly *Sorbo glabratae-Piceocoenetum aspleniocoenetosum*, where Norway spruce is dominant and accompanied by mountain ash (*Sorbus aucuparia*) and sycamore maple (*Acer pseudoplatanus*), and where the understory contains Blueberry (*Vaccinium myrtillus*; Vittoz, 1998).
The forest in the region Grande Rolat can be described as a long-term, closed forest (Kalwij, 2005; BFS, 1994). According to the foresters of the communities L'Abbaye (A. Croisier) and Le Chenit (R. Meylan), uneven-aged forestry is applied in Grande Rolat. Periodically selecting and harvesting individual trees or groups of trees prevents stands of the same age and size and lead therefore to a vertically structured forest (Schütz, 2002).

Many sycamore maples in the study area consisted of stool shoots. In most cases, all stool shoots of one tree were colonized by *L. pulmonaria.* Stool shoots serve for vegetative reproduction after the death of the originally standing tree.

2.3 Sampling design

The sampling design was based on the map of colonized and potential host trees within 251 circular 1-ha sampling plots that were randomly placed in the Parc Jurassien Vaudois (Kalwij *et al.*, in press). The study area included nine of these plots, four on the hilltop and five on the slope (appendix 5). Per plot, five colonized sycamore maples were randomly selected from the dataset of Kalwij. Coordinates and trunk diameter classes were gathered from the tree map. If there were less than five colonized sycamore maples per plot, all trees were sampled. In total, 41 colonized maple trees were sampled, 16 in the plots on the hilltop and 25 in the plots on the slope. Of these, 14 consisted of one or more stool shoots with *L. pulmonaria*.

In May and June 2005, the selected sycamore maples were located using a handheld GPS (Garmin 76C) with an external antenna. Pictures were taken of each colonized tree including stool shoots from all four wind directions to allow documentation of the spatial distribution pattern of *L. pulmonaria* on a trunk and to re-identify individual trees if necessary.
Lobaria pulmonaria was collected from each of the 41 sycamore maples including colonized stool shoots. To minimize damage to the sampled thallus but still have enough material for DNA extractions, only a single lobe tip of 6 cm^2 was taken, unless one third of the whole thallus would be exceeded (Scheidegger, 1995). If possible, thalli with apothecia or abundant isidia were avoided. If there were at least five thalli of sufficient size, five were selected as follows: one thallus between hip and eye level from 1) the North and 2) the South, one thallus from 3) the West and 4) the East at about four-meter height using a self-made harvest tool. The fifth thallus was taken from 5) another suitable direction and height on the trunk. If there were less than five thalli per tree, a sample was taken from each. Thus, thalli that lay one upstem the other were avoided because they are more likely to be of the same genotypes as run-off water on the tree surface may transport vegetative propagules that were established downstem. Armstrong (1981) found fragments and other propagules from *Parmelia sp*. in run-off water from a vertical, slate rock surface. This sampling design aimed therefore at minimizing the number of clonal thalli per tree.
Lobaria pulmonaria samples were taken from each of the colonized stool shoots of a sycamore maple (240 in total). DNA extraction and allele detection was done for all of them. However, to ensure a balanced statistic design, the samples of only one randomly chosen stool shoot per tree (161 in total) were used for statistical analyses.

2.4 Molecular analysis

The aim of the molecular analyses was to determine the alleles on six fungal microsatellite loci within the DNA of *L. pulmonaria* samples, LPu03, LPu09, LPu15, LPu16, LPu20 and LPu27 (Walser *et al.*, 2004). Microsatellites are "tandemly repeated sequences" that are considered neutral to selection (Jarne and Lagoda, 1996). The length of the repeat units was nine basepairs for LPu09 and two for the other microsatellite loci.
To isolate DNA, 45-55 mg (dry weight) of each cleaned thalli was liophilized for at least 12 hours and afterwards ground fine in a Retsch MM 300 Mixer Mill (Retsch, Haan, Germany) for two minutes at 30 Hz, step repeated if necessary. The DNeasy 96 Plant Kit (QIAGEN, Hilden, Germany) was used for DNA extractions following the slightly adapted manufacturer's protocol (appendix 6). Some extractions were carried out with another centrifuge (Eppendorf Centrifuge 5804) at lower centrifugal force g and other durations (given in italics).
For PCR reactions, the QIAGEN Multiplex PCR kit (QIAGEN, Hilden, Germany) was used. 1.7 µl of the QIAGEN Multiplex PCR Master Mix, 3.6 µl of Fluka water and 0.7 µl of one of the two 2 µM primer mixes (LPu03/09/15 or LPu16/20/27) were brought together and 1.0 µl DNA-Template was added. The amplification reactions were performed with a PTC-100 Programmable Thermal Controller (MJ Research, Waltham, USA). The steps (table 1) were programmed taking into account recommendations from the manual of the QIAGEN Multiplex PCR Master Mix and Walser *et al.* (2003, 2004).

Tab. 1: PCR steps for the primer mixes LPu03/09/15 and LPu16/20/27.

Steps	**LPu03/09/15**	**LPu16/20/27**
Activation	95°C for 15 minutes	95°C for 15 minutes
Denaturation	94°C for 30 seconds	94°C for 30 seconds
Annealing	55°C for 90 seconds	57°C for 90 seconds
Extension	72°C for 60 seconds	72°C for 60 seconds
Number of cycles	27 times to step "denaturation"	29 times to step "denaturation"
Final extension	60°C for 30 minutes	60°C for 30 minutes

From the PCR product, 0.5 µl was suspended in 12.2 µl HiDi Formamide. To avoid off-scaled peaks, the PCR's of LPu03/09/15 were first diluted with 7 µl of Fluka water. On ABI3100-*Avant* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), DNA fragments within the PCR products, labelled with fluorescent dyes, were detected. Alleles were sized with the help of 500 ROX Size Standard (0.2 µl per sample; Applied Biosystems, Foster City, CA, USA). On each 96-well plate prepared for ABI3100-*Avant*, one negative control and seven reference samples of Werth were run to ensure comparability of our results. For genotyping at which allele size and pattern were determined, GeneMapper 3.5 (Applied Biosystems, Foster City, CA, USA) was used.
From 240 sequenced thallus fragments, 161 were selected for statistical analyses. Thereof, 3 samples with missing microsatellite data had to be excluded due to poor DNA and missing sizing data.

The number of different alleles per plot and the allele sizes at the six microsatellite loci are shown in appendix 7.

2.5 Dendroecological analysis

To determine tree age, the 41 sycamore maples and colonized stool shoots were cored using an increment borer. The cores were extracted at approximately 50 cm above the root collar, high enough to avoid root material that would complicate age determination. If the increment borer did not intersect close enough to the pith, another core was taken at a higher level nearby. From one cored tree per plot, a second core was extracted about 10 cm above the first. The distance between the two boreholes was measured in order to determine the growth rate of the colonized sycamore maples.
To prepare the increment cores for tree ring analysis, they were first sanded with a belt sander and then cut using the WSL-Microtome, developed by Gärtner and Nievergelt. In addition, the plane surfaces were oiled in order to make the tree rings more visible. Under a binocular microscope and with the software program TSAP (Time Series Analysis and Presentation Program; Rinn, 1996), the tree rings were counted and tree-ring width (distance between tree rings) was measured. In cases where the pith was not intersected by the increment borer, a pith locator served to estimate missing tree rings (Bräker, 1981). A pith locator is a pattern of concentric circles with the same tree-ring width as the inner rings of the core. By fitting it to the shape of the inner rings, missing tree rings can be counted.
Since the cores were extracted at 50 cm above the root collar where the germination point of a tree is located, the uncounted tree rings between germination point and borehole should have been assessed according to the formula as described in Bolli (2004). To calculate uncounted tree rings, the distance between two boreholes of a tree is needed. However, the distance of only 10 cm was too small for a calculation. Therefore, the number of uncounted tree rings had to be estimated. According to Andreas Rigling, the number of uncounted tree rings was maximum five (personal communication). Because all cores were taken at the same height and an equal youth growth on slope and hilltop was assumed, the age of the cored sycamore maples on slope and hilltop could be compared.
Cores may lack tree rings. Missing tree rings originate from poor growth conditions. In this study, there were probably maximum two missing tree rings per core (Rigling, personal communication). Missing tree rings can be determined by cross-dating, where ring-width curves are compared with a generated reference curve (Fritts, 1976). This method allows detecting measurement errors, too. Measurement errors come from overlooked tree rings when they are more or less invisible or from erroneously counted tree rings. In this study, cross-dating was not possible due to time limitations. Nevertheless, generated mean ring-width curves of each the slope and the hilltop looked very similar. Thus, the number of missing rings and the accuracy of measurement is assumed to be the same on slope and hilltop and therefore, comparability of the age between slope and hilltop is given.
Of 41 cores, one core had to be excluded from subsequent statistical tests because it was rotten.

3 Data analyses

3.1 Hypotheses

In the following, specifications of the main questions in terms of alternative hypotheses H_A are given. Wherever the null hypotheses are assumed to be true instead of the alternative hypotheses, the hypotheses are marked with "H_0".

A 1. Differences between slope and hilltop

Habitat parameters

a	The number of sycamore maples per plot is not different between hilltop and slope.	H_0
b	The number of colonized sycamore maples per plot is less dense on the hilltop than on the slope.	H_A
c_1	The mean canopy cover is not different between hilltop and slope.	H_0
c_2	The number of trees per plot is not different between hilltop and slope.	H_0
d	The age of the colonized sycamore maples is not different between hilltop and slope.	H_0
e	The diameter at breast height (DBH) of the colonized sycamore maples is not different between hilltop and slope.	H_0

Spatial aggregation of host trees

f_1	The distance of the sycamore maples to the nearest colonized neighbour (NN) is not different between hilltop and slope.	H_0
f_2	The cluster index of the sycamore maples per plot is smaller on the hilltop than on the slope.	H_A
g	The distance of the colonized sycamore maples to the nearest colonized neighbour (NN) is larger on the hilltop than on the slope.	H_A
h	The distance of the uncolonized sycamore maples to the nearest colonized neighbour (NN) is smaller on the hilltop than on the slope.	H_A

Lobaria pulmonaria population parameters

i_1	The number of thalli per colonized sycamore maple is not different between hilltop and slope.	H_0
i_2	The number of thalli per plot is smaller on the hilltop than on the slope.	H_A
j_1	The number of thallus groups per colonized sycamore maple is not different between hilltop and slope.	H_0
j_2	The number of thallus groups per plot is smaller on the hilltop than on the slope.	H_A
k_1	The thallus area per colonized sycamore maple is not different between hilltop and slope.	H_0
k_2	The thallus area per plot is smaller on the hilltop than on the slope.	H_A

l The number of multilocus genotypes (G) per plot is smaller on the hilltop than on the slope. H_A

Population genetic parameters

m The percentage of G (M) per plot is smaller on the hilltop than on the slope. H_A

n_1 The minimum number of colonization events (C) per plot is fewer on the hilltop than on the slope. H_A

n_2 The minimum number of colonization events (C) per sycamore maple is fewer on the hilltop than on the slope. H_A

o_1 Gene diversity (H) per plot is not different between hilltop and slope. H_0

o_2 Genotype diversity (D) per plot is lower on the hilltop than on the slope. H_A

B 1. Comparisons between the study area and the other areas

Population genetic parameters

a_1 The number of multilocus genotypes (G) of the population in Northeast is more similar to G of the population of the undisturbed area East than to G of the populations of the disturbed areas West (charcoal) and North (logged & burnt).

a_2 Northeast and North share most multilocus genotypes (G).

b Gene diversity (H) in the population in Northeast is more similar to H in the population of the undisturbed area East than to H in the populations of the disturbed areas West (charcoal) and North (logged & burnt).

Spatial genetic structure

c A variogram of genotype diversity (D) shows spatial autocorrelation at short distances.

d A variogram of gene diversity (H) without accounting for recurrent genotypes shows spatial autocorrelation at short distances.

e A variogram of gene diversity H with accounting for recurrent genotypes shows no spatial autocorrelation.

B 2. Connectedness of the four areas

a A molecular variance analysis (AMOVA) reveals low genetic differentiation between the areas Northeast, East, West and North.

C 1. Clonal propagation within sycamore maples

a Genotype diversity (D) on single trees is small.

b Genetic differentiation (AMOVA) on single trees is low.

3.2 Failure to reject a null hypothesis H_0

If a test fails to reject a null hypothesis, there are two possible interpretations.
On the one hand, the null hypothesis may be false, but the sample size was too small to indicate significance. There is a lack of power. Not rejecting H_0 when H_0 is false but a given alternative hypothesis H_A is true is called type II error. In power analyses, the probability of making a type II error, i.e. the power, is calculated. To calculate the power of a test, the effect size is required. The larger the effect size, the greater the power of a test.
On the other hand, the data may be consistent with the null hypothesis. There is a lack of effect. The more an experiment produces data consistent with a null hypothesis, the more an effect size approaches zero (Randall, 2004). Thus, effect sizes may be used in cases where null hypotheses have to be "proved".

For each tested hypotheses, the effect size was additionally calculated.
The effect size for two independent groups is the standardized mean difference (Cohen, 1988):

$$d = \frac{m_A - m_B}{\sigma}$$

m_A, m_B: population means
σ: standard deviation of either population, since they are assumed equal

In practice, the pooled standard deviation (σ_{pooled}) is commonly used to estimate the standard deviation (Rosnow and Rosenthal, 1996):

$$\sigma_{pooled} = \sqrt{\frac{(N_A - 1)\,\sigma^2 + (N_B - 1)\,\sigma^2}{N_A + N_B - 2}}$$

N_A, N_B: sample sizes

According to Cohen (1988), d = 0.2 is a small, d = 0.5 is a medium and d = 0.8 is a large effect size.

3.3 Definitions

The tree map of Kalwij *et al*. (in press) was used to calculate the number of the sycamore maples per plot (density) on the one hand and of only colonized sycamore maples per plot on the other hand. Trees of trunk width <10 cm were not mapped by Kalwij.
Canopy cover and number of trees per plot were used as a measure for light conditions. For each sampled sycamore maple, the mean canopy cover within a radius of 7.5 meters was derived from a LiDAR-based boolean map of "tree" (vegetation exceeding 1.50 m) and "no tree", in collaboration with Kalwij. LiDAR data (Light detection and Ranging) are airborne laser scanning data. The number of trees per plot was also derived from LiDAR data, where all trees from 1.50 m were detected (Kalwij, 2005).
The age of the colonized sycamore maples was derived from cores (see chapter 2.5) and the diameter was measured at breast height at 1.30 m (DBH). Additionally, the cumulative tree width was derived from summing up the tree-ring widths of each year, while the first 27 years had to be excluded due to uncertain data. This variable contains the age (number of tree rings) as well as the annual growth (distance between tree rings).

The spatial aggregation of sycamore maples in the nine plots was calculated by measuring the distance to the nearest colonized neighbour (NN) for each colonized or uncolonized sycamore maple with the kind help of Kalwij. Data were corrected for edge effects: sycamore maples closer to the edge of a 1-ha plot than to a colonized tree were excluded. All stool shoots of a sycamore maple were accounted as one single tree. To characterize the spatial aggregation of the sycamore maples, a cluster index calculated by Kalwij was used (Kalwij *et al*., in press).

For each sampled sycamore maple, the total number of thalli per tree was counted. If there were more than 24 thalli per tree, the following thallus number classes were used: 25-50, 51-75, 76-100, 101-150, 151-200, 200-300 and 300-400 and the mean class value per tree was calculated. The number of thalli at tree level was subsequently summed up to the plot level.
Furthermore, the total number of thallus groups per tree was counted and summed up to the plot level. Thallus groups were defined as patches of aggregated, overlapping thalli.
The total thallus area of *L. pulmonaria* covering the total bark surface of a tree was measured in dm^2 and then summed up to the plot level. If the thallus area was larger than 1 dm^2, the number of size-DIN A4 sheets covered by *L. pulmonaria* was counted. Since one A4 sheet measures 6.2 dm^2, the sum of the thallus area in dm^2 could be estimated from the counted number of A4 sheets.
For each sycamore maple, it was additionally recorded if apothecia were present or absent. Apothecia on thalli high above could not be detected.

The multilocus genotypes (G) were composed of the alleles on the six fungal microsatellite loci. G is the number of multilocus genotypes per plot. The calculation of G allowed counting the number of shared multilocus genotypes between plots. M, calculated by dividing G by the number of sampled thalli, is the percentage of G. A low M means few different multilocus genotypes but a lot of clonal thalli among the samples.
C is the minimum number of colonization events at plot or at tree level, calculated as the "number of alleles at the most variable locus" (Walser *et al*., 2003). Since only 5 trees per plot and 5 thalli per tree were sampled, C may well be underestimated.

H is the unbiased estimate of the expected heterozygosity per plot, averaged over all six loci (Walser *et al*., 2003). The formula was based on Nei (1978) but rewritten for haploid individuals. According to Wagner *et al.* (2005), H is the "probability that two gene copies sampled with replacement differ at locus l":

$$H_l = \frac{N}{N-1}\left[1 - \sum_k p_k^2\right]$$

N: number of gene copies
k: number of different alleles

Where possible, the number of collected thalli per tree was 5, as planned. Since some sycamore maples had less than 5 thalli, H had to be calculated with resampling: one thallus per tree was randomly selected and the mean of H from 100 permutations calculated.
Similar to H, D is the "probability of sampling two individuals of different multilocus genotypes" (see Wagner *et al.*, 2005):

$$D = \frac{N}{N-1}\left[1 - \sum_g p_g^2\right]$$

g: number of different multilocus genotypes

A variogram shows a distance-dependent estimate of the population variance (Wagner *et al.,* 2003). Three different variograms were calculated: 1) A variogram of D reflecting the clonal component of the population, 2) a variogram of H without weighting for recurrent genotypes reflecting the overall spatial genetic structure and 3) a variogram of H with weighting for recurrent genotypes reflecting the sexual component. As in Werth *et al*. (subm.), the distance classes went from 0 m, where pairs of thalli from the same tree were compared, up to 450 m. 450 m corresponded to ⅔ of the maximum distance between the plots. The lag distance was 50 m.

Analyses of molecular variance, AMOVA, split the total variance into covariance components according to hierarchical levels: groups, populations within groups and individuals within populations (Excoffier, 2000). These covariance components are used to compute fixation indices Φ (Wright, 1965). An AMOVA reveals genetic differentiation between the sampled populations. The lower Φ, the lower genetic differentiation and the higher gene flow.
For the studied *L. pulmonaria* population in the area Northeast, an AMOVA was done at three different levels: on trees, among trees within plots and among plots. An AMOVA was also calculated at area level: within plots, among plots within the areas Northeast, East, West and North and among the areas. Additionally, the number of migrants, Nm, was calculated showing the estimated number of migration events per generation between the areas.

3.4 Calculations

To compare hilltop to slope, nonparametric Mann-Whitney rank tests were performed in SPSS 11.5.1 (Anonymous, 2002). Counting data first were square-root transformed.

To calculate G, M, C, H and variograms of H and D, R 2.0.1 was used (Ihaka and Gentleman, 1996). The R-codes were programmed by Werth and Wagner (Werth *et al.*, subm.; Wagner *et al.*, 2005).

All correlations were tested with a Pearson correlation. Additionally, R^2 was calculated.

Analyses of molecular variance (AMOVA, Excoffier *et al.*, 1992) were performed in ARLEQUIN 2.0 (Schneider *et al*., 2000).

4 Results

4.1 Differences between slope and hilltop

4.1.1 Habitat parameters

As the tree map of Kalwij *et al*. (in press) suggested, the number of sycamore maples per plot (density) in the study area Northeast was not significantly different between slope and hilltop (table 2). Since the effect size d was small, the null hypothesis H_0 was assumed to be true (chapter 3.1). On average, there were 30 sycamore maples per plot. However, on the slope were significantly more colonized sycamore maples.
The light conditions, derived from mean canopy cover and tree density per plot, were not significantly different between slope and hilltop, as assumed. The small effect size d confirmed H_0. On average, canopy cover per plot was 59% and there were 228 trees per plot.
Regarding the age of the colonized sycamore maples, there was no significant difference between hilltop and slope. The effect size d was medium. The mean age was about 119 years. There may be an additional 7 years if we take into account missing tree rings and a rough estimate for the years between germination point and borehole. Thus, the colonized sycamore maples were, on average, approximately 126 years old.
The diameter at breast height (DBH) did not differ significantly, either. The effect size d was small. On average, the DBH came to 25 cm. Figure 1 shows the number of colonized and uncolonized sycamore maples per DBH class. Age had a significant effect on DBH, but the correlation of age with DBH was very weak (figure 2).

Tab. 2: Mann-Whitney rank tests for differences between slope and hilltop within the study area Northeast in the Swiss Jura Mountains. P-values, effect sizes, means, levels of comparison and sample sizes of different measure data regarding habitat parameters, spatial aggregation of host trees and *Lobaria pulmonaria* population parameters. The significance level is 5%.

		P-value	**Effect size**	**Mean**		**Level of comparison**	**Sample size**	
				slope	hilltop		slope	hilltop
Number of sycamore maples per plot	H_0	0.905	0.16	31	28	plot	5	4
Number of colonized sycamore maples per plot	HA	0.032	1.79	17	5	plot	5	4
Mean canopy cover	H0	0.368	0.15	60%	58%	tree	232	143
Number of trees per plot	H0	0.730	0.18	227	229	plot	5	4
Age of colonized sycamore maples	H0	0.404	0.44	122	128	tree	24	16
Diameter at breast height (DBH)	H0	0.320	0.29	25	23	tree	25	16
Sycamore maples – nearest colon. neighbour (NN)	H0	0.001	0.74	10	15	tree	84	48
Cluster index of sycamore maples per plot	HA	0.730	0.02	0.80	0.79	plot	5	4
Colonized sycamore maples – NN	HA	1.000	0.13	8	9	tree	43	7
Uncolonized sycamore maples – NN	HA	0.015	0.65	11	16	tree	41	41
Number of thalli per tree	H0	0.843	0.002	59	71	tree	25	16
Number of thalli per plot	HA	0.556	0.12	296	284	plot	5	4
Number of thallus groups per tree	H0	0.947	0.12	20	22	tree	25	16
Number of thallus groups per plot	HA	0.413	0.33	100	84	plot	5	4
Thallus area per tree	H0	0.271	0.36	59	36	tree	25	16
Thallus area per plot	HA	0.413	0.60	301	116	plot	5	4

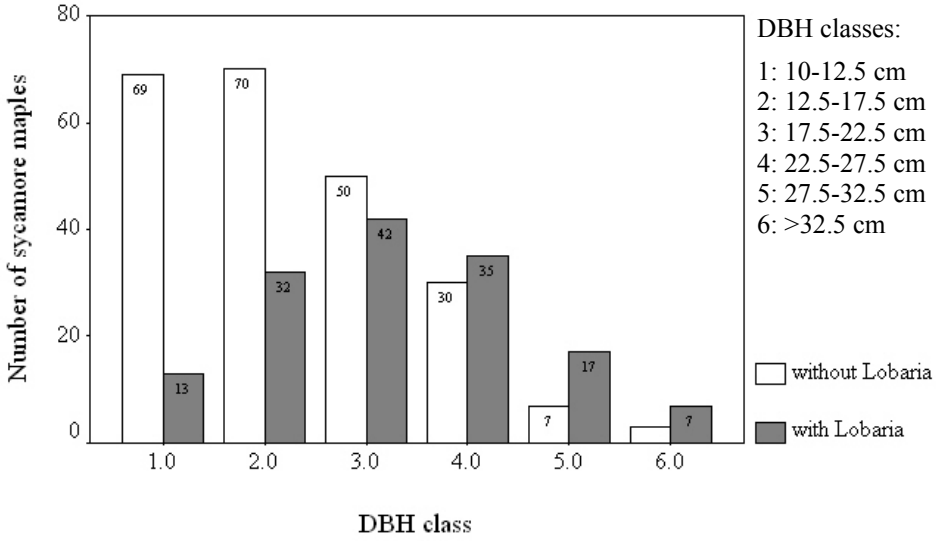


Fig. 1: The number of by *Lobaria pulmonaria* colonized and uncolonized sycamore maples per DBH class (diameter at breast height) in the study area Northeast, Swiss Jura Mountains.

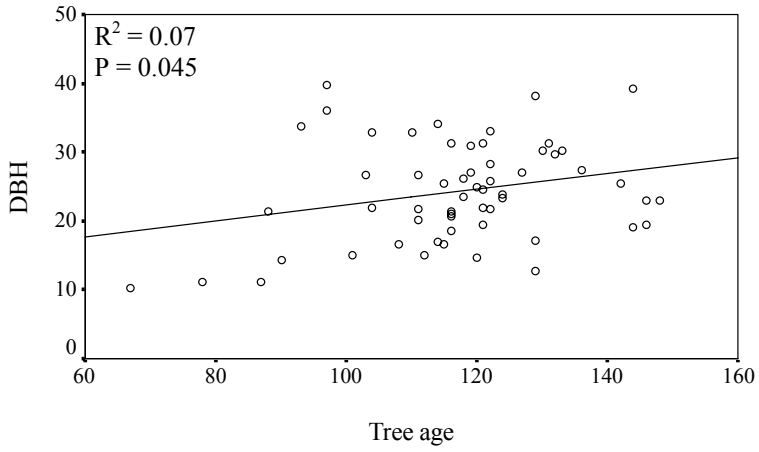


Fig. 2: Correlation of age with diameter at breast height (DBH) of the sampled sycamore maples in the study area Northeast, Swiss Jura Mountains. The significance level is 5%.

4.1.2 Spatial aggregation of host trees

The distance from a sycamore maple to its nearest colonized neighbour (NN) was significantly different between slope and hilltop (table 2). Despite this, the sycamore maples were not more clumped on the slope. The distance from each uncolonized sycamore maple to the nearest colonized neighbour was significantly smaller on the slope. In contrast, the distance from each colonized sycamore maple to the nearest colonized neighbour did not differ significantly. On average, sycamore maples with *L. pulmonaria* were located 8 m away from each other.

4.1.3 *Lobaria pulmonaria* population parameters

Regarding the density of the *L. pulmonaria* population at tree and plot level, there was no significant difference between slope and hilltop (table 2). The small effect size d indicated consistence with H_0. On average, there were 64 thalli on each tree. The sampled sycamore maples per plot were colonized by 291 thalli, on average. Of the 41 sampled sycamore maples in the study area, 2 (5%) had fertile thalli.
No significant difference was found regarding the aggregation of thalli. The effect size d at tree level was small, too. On average, there were 20 thallus groups at tree level and 93 at plot level, respectively.
Regarding the thallus area, slope and hilltop did not differ at tree and plot level. The effect size d at tree level was small. Single sycamore maples were covered with 50 dm^2 *L. pulmonaria*, on average, whereas at plot level, the thallus area was 228 dm^2. The thallus area per tree was only slightly correlated with the variables age (not significant) and DBH (significant, appendix 8). Therefore, thallus area could be used as a tree size-independent measure for *L. pulmonaria* cover on a trunk.

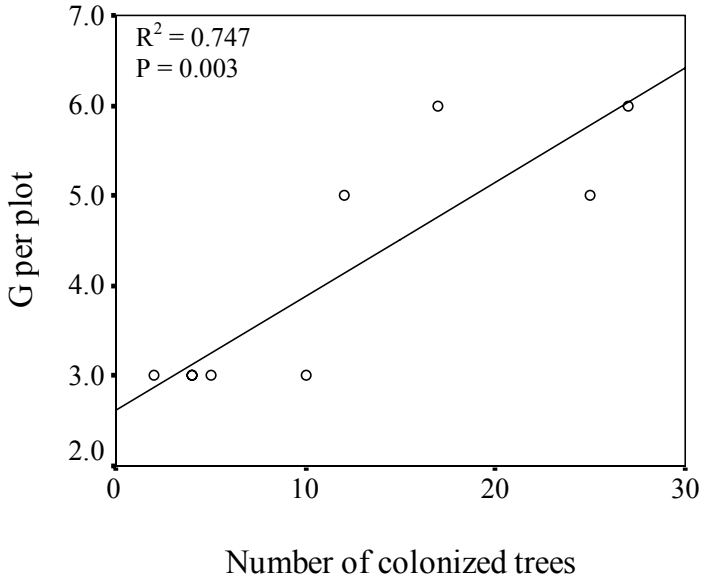
4.1.4 Population genetic parameters

The number of multilocus genotypes per plot, G, was higher on the slope than on the hilltop (table 3), possibly due to more colonized sycamore maples on the slope (table 2; figure 3a). In contrast, the correlation of the number of sampled sycamore maples per plot and the number of sampled thalli per plot, respectively, with G was week and not significant (figure 3b and c). The exact p-value of a Mann-Whitney rank test with G was 0.063, thus, the null hypothesis could not be rejected. However, the asymptotic p-value of 0.030 confirmed the alternative hypothesis. To avoid type II errors due to small sample sizes on slope and hilltop, the effect size was calculated (table 3). Compared to the effect sizes d of Cohen (1988), the effect size of G was very large. Thus, G was considered marginally significant. Contrariwise, the percentage of multilocus genotypes per plot, M, was not significant different between slope and hilltop.
At plot level, the number of colonization events, C, was significantly different between slope and hilltop whereas at tree level, there was no significant difference (table 3). C per sampled sycamore maple ranged from one to maximum three (figure 4). It was only very slightly or even not correlated at all with age, DBH, cumulative tree width or thallus area. None of the correlations was significant (figure 5).
The unbiased gene diversity (H) and genotype diversity (D) were both not significantly different between the plots from slope and hilltop (table 3). This was expected for H and could be confirmed by a small effect size. The average probability to draw two different alleles out of the Northeast's allele pool when sampled with replacement was 44% and the probability of sampling two different multilocus genotypes was 55%, respectively.

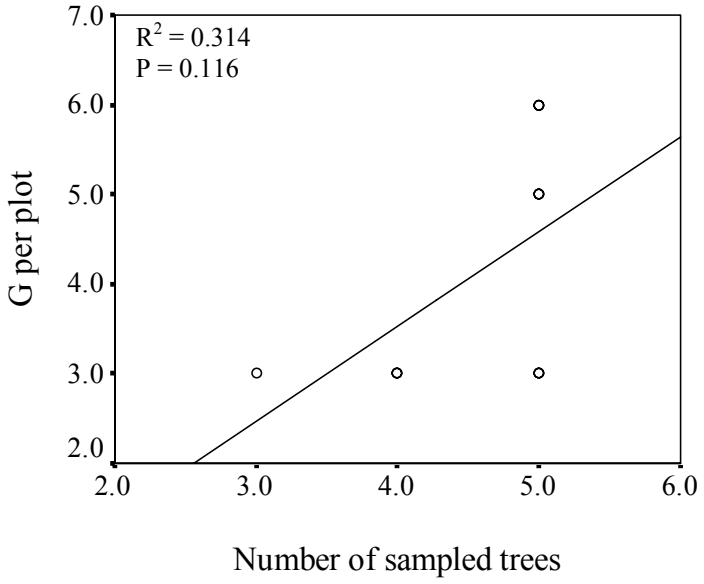
Tab. 3: P-values of the Mann-Whitney rank tests, effect sizes, means, levels of comparison and sample sizes of the number of multilocus genotypes per plot (G), percentage of multilocus genotypes per plot (M), unbiased gene diversity per plot (H), genotype diversity per plot (D) and colonization events (C) at plot and tree level of a *Lobaria pulmonaria* population. The study area Northeast in the Swiss Jura Mountains was divided up in plots on slope and hilltop. The significance level is 5%.

	P-value		**Effect size**	**Mean**		**Level of comparison**	**Sample size**	
	exact	asymptotic		slope	hilltop		slope	hilltop
G	0.063	0.030	2.20	3	5	plot	5	4
M	0.730	0.712	0.29	0.24		plot	5	4
H	0.556	0.462	0.15	0.44		plot	5	4
D	0.413	0.327	0.72	0.55		plot	5	4
$\mathbf{C_{plot}}$	0.032	0.023	2.36	3	4	plot	5	4
$\mathbf{C_{tree}}$	0.217	0.139	0.54	1		tree	24	16

3a


R^2 = 0.747
P = 0.003
G per plot
7.0
6.0
5.0
4.0
3.0
2.0
0
10
20
30
Number of colonized trees

3b


R^2 = 0.314
P = 0.116
G per plot
7.0
6.0
5.0
4.0
3.0
2.0
2.0
3.0
4.0
5.0
6.0
Number of sampled trees

3c

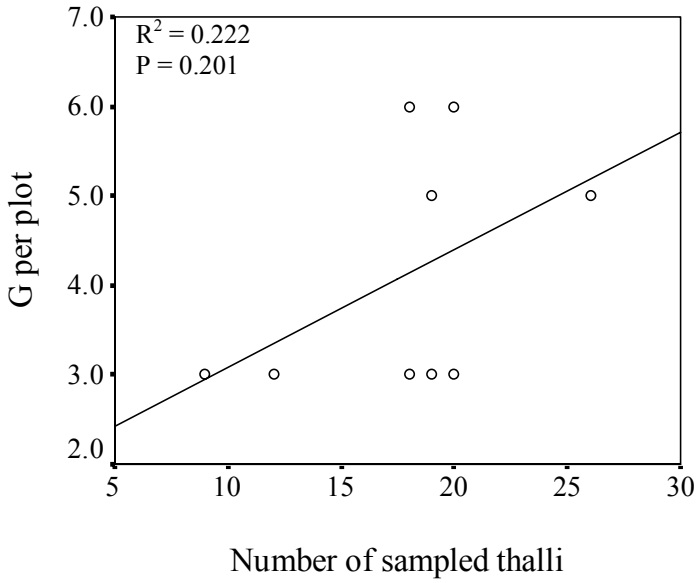
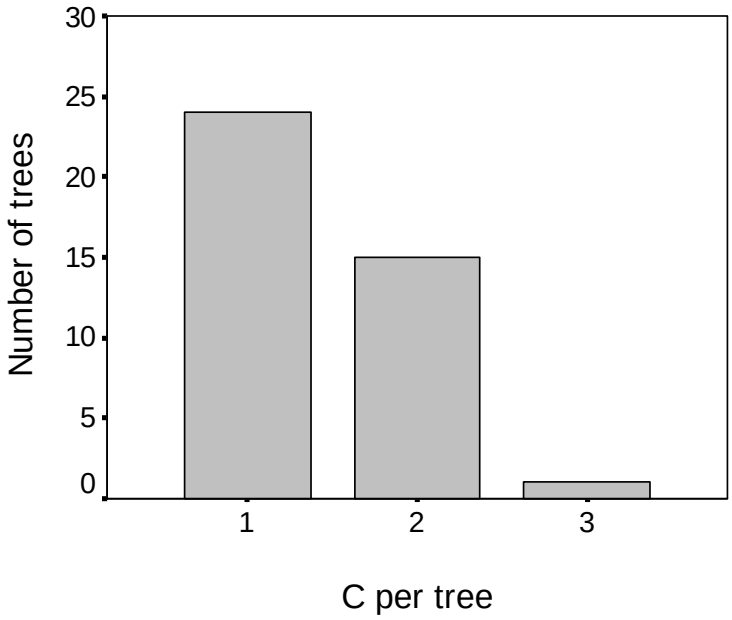


Fig. 3: Correlation at plot level of a) the number of colonized sycamore maples with the number of multilocus genotypes (G), b) the number of sampled sycamore maples with the number of multilocus genotypes (G) and c) the number of sampled thalli with the number of multilocus genotypes (G) in a *Lobaria pulmonaria* population in the study area Northeast, Swiss Jura Mountains. The significance level is 5%.

4a



4b

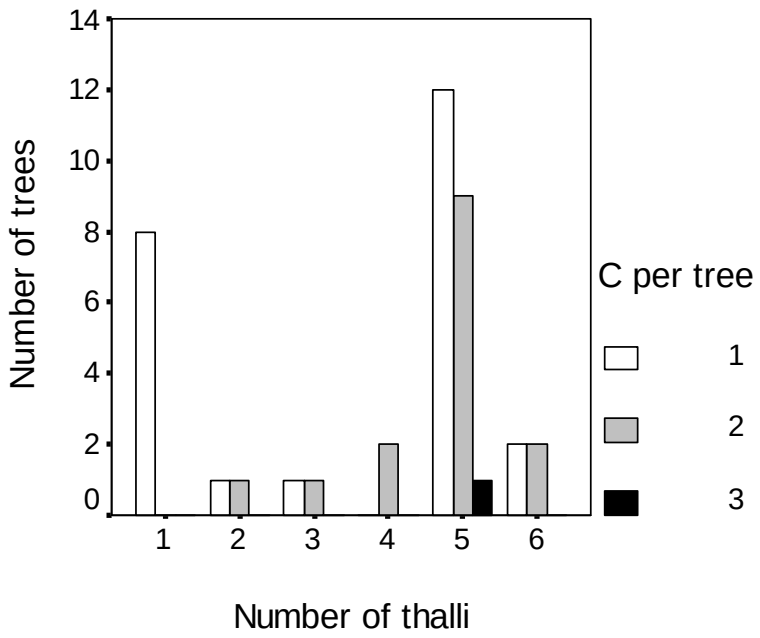
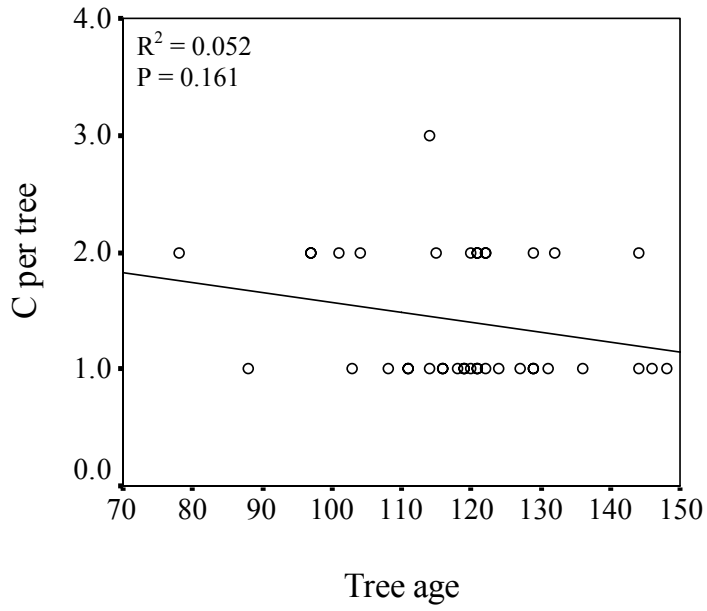
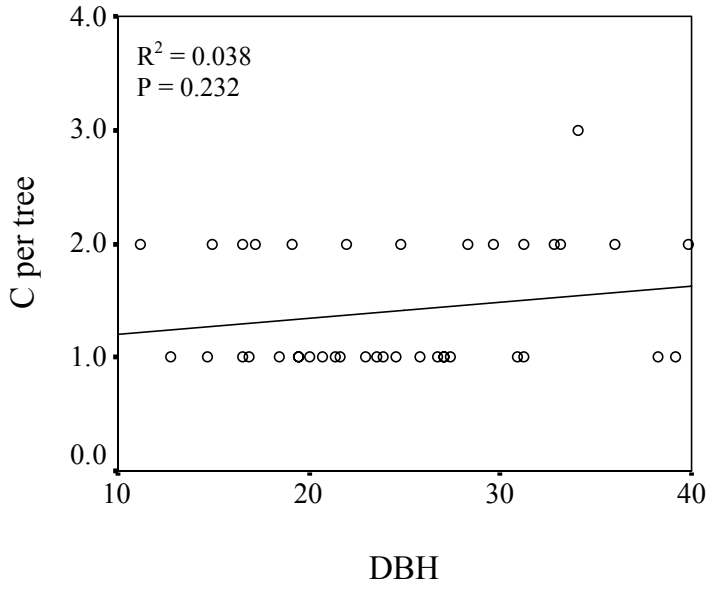


Fig. 4: In 4a), the number of sycamore maples, colonized by *Lobaria pulmonaria*, per different number of colonization events (C) is shown. In 4b), the number of sampled thalli per tree was taken into account.

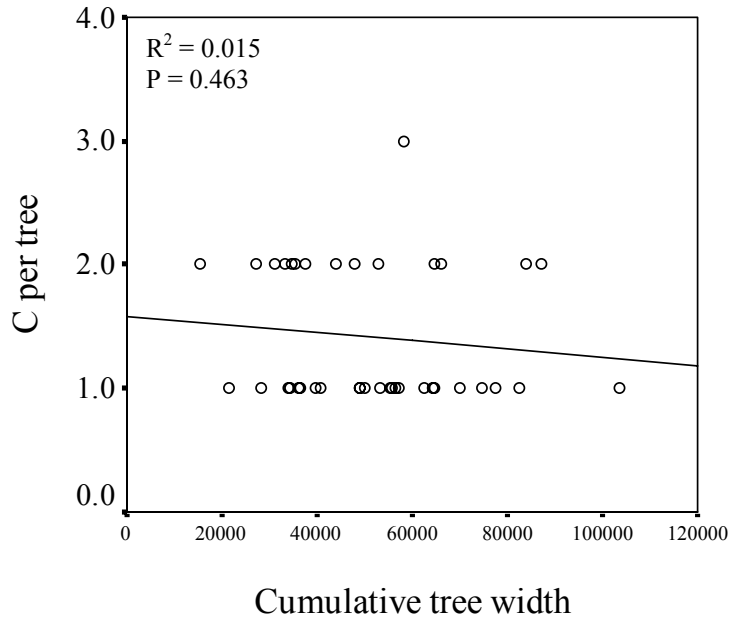
5a


R2 = 0.052
P = 0.161
C per tree
4.0
3.0
2.0
1.0
0.0
70
80
90
100
110
120
130
140
150
Tree age

5b


R2 = 0.038
P = 0.232
C per tree
4.0
3.0
2.0
1.0
0.0
10
20
30
40
DBH

5c



5d

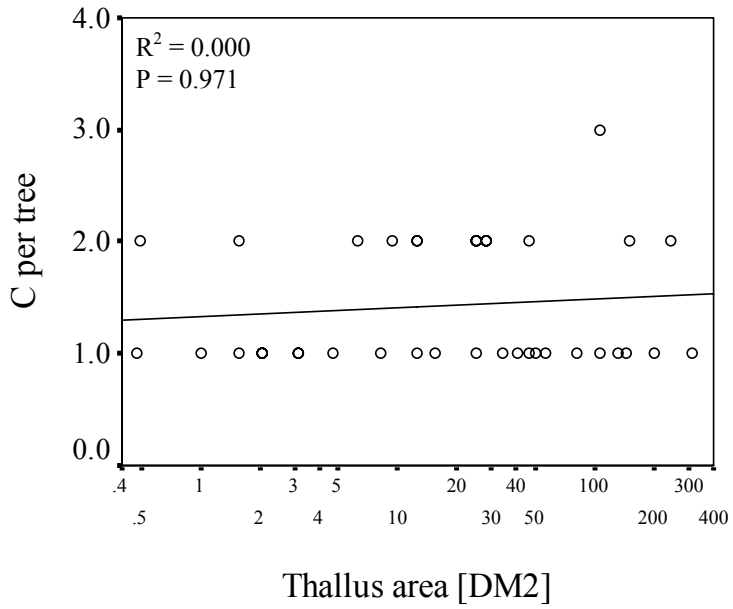


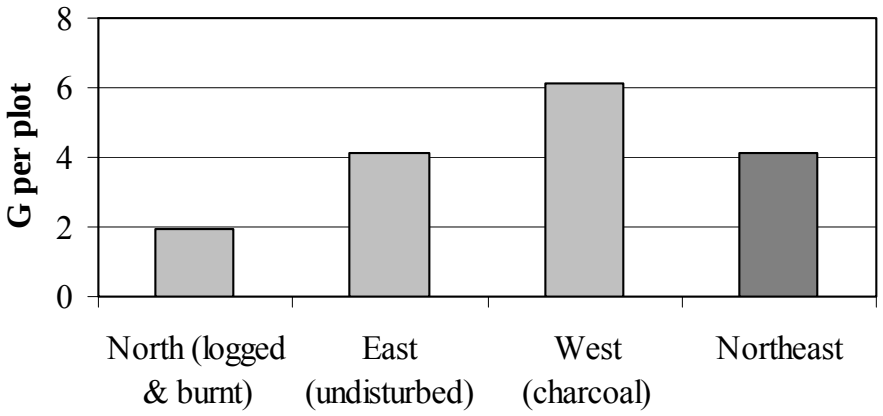
Fig. 5: Correlation of a) tree age, b) diameter at breast height (DBH), c) cumulative tree width and d) *Lobaria pulmonaria* thallus area with the number of colonization events (C) per sycamore maple in the study area North-east, Swiss Jura Mountains. The significance level is 5%.

4.2 Comparisons between study area and the other areas

4.2.1 Population genetic parameters

Figure 6 allows comparisons of the number of multilocus genotypes G per plot and the unbiased gene diversity H per plot between the population in the study area Northeast and the populations in the areas West (charcoal), North (logged & burnt) and East (undisturbed) of Werth *et al.* (subm.). Molecular analysis showed similar values of G and H for the populations Northeast and East. G and H were higher in the western, but smaller in the northern population.
The area Northeast shared one multilocus genotype with the area North, but none with the area East (appendix 9).
In contrary to this study, beeches as host trees were included in the study of Werth *et al*. (subm.). However, since colonized beeches are rare in this region (Kalwij *et al*., in press), the results with or without beeches show little variation.

6a



6b

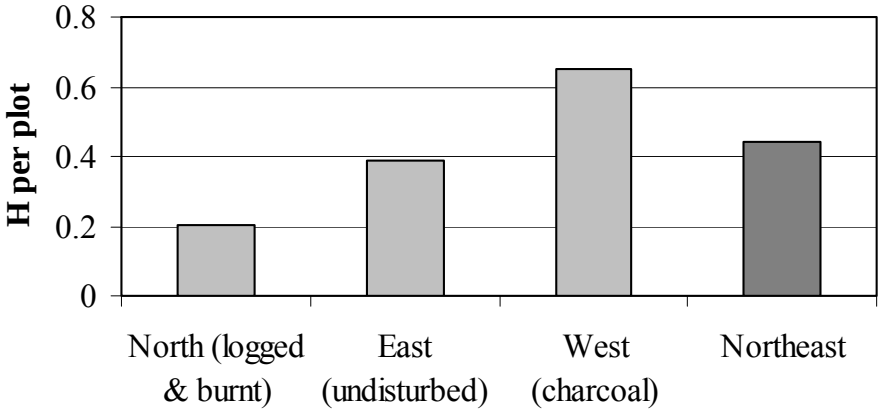
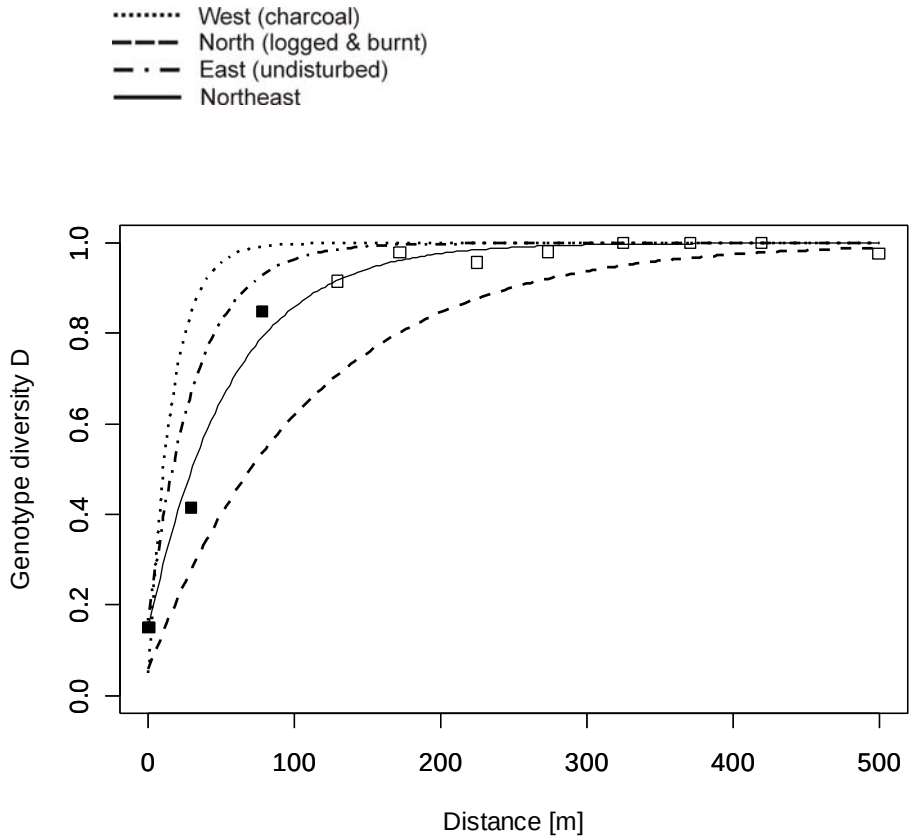


Fig. 6: Comparisons between *Lobaria pulmonaria* populations of four areas in the Swiss Jura Mountains in terms of a) the number of multilocus genotypes per plot (G) and b) the unbiased gene diversity (H). The 95% confidence intervals are not shown.

4.2.2 Spatial genetic structure

The variograms of D and H (figure 7) of the population Northeast were most similar to those of the population East regarding shape and value of the curves. However, significance tests for differences between variograms are not available.
In the populations Northeast and East, the variogram of D (figure 7a), reflecting the clonal component of the population, showed spatial autocorrelation at short distances. Up to 75 m, D increased significantly. The first distance class was significant with a value of D = 0.15 in Northeast and D = 0.20 in East. Thus, the probability of sampling two individuals of different multilocus genotypes from a tree in Northeast and East was 15% and 20%, respectively.
Similar to the areas East, West and North, the variogram of H with accounting for recurrent genotypes (figure 7b), reflecting the sexual component, showed no significant spatial autocorrelation for the population in the study area Northeast. Without accounting for recurrent genotypes (figure 7c), however, the first distance class of Northeast was significant with a low value of H = 0.1. In the area East, the first distance class was significant, too, with a similar value of H = 0.1, but the significant autocorrelation extended to 50 m.

7a



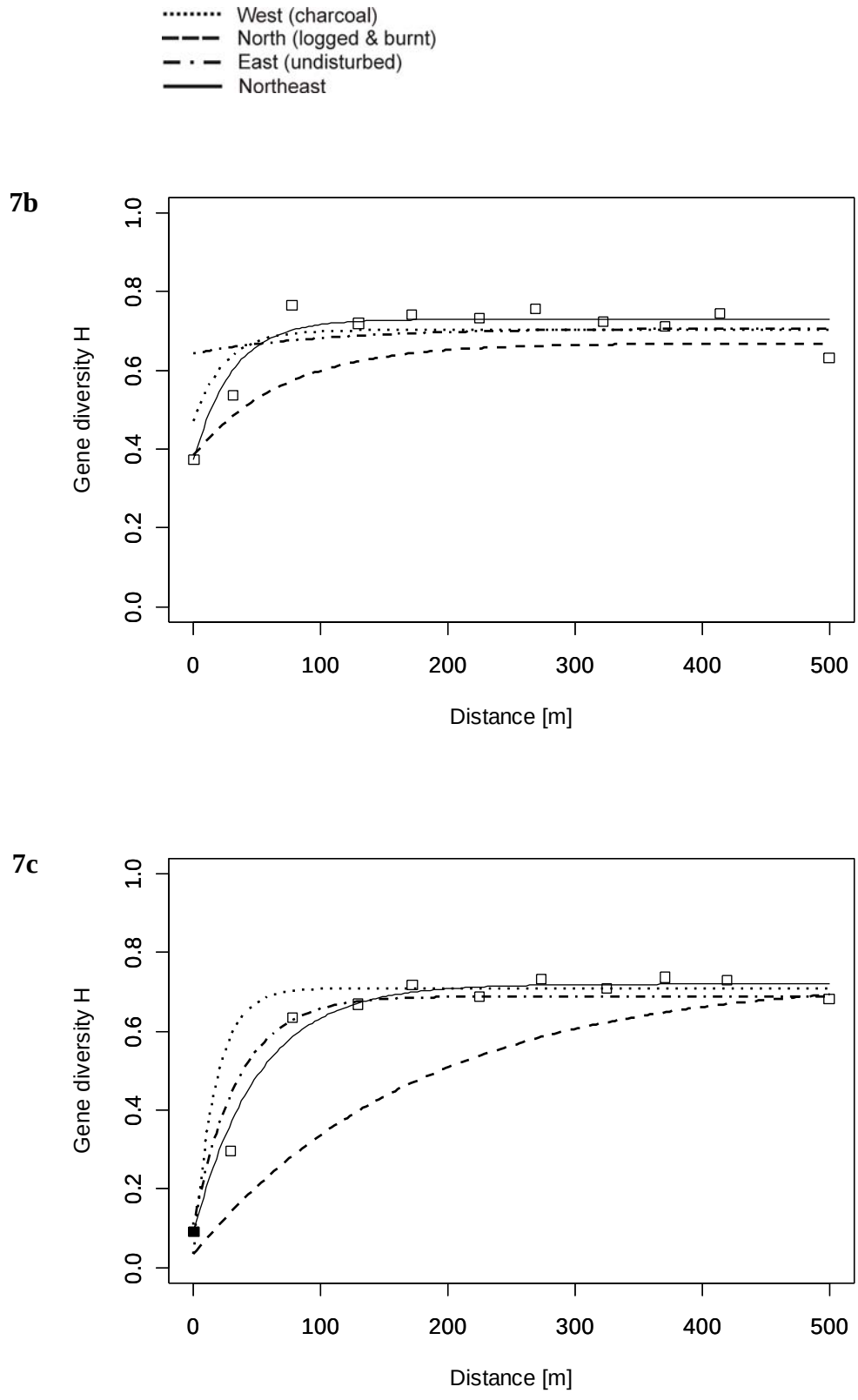


Fig. 7: Variograms of a) genotype diversity (D), b) gene diversity (H) with accounting for recurrent genotypes and c) gene diversity (H) without accounting for recurrent genotypes within *Lobaria pulmonaria* populations of four areas in the Swiss Jura Mountains. Filled symbols indicate significant spatial autocorrelation at the 5% level in the area Northeast.

4.3 Connectedness of the four areas

A molecular variance analysis (AMOVA) showed that most of the molecular variance was found among plots within the four areas Northeast, East, West and North and within plots. Among the areas, the percentage of explained variance was very small and non-significant (table 4).

Tab. 4: Analysis of molecular variance of *Lobaria pulmonaria* populations in 46 plots and 4 areas in the Swiss Jura Mountains. SS: sum of squares; d.f.: degrees of freedom; % variance: percentage of explained variance; Φ-statistics: fixation indices. An asterisk (*) indicates significance at the 5% level.

Source of variation	d.f.	SS	% variance	Φ-statistics
Among areas	3	87.6	0.92	0.009
Among plots within areas	42	923.8	49.22	0.497*
Within plots	932	943.1	49.86	0.501*
Total	977	1954.4		

Figure 8 shows pairwise F_{ST} values (in bold), which are analogous to the fixation indices Φ, and the estimated numbers of migrants per generation, N_m (in italics). Statistically significant, low genetic differentiation between the four areas indicated high gene flow. Gene flow was highest between Northeast and the undisturbed area East and lowest between Northeast and the disturbed areas West (charcoal) and North (logged & burnt). The estimated numbers of migrants per generation between the areas were highest between Northeast and East.

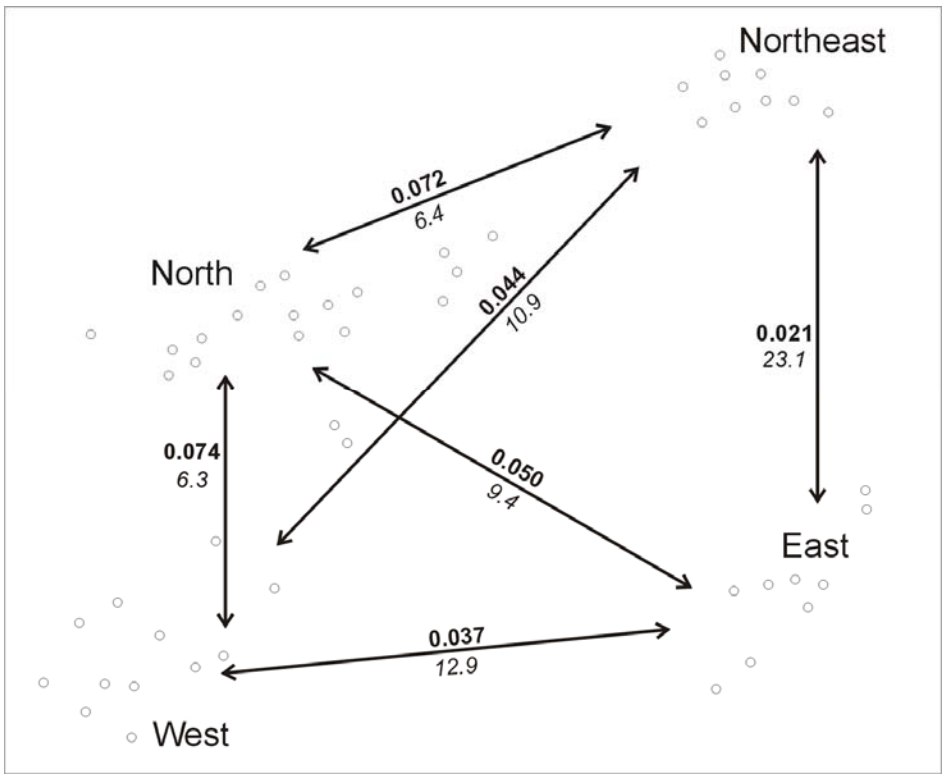


Fig. 8: Pairwise F_{ST} values (in bold) show gene flow between *Lobaria pulmonaria* populations in four areas in the Swiss Jura Mountains. All values are significant. The estimated number of migrants per generation (N_m) is given in italics.

4.4 Clonal propagation within sycamore maples

Genotype diversity D within the sampled sycamore maples was very small, only 15% (see chapter 4.2.2).
An AMOVA was performed to calculate the gene pool within trees. The AMOVA revealed that most of the molecular variance was found among sycamore maples within plots. Among plots and on single sycamore maples, molecular variance explained a significant, only small amount of the total variance. The fixation indices Φ among trees within plots and on single trees were very high and therefore, genetic differentiation was low (table 5).

Tab. 5: Analysis of molecular variance in the 9 plots of the study area Northeast, Swiss Jura Mountains. The plots contained 41 sycamore maples, colonized by *Lobaria pulmonaria*. SS: sum of squares; d.f.: degrees of freedom; % variance: percentage of explained variance; Φ-statistics: fixation indices. An asterisk (*) indicates significance at the 5% level.

Source of variation	d.f.	SS	% variance	Φ-statistics
Among plots	1	42.2	20.95	0.210*
Among trees within plots	37	236.8	66.66	0.843*
Within trees	119	33.7	12.39	0.876*
Total	157	312.7		

5 Discussion

This diploma thesis aimed to answer four main questions regarding a *Lobaria pulmonaria* population in the Swiss Jura Mountains (appendix 2).
First, comparisons were done within the *L. pulmonaria* population that is spatially distributed on a slope and a hilltop in the study area Northeast. As a tree map revealed, there were more colonized sycamore maples on the slope than on the hilltop, while the host tree density was not significantly different (Kalwij *et al*., in press; appendix 4). Does the difference in colonized trees reproduce at genetic level, indicating two different populations of *L. pulmonaria* in the study area Northeast? Can possible differences be explained by spatial aggregation of the host trees or habitat parameters?
Molecular analysis suggested that the study area Northeast contains only one *L. pulmonaria* population. Thus, taking a second step and comparing this population to the populations of the undisturbed area East and the disturbed areas West (charcoal) and North (logged & burnt) of Werth *et al.* (submitted) was legitimate. Comparisons of population genetic parameters and spatial genetic structure should reveal whether or not the study area Northeast is an undisturbed rather than a disturbed area.
The third main question dealt with the connectedness of the four areas. Assumed historical gene flow was used as a measure of connectedness.
Finally, the clonal propagation of *L. pulmonaria* at tree level after few colonization events was investigated.

5.1 Differences between slope and hilltop

Molecular analysis showed that gene diversity H and genotype diversity D at plot level were not significantly different between slope and hilltop. However, more multilocus genotypes G per plot were found on the slope than on the hilltop (tab. 3). A possible explanation could be the higher number of colonized sycamore maples on the slope as G increased significantly with the number of colonized trees per plot (tab. 2, fig. 3).

On the slope, the minimum number of colonization events C per plot was higher than on the hilltop (tab. 3). Because slope and hilltop did not differ in the density of the sycamore maples (tab. 2), we tested if the spatial aggregation of the sycamore maples could lead to a different minimum number of colonization events C. Dispersal of propagules has been shown to be more effective in a clumped host population than in a randomly distributed population (Gu, 2001). However, the cluster index calculated by Kalwij *et al*. (in press) did not show a higher aggregation of sycamore maples on the slope than on the hilltop (tab. 2). Compared to the hilltop, the mean distance between the sycamore maples and the nearest colonized neighbour (NN) on the slope was significantly smaller, indeed. But the marginal difference of five meters probably did not affect the number of colonization events. There might be other factors than host tree aggregation affecting C.
Tree surface and duration of exposure are assumed to explain C. Stand structural parameters such as diameter at breast height (DBH) and age therefore both influence the probability of colonization. The older and thicker a tree, the more colonization events may occur (Riiali *et al*., 2001). However, we found neither significant correlation of C per tree with age or DBH, nor of C per tree with the cumulative tree width containing both variables (fig. 5).
Alternatively, microclimate may affect germination of propagules as light conditions and air moisture are considered to be important factors for lichen growth (McKenzie *et al*., 2001).

We found no evidence for difference in light conditions, measured by local tree cover, between slope and hilltop (tab. 2). As the colonized sycamore maples were densely covered with many large thalli, the *L. pulmonaria* population was relatively luxuriant. Hence, light conditions for growth may be considered beneficial in the study area Northeast. Regarding humidity, differences between slope and hilltop could originate from topographical differences. It is possible that wind transports humid air onto the slope being downwind or that a cold air basin is formed in the little valley, bordered by the slope.

5.2 Comparisons between Northeast and East, West and North

Molecular analysis revealed that the *Lobaria pulmonaria* population in the study area Northeast bear resemblance to the population of East that was considered undisturbed and autochthonous (Werth *et al*., subm.). As figure 6 shows, the number of multilocus genotypes (G) as well as the unbiased gene diversity (H) had very similar values. Hence, there is evidence that the north-eastern population is undisturbed and autochthonous, too.
Similarities between Northeast and East regarding spatial genetic structure, i.e., shape and value of the variograms of gene diversity (H) and genotype diversity (D), were also observed (fig. 7). Reflecting the clonal component of the population, the variograms of D showed significant spatial autocorrelation at short distances in both studies. As the distance from a tree increased, the probability of sampling two individuals of different multilocus genotypes also increased. Thus, the dispersal of clonal propagules was greatest just around the source tree and then decreased with increasing distance. This implies that clonal propagules are restricted to short distances as many studies have suggested. Walser *et al*. (2001) detected a local dispersal range up to 50 m away from the source tree within five days. Other authors used mainly indirect methods such as population structure (Dettki *et al*., 2000; Walser, 2004) or transplantation experiments (Sillett *et al*., 2000, Hilmo 2001) to determine dispersal distances during certain periods.
On the contrary, the variogram of H with down weighted recurrent genotypes, reflecting the sexual component, showed no significant spatial autocorrelation in all four areas. With increasing distance from the source tree, the variance of H did not increase significantly. H seemed to be independent from distance. This indicates that the dispersal of ascospores may not be restricted to short distances. Moreover, they were believed to be more suitable for long-distance dispersal than vegetative propagules which were effective at short distances (Bailey, 1976).

5.3 Connectedness of Northeast with East, West and North

Molecular variance analysis revealed high gene flow between Northeast and the areas East, West and North of Werth *et al.* (subm.) in terms of F_{ST} values (tab. 4; fig. 8). F_{ST} values may indicate historical gene flow. In this study, the low genetic differentiations between Northeast, East, West and North are assumed to originate from a time where these areas were still connected to one forest containing a big *Lobaria pulmonaria* population. The pasture of about 1 km which separates the area East from the areas West and North was established in the 12th century (Sjögren, 2005). Before, the landscape was probably connected. However, there is no evidence that this pasture represented an effective boundary to past gene flow (Werth, 2005).

Assignment tests by Werth (2005) revealed that the populations of East, West and North can be assigned to the same three allele pools. This result may indicate that East, West and North are subpopulations of a once bigger population.

5.4 Colonization of sycamore maples and clonal propagation

Although the sampling strategy was designed in order to maintain five different thalli per trunk, i.e., to maximize the number of multilocus genotypes (G), the number of colonization events C per sycamore maple was small. Even in cases where five thalli could actually be sampled per sycamore maple, only one or two colonizers successfully established on the trunk (fig. 4). Despite a rather small C per sampled tree, there was a high number of thalli per tree, indicating that once a propagule has been established, reproduction and further local dispersal on the host tree is very effective (tab. 2).
Clonal propagation may be an effective reproduction strategy. The variogram of the multilocus genotype diversity D showed that on a single tree, the probability of sampling two individuals of different multilocus genotypes is less than about 15% (fig. 7). Thus, most of the sampled thalli per tree were of the same genotype. An AMOVA revealed that genetic differentiation within single trees was much lower than between trees. On single sycamore maples, the percentage of explained variance was only 13% and the fixation index was very small (tab. 5). The low genetic differentiation within single trees is evidence that propagation on single trees mainly occurs vegetatively.
This finding agrees with one of the three different colonization scenarios of Walser *et al.* (2003) that expects no or low genetic variation on a tree if after a successful colonization, the thallus acts as a founder. Vegetative dispersal may lead to dense *L. pulmonaria* populations on trees and is assumed to be more effective than colonization of trees.
Vegetative dispersal on trees may occur through stem flow that transports propagules downwards on trunks, but also through splashes resulting in lateral dispersal (Bailey, 1966; Armstrong, 1981). Animals may act as vectors on trunks, too. For example, Bailey (1970) found ants carrying soredia. Viable ascospores were detected in faecal pellets of lichenivorous mites (Meier *et al*., 2002). Propagules of *Lobaria amplissima* may even germinate out of snails' excrements (C. Scheidegger, personal communication). While water and certain animals are important for local dispersal, wind may be a vector for long-distance dispersal (Walser *et al.*, 2001).

Whether colonization events are frequent in a population depends on the production of vegetative propagules and ascospores. There is evidence that ascospores can only be formed in dense and diverse populations (Zoller *et al*. 1999; Walser *et al*., 2003). In the study area, only 5% of the sampled sycamore maples had fertile thalli, indicating a low production of ascospores. However, it is not only important how many propagules reach a host tree, but also if establishment is successful and if the microclimate (see chapter 5.1) is favourable for growth.
Substrate quality in terms of bark texture plays a major role in the entrapment and establishment of propagules. Armstrong (1981) observed colonization of smooth bark and rocks first by crustose lichens and later, when larger cracks are formed, by *Parmelia sp.* Trees with thick trunks usually have a more cracked bark than thinner ones and therefore, the DBH can be used as an indicator for substrate quality. According to Kalwij *et al*. (in press), the proportion of colonized sycamore maples increased considerably with increasing DBH, possibly due to improved substrate quality. In this study, the proportion of colonized sycamore maples of the two DBH classes, to which most trees belong, was 16% and 31%, respectively (fig. 1).
Competition can hinder a successful establishment of propagules. In this study, intra-specific competition as well as inter-specific competition between *L. pulmonaria* and moss or crustose lichens was not investigated because uncolonized host trees were not included in the sampling design.

6 Conclusions and perspectives

The *Lobaria pulmonaria* population of the study area Northeast was considered as one population without subdivision in slope and hilltop since gene diversity (H) and genotype diversity (D) per plot did not differ (tab. 3). Therefore, it could be compared to the undisturbed area East and the disturbed areas West (charcoal) and North (logged & burnt) of Werth *et al.* (subm.).
However, the minimum number of colonization events (C) per plot was significantly higher on the slope than on the hilltop, probably due to more beneficial air moisture. The number of multilocus genotypes (G) per plot was higher on the slope, too, by reason of a higher number of colonized sycamore maples (tab. 2 and 3).
Regarding population genetic parameters such as the number of multilocus genotypes (G) and gene diversity (H) per plot, the *L. pulmonaria* population of the study area Northeast was most similar to the undisturbed area East of Werth *et al.* (subm., fig. 6). Therefore, we assume that the study area Northeast is undisturbed, too. The spatial genetic structure in the northeastern population also seemed to be very similar to the eastern population (fig. 7). However, differences between variograms could not be tested because significance tests are still lacking.

High gene flow between the areas Northeast, East, West and North probably shows historical gene flow indicating the connectedness of the areas in the past (fig. 8). If the landscape contained one ample *L. pulmonaria* population in former times, the areas Northeast, East, West and North would have been assigned to the same allele pool. According to Werth (2005), the populations of East, West and North contain the same three allele pools. Thus, they may be remnants of a once bigger population. To decide if the *L. pulmonaria* population of Northeast is another subpopulation of the big population, further assignment tests should be performed.
There is evidence that *L. pulmonaria* populations on the sampled sycamore maples were dense, despite a small number of colonization events (C) per tree (fig. 4). The luxuriant populations on the sycamore maples probably developed due to clonal propagation and rapid dispersal within trees. Thus, propagation and local dispersal seemed to be more effective than the colonization of a tree per se. It would be interesting to know how many colonization events actually occur on trees, if more sycamore maples per plot, more thalli per sycamore maple and thalli from higher than four meters were sampled.
Recombination is assumed to occur only between genotypes from the same trees (C. Scheidegger, personal communication). Thus, fertile thalli on trees indicate recombination within trees. Since recombination enhances gene diversity (H), trees with fertile thalli should have a high gene diversity. In contrast to H, C is not affected by recombination. Further investigations might enlighten the interaction of fertility and H at tree level.

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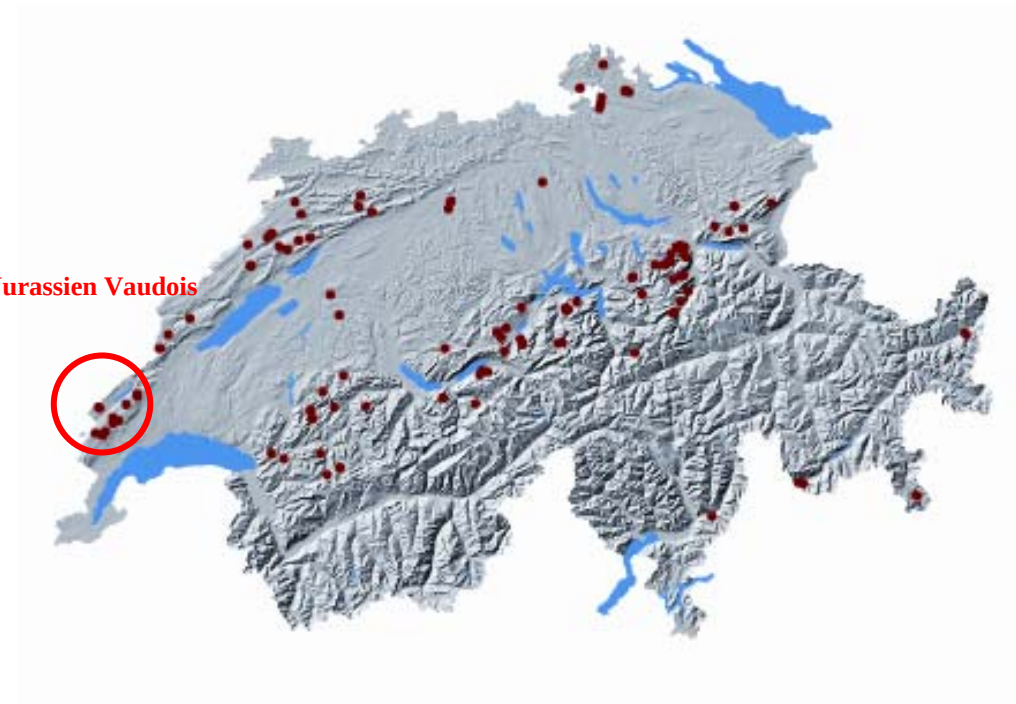
Appendix

Appendix 1: *Lobaria pulmonaria*

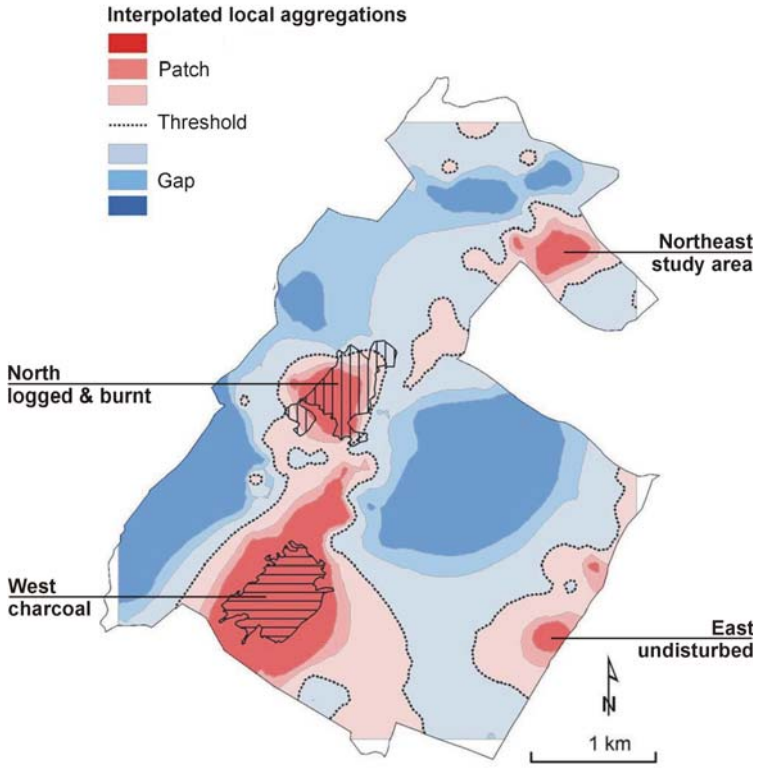
App. 1: The epiphytic lungwort *Lobaria pulmonaria* on a sycamore maple.

Appendix 2: Distribution of *Lobaria pulmonaria* in Switzerland



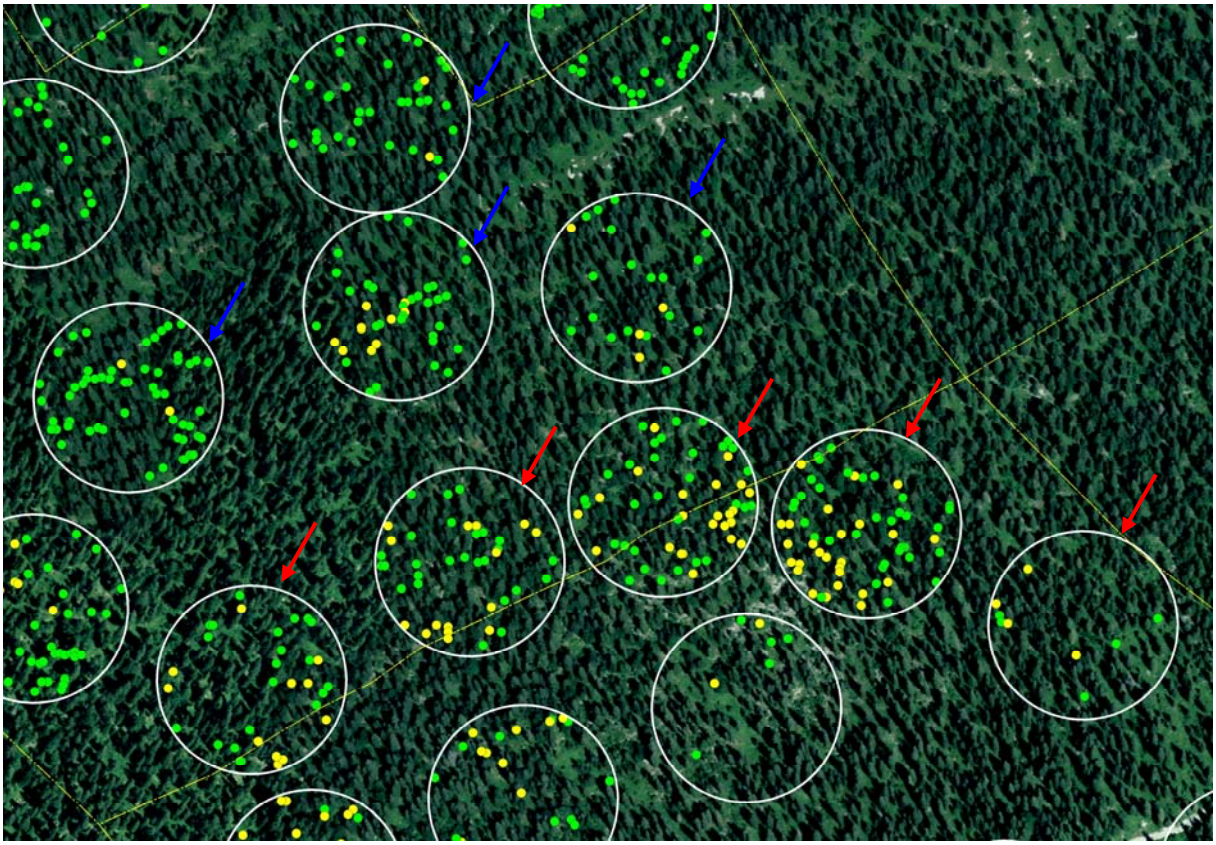
App. 2: Current distribution of *Lobaria pulmonaria* populations in Switzerland. The study area Northeast was located in the Parc Jurassien Vaudois in the Jura Mountains. Source: Stofer, S., Scheidegger C., Dietrich M., Frei M., Groner U., Keller C., Roth I., Sutter F., Zimmermann, E (2003). SwissLichens. http://www.swisslichens.ch

Appendix 3: Four *Lobaria pulmonaria* "hot spots"



App. 3: Study area of Kalwij (2005) in the Swiss Jura Mountains. Hot spots (patches) of *Lobaria pulmonaria* populations are shown in red. The area East was considered undisturbed, the areas West and North were considered disturbed (Kalwij, 2005; Werth *et al.*, subm.). North-east was the study area of this diploma thesis.
Figure adapted from Kalwij, 2005.

Appendix 4: Colonized and potential host trees of *Lobaria pulmonaria*



App. 4: Green points: potential host trees, i.e., sycamore maple and beech, of *Lobaria pulmonaria*. Yellow points: sycamore maples and beeches colonized by *Lobaria pulmonaria*. The study area consisted of 9 plots. Blue arrows: plots on the hilltop, red arrows: plots on the slope.
Figure adapted from Kalwij, 2005.

Appendix 5: Sampling plots

App. 5: Sampling plots in the study area Northeast, Swiss Jura Mountains.

Plot ID	Communities	Plot coordinates (xy)		Plot position	Elevation	Sampled maple trees	Collected thalli	Analyzed thalli
Wer126	Le Chenit	507044	157178	slope	1362.46	5	41	26
Wer208	Le Chenit	507175	157249	slope	1366.40	5	30	20
Wer178	L'Abbaye	507291	157285	slope	1357.79	5	18	18
Wer177	L'Abbaye	507413	157272	slope	1347.45	5	43	19
Wer198	L'Abbaye	507543	157211	slope	1341.75	5	45	20
						25	**177**	**103**
Wer225	Le Chenit	506970	157348	hilltop	1381.16	4	12	12
Wer128	Le Chenit	507132	157403	hilltop	1387.30	5	19	19
Wer162	Le Chenit	507275	157414	hilltop	1384.27	4	23	18
Wer214	Le Chenit	507118	157516	hilltop	1384.53	3	9	9
						16	**63**	**58**
Total						**41**	**240**	**161**

Appendix 6: Extraction Protocol

1. Lyophilise about 50 mg of fresh material in 2ml Eppendorf tube (or in blue 96 well) containing 1 steel or glass bead.
2. Disrupt plant material in Retsch Mixer Mill (MM 300); 2 min at 30.00 amplitude (=30 Hz).
3. Combine Buffer AP1, RNase A and Reagent DX according to table.

	Per sample		Mix for 2*96 samples (based on 220 samp.)	
	Embryo	Leaf / Lichen	Embryo	Leaf / Lichen
Buffer AP1 (65°C)	450 µl	600 µl	99 ml	132 ml
RNase A	1 µl	1 µl	220 µl	220 µl
Reagent DX* (65°C)	1 µl	1 µl	220 µl	220 µl

* antifoaming agent

4. Incubate for 5 min and then ensure the samples are properly suspended (vortexing may be necessary)
5. Centrifuge (Sigma 4K15) the samples for 5 min at 5600 g to pellet cell debris *(18 min at 3700 g)*.
6. Using 102 mm long tips, transfer 400 µl of supernatant to a new set of labeled collection microtubes.
7. Add 130 µl Buffer AP2 to each lysate and close the tubes with caps provided. Place the clear cover over the rack of collection microtubes and shake vigorously for 15 sec. Buffer AP2 precipitates proteins and polysaccharides.
8. Centrifuge the microtubes for 10 sec at 1500 g (short spin) to collect drops from the caps. Incubate for 10 min (up to 30 min) at –20°C.
9. Centrifuge for 5 min at full speed (5600 g) *(10 min at 3700 g)*.
10. Using 102 mm long tips, transfer 400 µl of each supernatant to a new set of labeled collection microtubes.
11. Add 1.5 vol. (typically 600 µl) Buffer AP3/E. Close the collection microtubes with new caps. Place the clear cover over the rack of collection microtubes and shake vigorously for 15 sec. Buffer AP3/E promotes binding of the DNA to the silica gel membrane.
12. Centrifuge the rack for 10 sec at 1500 g (short spin) to collect drops from the caps. Remove and discard caps.
13. Place a DNeasy 96 plate on top of a square-well block. Label the plate for later sample identification.
14. Using 102 mm long tips, carefully apply 1 ml of each sample to each well.
15. Seal the plate with tape sheet. Centrifuge for 4 min at full speed (5600 g) *(3700 g)*. Centrifuge for a further 4 min if not all of the lysate passed through the membrane.
16. Remove the tape sheet. Add 800 µl of Buffer AW to each well. Buffer AW removes further impurities e.g. polysaccharides and polyphenols.
17. Seal the plate with tape sheet. Centrifuge for 15 min at full (5600 g) *(3 min at 3700 g)* to dry the membranes.

18. To evaporate any remaining EtOH, orientate the DNeasy membranes of labeled elution tubes and put in 65°C oven for no more than 5 min.
19. Elute the DNA by adding 100 µl of Buffer AE to each well. Seal the plate with tape sheet. Incubate for 1 min at room temperature. Centrifuge at full speed (5600 g) *(3700 g)* for 2 min.
20. Repeat step 19 with a further 100 µl of Buffer AE.
21. Short-term storage (max. 2 days) of samples at 4°C. For longer term storage, freezing at –20°C is recommended.

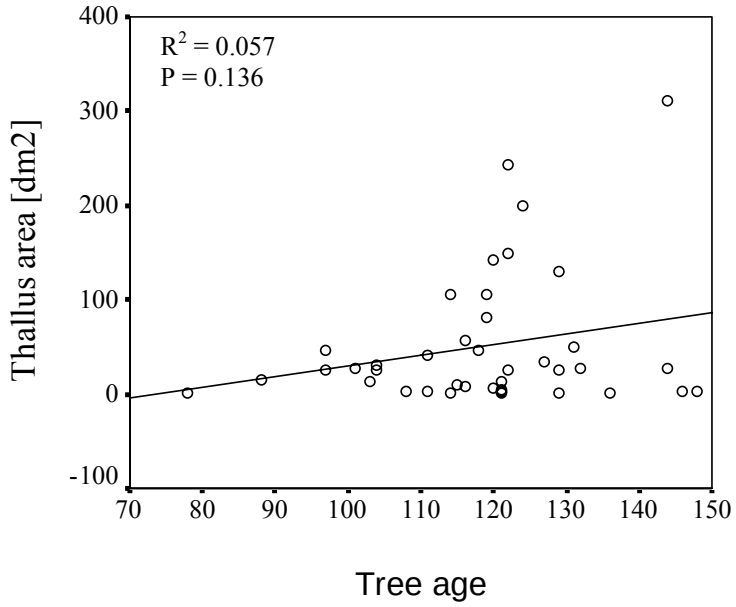
Appendix 7: Number of different alleles and allele sizes

App. 7: Number of different alleles and allele sizes at six microsatellite loci within the *Lobaria pulmonaria* population in the study area Northeast, Swiss Jura Mountains. The plots are distributed on slope and hilltop.

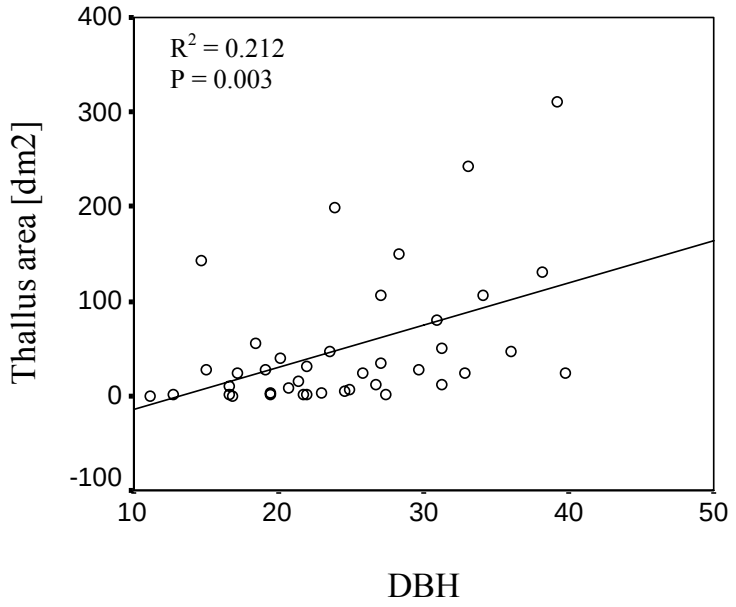
Plot	**Location**	**LPu03**	**LPu09**	**LPu15**	**LPu16**	**LPu20**	**LPu27**
Wer214	hilltop	186	200	159	195	207	195
				163	209	215	199
Wer225	hilltop	186	200	159	193	195	195
			389	161	203	205	199
			434	163	209	207	
Wer128	hilltop	186	200	163	209	207	197
						209	199
Wer162	hilltop	186	182	153	193	207	195
		188	200	159	207	209	199
		190	209			211	
Number of different alleles	**hilltop**	**3**	**5**	**4**	**5**	**6**	**3**
Wer177	slope	186	200	153	193	187	193
		188	209	161	197	199	195
			389	163	211	205	197
						215	
Wer178	slope	180	200	159	193	197	193
		186	209	161	195	203	197
		188	434		197	205	
			452			207	
						215	
Wer198	slope	186	380	161	193	205	195
			389		203	209	
			416				
Wer208	slope	186	200	153	193	203	193
			209	159	197	205	195
			398	161	203	207	
			425		205	209	
						211	
Wer126	slope	186	182	177	203	207	195
		188	209	161	205	209	
			398	169			
			407				
Number of different alleles	**slope**	**3**	**11**	**6**	**6**	**9**	**3**
Total number of different alleles		**4**	**11**	**6**	**8**	**10**	**4**

Appendix 8: Correlation of age and DBH with thallus area

a

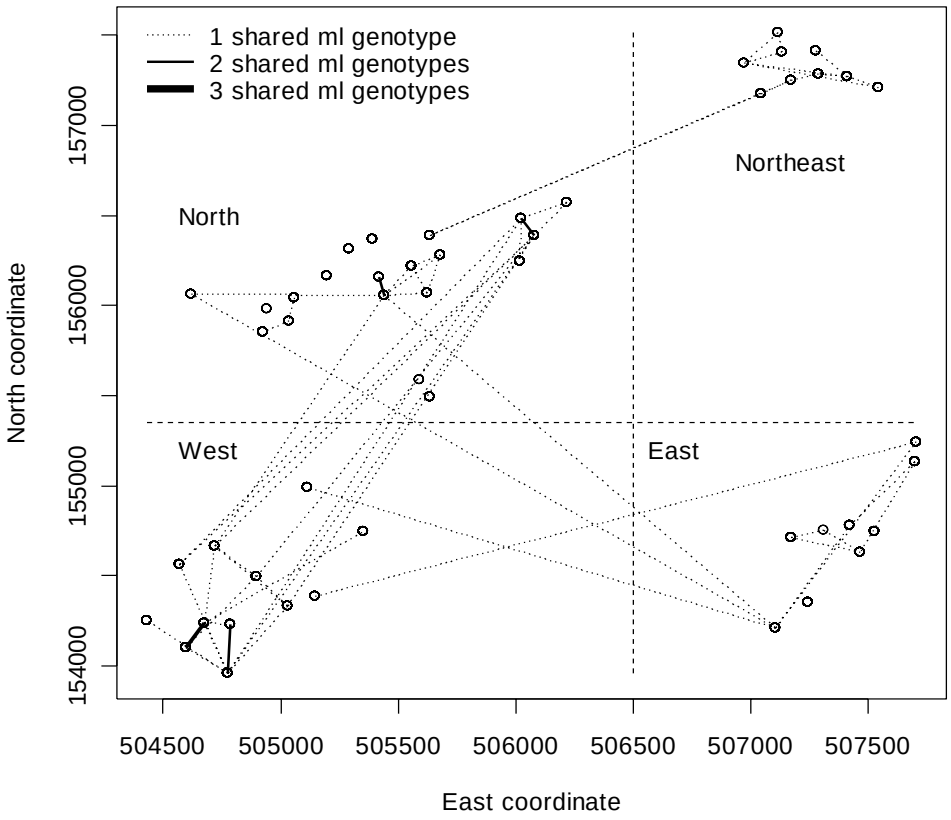


b



App. 8: Correlation of a) age and b) diameter at breast height (DBH) of the sampled sycamore maples with thallus area [dm^2] per tree of *Lobaria pulmonaria* in the study area Northeast, Swiss Jura Mountains. The significance level is 5%.

Appendix 9: Shared multilocus genotypes between plots



App. 9: Map of 1ha-plots in four areas in the Swiss Jura Mountains. The lines show shared multilocus genotypes of *Lobaria pulmonaria* between the plots.