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Abstract

Lichens associated with old forest are commonly assumed to be negatively affected by tree logging or natural disturbances. However, in this study performed in a spruce-dominated sylvopastoral landscape in the Swiss Jura Mountains, we found that genetic diversity of the epiphytic old-forest lichen *Lobaria pulmonaria* depends on the type of disturbance. We collected 923 thalli from 41 sampling plots of 1 ha corresponding to the categories stand-replacing disturbance (*burnt*), intensive logging (*logged*) and uneven-aged forestry (*uneven-aged*), and analysed the thalli at six mycobiont-specific microsatellite loci. We found evidence for multiple independent colonisations of demes located in burnt and logged areas. Using spatial autocorrelation methods, the spatial scale of the genetic structure caused by the clonal and recombinant component of genetic variation was determined. Spatial autocorrelation of genotype diversity was strong at short distances up to 50 m in *logged* demes, up to 100 m in *uneven-aged* demes, with the strongest autocorrelation up to 150 m for *burnt* demes. The spatial autocorrelation was predominantly attributed to clonal dispersal of vegetative propagules. After accounting for the clonal component, we did not find significant spatial autocorrelation in gene diversity. This pattern may indicate low dispersal ranges of clonal propagules, but random dispersal of sexual ascospores. Genetic diversity was highest in logged demes, and lowest in burnt demes. Our results suggest that genetic diversity of epiphytic lichen demes may not necessarily be impacted by stand-level disturbances for extended time periods.

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Introduction

Genetic diversity of natural populations, an important component of biodiversity, may be affected by disturbance (Bradshaw 2004; Baucom *et al.* 2005; Volis *et al.* 2005). In forested ecosystems, wind-throws and fires belong to the natural disturbance regime. In many parts of Central Europe, human influence has added various types of forest disturbances (Bradshaw 2004). For instance, the traditional sylvopastoral landscapes found in many mountainous areas of Europe are regularly disturbed by cattle grazing or timber logging (Kirby *et al.* 1995; Gillet *et al.* 2002). At the same time, however, these systems often have a high species richness (Vittoz 1998) and may harbour e.g. populations of endangered old-forest species such as the epiphytic lichen *Lobaria pulmonaria* (Zoller *et al.* 1999).

If a disturbance event leads to a significant reduction in the habitat of a particular population, the local population size generally declines. This may lead to a population bottleneck, involving an instant loss of rare alleles (Hartl & Clark 1997). If population size remains small over an extended time period, rare alleles may further be lost due to genetic drift, leading to a continuing decrease of genetic diversity over time (Nei *et al.* 1975), particularly in terms of allelic richness (Widmer & Lexer 2001). Subsequently, other population genetic consequences of small population sizes may occur, as the population enters an extinction vortex (Tanaka 2000; Frankham *et al.* 2002). In self-incompatible organisms such as some plants or fungi, the likelihood that compatible mating types are present is low in very small populations, and their absence may prevent sexual reproduction (Vekemans *et al.* 1998; Zoller *et al.* 1999; Taylor *et al.* 2000; Walser *et al.* 2004). On the other hand, if disturbance has caused the local extinction of a species, the habitat is open for recolonisation during forest succession. In many cases, the habitat is recolonised by only a few founders, leading to low genetic diversity in the new population (founder event; Hartl & Clark 1997). However, as new individuals (or gametes in plants and fungi) enter the population over time, genetic diversity increases and the footprints of severe bottlenecks or of founder events are successively erased.

In several insect-pollinated woody plant species, gene flow was not reduced under silvicultural management (Nason & Hamrick 1997; Sork *et al.* 2005), and in a wind-pollinated tree species, habitat fragmentation even led to facilitated pollen movement and thus increase gene flow, explaining why high genetic diversity was found in remnant populations (Bacles *et al.* 2005). However, when comparing continuous and naturally fragmented populations in a tropical tree species, fragmented populations exhibited slightly lower genetic diversity (Pither *et al.* 2003). Studies of vascular epiphytes have shown lower genetic diversity of disturbed, spatially isolated populations following forest fragmentation (Gonzalez-Astorga *et al.* 2004; Trapnell & Hamrick 2005). An interesting question is if cryptogamic epiphytes, e.g. bryophytes, lichens, and fungi, also exhibit lower genetic diversity in disturbed populations, even though they mostly have smaller, probably more easily wind-dispersed diaspores than some vascular epiphytes (Bailey 1976).

Lichens have a long generation time, which is one reason why they are considered sensitive to disturbances such as logging (Gilbert 1977; Seaward 1982). Consequently, random sampling effects such as bottlenecks or founder events in the population history should be imprinted in the genetic diversity of lichens for a comparatively long period. Recent studies in population genetics of lichens have mainly focused on large spatial scales, comparing populations among regions and continents

(Högberg *et al.* 2002; Myllys *et al.* 2003; Printzen & Ekman 2003; Printzen *et al.* 2003; Walser *et al.* 2005). Very little is known about genetic diversity, gene flow, and population dynamics of lichens within landscapes, and about the effect of disturbances on epiphytic lichen populations. To overcome this gap in knowledge, we performed a population genetic study at the landscape scale focussing on the comparison of demes of an epiphytic lichen within a sylvopastoral landscape where stand-replacing disturbances had been reconstructed (Kalwij *et al.* in press). Our model organism, the epiphytic lichen *L. pulmonaria*, is described as an indicator lichen of forest canopy continuity in Europe (Rose 1992). The species is also thought to be dispersal-limited (Scheidegger *et al.* 1995; Walser *et al.* 2001). Since the haploid fungus of *L. pulmonaria* produces both vegetative and sexual propagules (Yoshimura
10   1971), its genetic structure consists of a clonal as well as a recombinant component. Both propagule types, vegetative soredia containing both the haploid fungus and the green algal component and sexual fungal ascospores, are wind dispersed (Werth, S. unpublished data; Walser *et al.* 2001). *L. pulmonaria* has a generation time of the clonal component of 35 years or longer in central Europe (Scheidegger & Goward 2002). Owing to forest management with short rotation cycles and to air pollution, *L. pulmonaria* has suffered a severe decline in Central Europe during the last decades. The species is considered vulnerable in the Red List of Switzerland (Scheidegger *et al.* 2002) and is endangered (Hallingbäck & Martinsson 1987; Wirth 1995; Wirth *et al.* 1996; Türk & Hafellner 1999; Søchting & Alstrup 2002) or extinct (Aptroot *et al.* 1999) in other European countries.

In our study area, a sylvopastoral landscape dominated by spruce forests (*Picea abies* L.) and
20   wooded pastures in the Swiss Jura Mountains with a spatial extent of 46°30'-34'N and 06°10'-16'E at an elevation of 1300-1450 m, *L. pulmonaria* exhibits large population sizes. The local carrier trees of *L. pulmonaria*, sycamore maple (*Acer pseudoplatanus* L.), and beech (*Fagus sylvatica* L.), are scarce and irregularly distributed within the mixed forest patches (Kalwij *et al.* in press).

Forests in the entire study area are subject to tree-level forest management, i.e., unevenaged forestry, where individual trees of all size classes are harvested in order to maintain a constant size class distribution (category: *uneven-aged*). Two forest areas in the north and west of the study area (Fig. 1) were additionally affected by 19th century disturbances (categories: *burnt* and *logged*; Kalwij *et al.* in press). Large population sizes of *L. pulmonaria* were found in all three disturbance categories (Kalwij *et al.* in press). This raises the questions of how the recolonisation process of such
30   differentially disturbed habitats by *L. pulmonaria* took place, and how it affected local genetic diversity.

Specifically, this paper addresses the following questions: (1) Were demes of *L. pulmonaria*, defined as assemblages of thalli within 1-ha sampling plots, affected by a stand-replacing disturbance founded by only a few independent immigration events? (2) What is the effect of stand-level disturbances on genetic diversity in *L. pulmonaria* demes and on genetic distance among demes, and does it depend on deme size in terms of the number of colonised trees? (3) Is there spatial genetic structure, and if so, does this structure differ between the three disturbance types? (4) Does the spatial genetic structure differ between clonal and recombinant components, and, hence, what are the dispersal ranges of clonal vs. recombinant propagules? We discuss the results in the context of conservation
40   strategies for *L. pulmonaria*.

Methods

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Sampling design

We investigated 41 1-ha plots, where the occurrence of *L. pulmonaria* and its carrier trees, maple and beech was known from Kalwij *et al.* (in press). Kalwij *et al.* (in press) delineated two stand-level disturbances: *(i)* Intensive, stand-level logging between 1850 and 1900, possibly selective for *P. abies*, for charcoal production (in the following referred to as *logged*; Fig. 1) and *(ii)* large-scale, stand-replacing logging in 1870, followed by wind throw and, in the next year, a two-week fire (in the following named *burnt*; Fig. 1). Within the *burnt* area, single old trees were present which survived the stand-replacing disturbance (J. Bolli, unpublished data). We refer to plots under uneven-aged forestry as *uneven-aged*. From the *logged* area, nine plots were chosen and from the *burnt* area, 12 plots were investigated. Twenty plots were classified as *uneven-aged* (Fig. 1).

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A hierarchical random sample of 923 thalli was collected from the 41 plots. Within each plot, all carrier trees of *L. pulmonaria* found by Kalwij *et al.* (in press) were searched for *L. pulmonaria*. A maximum of 24 thalli per deme were randomly selected from different trees. If there were fewer than 24 trees colonised by *L. pulmonaria*, multiple thalli were sampled from the same tree (for statistical treatment of unbalanced sampling see below). If there were fewer than 24 thalli in a plot, every thallus found was included. From each thallus, we collected a single lobe tip of a minimum area of 3 cm^2.

Molecular analysis

Sample preparation for DNA extractions followed Walser *et al.* (2003). Total DNA was isolated using the DNeasy 96 plant kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

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Six unlinked (Walser *et al.* 2004) fungal microsatellite loci (Walser *et al.* 2003) were analysed. A modified set of PCR primers (table 3 in Walser *et al.* 2004) was used in two multiplex PCR reactions of 10 µL each. The first multiplex PCR contained 1 µL genomic DNA, 25 nM of the primers for *LPu03* and *LPu09*, 125 nM of the primers for *LPu15*, 1× PCR buffer (Sigma, Saint Louis, MO, USA), 1.87 mM MgCl$_2$, 50 µM of dNTPs (Promega, Madison, WI, USA), and 0.75 U DNA polymerase (Sigma, Saint Louis, MO, USA). The second multiplex differed in that primer concentration was 125 nM for all of the six primers used (loci *LPu16, LPu20, LPu27*). We used the fluorescent labelling of primers proposed by Walser et al (2004). The amplification protocol of both multiplex amplification reactions began with an initial denaturation at 94°C for 120s, 29 cycles of denaturation at 94°C for 60s, annealing at 57°C for 60s and extension at 72°C for 60s, followed by a final extension at 72°C for 45 minutes. All amplification reactions were performed with a PTC-100 thermal cycler (MJ Research, Waltham, USA). Fragment sizes of PCR products were determined on an ABI3100-avant automatic sequencer (Applied Biosystems, Foster City, CA, USA). Alleles were sized with an internal size standard (ROX 500, Applied Biosystems, Foster City, CA, USA). A reference sample of *L. pulmonaria* with known allele sizes was run on each plate to check repeatability of the allele sizing. Genotyping was done using GENOTYPER 2.1 (Applied Biosystems, Foster City, CA, USA).

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Data analysis

There were missing values for 13 thalli, and in 15 thalli, multiple alleles were found for at least one locus. These latter 15 samples most likely consisted of more than one genetic individual of the haploid mycobiont. Alternatively, by chance, fertile dikaryotic and diploid hyphae within thalli might have been used for extraction. We thus excluded 28 thalli from statistical analyses (895 thalli remaining).

On every tree from which we collected *L. pulmonaria*, we estimated the total thallus area of *L. pulmonaria* in cm^2. If the cumulative thallus area was larger than 1 dm^2, we estimated it as the number of DINA4-size sheets covered by *L. pulmonaria* on the tree. Furthermore, we roughly estimated the number of thalli on each tree from which we collected *L. pulmonaria*, counting up to 24 thalli or, for

10 larger numbers, using the following thallus number classes: 25-50, 51-75, 76-100, 101-150, 151-200, 200-300, 300-400. We calculated the sum of the number of thalli or of the median class value over all trees on which we had sampled *L. pulmonaria* per plot (deme census size), as well as the sum of the thallus area per plot (deme thallus area). Note that by this procedure, thallus number and thallus area were underestimated in plots where more than 24 trees were present, which was the case in three *uneven-aged* plots with 34, 30 and 26 trees colonised and in one *logged* plot with 104 trees colonised by *L. pulmonaria*.

Our sampling design was unbalanced for the number of thalli sampled per tree, which differed among plots. To overcome this problem, we based our analyses on averaged values from 1000 reduced data sets, created by randomly selecting one thallus per tree.

20 We estimated the minimum number of immigration (including original colonisation) events (*C*) per deme as the number of alleles at the most variable locus (Walser *et al.* 2003). To test for a deviation of *C* per disturbance category from uniformity, we computed a χ^2-test in R (Anonymous 2004).

To quantify genetic diversity of demes for a comparison of different disturbance categories, we calculated Nei's unbiased gene diversity (*H*; Nei 1978) for each deme as $H = \frac{1}{r}\left(\sum_{k=1}^{r} h_k\right)$, where

$h_k = \frac{n}{n-1}(1 - \sum_{i=1}^{m_k} x_{ik}^2)$, x_{ik} is the frequency of the *i*th allele at the *k*th locus in a subpopulation, m_k is the number of alleles at the *k*th locus, and *r* the number of loci analysed. Furthermore, we calculated the number of multilocus genotypes (*G*) and the percentage of multilocus genotypes per deme (*M*), i.e. the number of multilocus genotypes divided by the number of thalli. For the calculation of *H, G* and *M,* we

30 wrote our own code in the R statistical package for haploid data (Anonymous 2004). We performed analysis of covariance (ANCOVA) of *G, H* and *M* as a function of the log-transformed number of *L. pulmonaria* carrier trees per deme as covariate and disturbance category as factor (three levels). Histograms of the residuals were inspected visually in order to check if they were close to normal distribution, and otherwise, a transformation of the response variable was performed to ensure approximately normally distributed residuals. To test for homogeneity of variance among disturbance categories, Levene's test was preformed in R (library Rcmdr, xxx).

To assess if there were major differences in genetic distance among disturbance categories, we calculated Nei's minimum genetic distance between demes (D_m; Takezaki & Nei 1996) as $D_m = (J_a + J_b)/2 - J_{ab}$, where J_a and J_b are the average identity over loci within subpopulation a and b, respectively, calculated as $J_a = \frac{1}{r}\sum_j^r \sum_i^{m_j} x_{aij}^2$ and $J_b = \frac{1}{r}\sum_j^r \sum_i^{m_j} x_{bij}^2$, where J_{ab} is the average identity of subpopulations a and $b,$ calculated as $J_{ab} = \frac{1}{r}\sum_j^r \sum_i^{m_j} x_{aij} x_{bij}$. Here, x_{aij} and x_{bij} are the frequencies of the ith allele at the jth locus in subpopulation a and b, respectively. D_m was calculated with a self-written code in R (Anonymous 2004). To account for the unbalanced sampling design, we used an averaged value of D_m based on 1000 reduced data sets (see above). Principal coordinate analysis (PCoA) of genetic distance D_m was calculated for a two-dimensional ordination space with the library stats (function cmdscale; R Development Core Team 2004) in R in order to assess if there were major differences in genetic distance among disturbance categories.

To test if there was spatial genetic structure in *L. pulmonaria* within the study area and if the structure differed between disturbance categories, we calculated three different types of variograms for each level of disturbance using the methods described in Wagner *et al.* (2005): (1) a variogram of gene diversity H without accounting for recurrent genotypes, representing the overall spatial genetic structure, (2) a variogram of genotype diversity D, the probability of sampling a different multilocus genotype (Wagner *et al.* 2005), reflecting the clonal component, and (3) a variogram of H weighted for recurrent genotypes, reflecting the sexual component. The weighting procedure is described in detail in Wagner *et al.* (2005). A lag distance of 50 m was chosen, with the first distance class containing only pairs of samples from the same tree and the last distance class containing all pairs of samples separated by more than 450 m. Statistical significance of spatial autocorrelation was assessed for each variogram and each distance class using a one-sided permutation test with 100 permutations (Wagner *et al.* 2005). Exponential variogram models were fitted with the library gstat in R (Pebesma 2004).

Results

Non-spatial analysis

Deme census sizes of *Lobaria pulmonaria* in terms of the mean number of thalli were highest in the *burnt* area, followed by the *logged* area (Table 1). The mean cumulative thallus area per plot was largest in the *burnt* area (Table 1).

The minimum number of immigration events C per deme ranged from one to eight. There was no statistically significant association between C and disturbance ($\chi^2_{14} = 16.6$, $P > 0.05$).

Among the 895 *L. pulmonaria* samples analysed, we found 176 multilocus genotypes ($M = 19.7$ %). Levene's tests of homoscedasticity showed that variances were homogeneous among treatment groups for H (df=2, $F=1.94$, $P=0.157$), G (df=2, $F=1.32$, $P=0.280$), and M (df=2, $F=2.22$, $P=0.123$). There was no statistically significant interaction between disturbance category and the

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number of colonised trees per plot in the analyses of covariance on *M* and *H*; thus, the statistical models without interaction terms are shown (Table 2). There was a significant effect of disturbance on *H* ($P = 0.012$; Table 2) and on *M* ($P = 0.005$; Table 2), which were highest in *logged* demes, intermediate in *uneven-aged* demes, and lowest in *burnt* demes (Fig. 2). Gene diversity *H* increased significantly with the number of colonised trees per plot, whereas the proportion of multilocus genotypes *M* decreased (Fig. 3). In *burnt* demes, the number of multilocus genotypes *G* did not increase significantly with the number of trees colonised by *L. pulmonaria* (Fig. 3a).

Demes belonging to the same disturbance category did not represent distinctive clusters with a noteworthy difference in genetic distance to other disturbance categories (Fig. 4). The *logged* demes represented a small cluster, and were generally more similar to each other than demes of the other two categories. Contrary, *Logged* and *burnt* demes showed a larger variance in genetic distance (Fig. 4).

Spatial analysis

At short distances, significant spatial autocorrelation in genotype diversity *D* was detected in all disturbance categories (Fig. 5). Spatial autocorrelation of genotype diversity was significant at distances up to 50 m in *logged* demes, up to 100 m in *uneven-aged* demes, and up to 150 m in *burnt* demes. Genotype diversity was substantially reduced in *burnt* demes which showed the strongest spatial autocorrelation (Fig. 5).

Without accounting for recurrent multilocus genotypes, significant spatial autocorrelation in gene diversity *H* extended up to 100 m in *burnt* demes, up to 50 m in *uneven-aged* demes, and up to 0 m (i.e., within trees) in *logged* demes (Fig. 6a). As in the variogram of *D*, the variogram of *H* of *burnt* demes showed stronger spatial autocorrelation than the other disturbance categories (Fig. 6a).

When the effect of recurrent multilocus genotypes was accounted for (Fig. 6b), a low degree of spatial autocorrelation was only found in *burnt* demes in the first two distance classes and in the first distance class in *logged* demes. Most notably in Fig. 6, the value for the first distance class changed from ~0.1 to ~0.4 when recurrent genotypes were accounted for, indicating that much of the spatial autocorrelation was due to the clonal component. Note that due to the weighting of recurrent multilocus genotypes, the effective number of pairs per distance class was reduced from a range of formerly 1000 to 4000 pairs to 10 to 40 effective pairs. Therefore, the power of the significance test was low, and non-significance has to be interpreted carefully.

Discussion

The main objectives of this study were to determine if demes affected by stand-replacing disturbance (*burnt*) were founded by multiple immigration events, to assess spatial genetic structure, to evaluate dispersal ranges for the clonal and sexual component of genetic variation, and to test the effect of stand-level disturbances on genetic diversity of *L. pulmonaria*.

Minimum number of immigration events

We found no significant differences when we compared the minimum number of immigration events in demes among disturbance categories. *Burnt* and *logged* demes of *L. pulmonaria* were founded by independent immigration events from multiple sources, followed by rapid clonal spread at the deme level.

Spatial genetic structure and dispersal ranges

The clonal component had the largest impact on spatial genetic structure, as little spatial autocorrelation remained when clones were accounted for, implying that spatial autocorrelation owing to the sexual component of genetic variation was small by comparison. The variogram curve of genotype diversity of the *burnt* area was flatter, with significant spatial autocorrelation extending to a
10 larger distance than those of the two other categories. Hence, the clonal component had a larger spatial extent in the *burnt* demes, suggesting substantial clonal spread. For instance, the more extensive clonal growth may have resulted from the lower original density of the lichen following fire and subsequent recolonisation by relatively few genotypes. Variogram analysis of gene diversity H and of genotype diversity D nevertheless showed that spatial autocorrelation due to clonal dispersal or selfing was confined to short distances. This implies that within the time since the stand-replacing disturbance in *burnt* demes (130 years), the majority of propagules resulting from vegetative propagation or selfing established within 150 meters' distance from their origin. The variogram analysis showed clearly that the range of clonal propagules was lower than that of ascospores (Fig. 6). As clonal propagules were abundant in the study area – almost every thallus we found exceeding 10 cm in diameter contained
20 soredia (Scheidegger & Goward 2002) – they may be important for the rapid colonisation of trees within forest stands at short distances. In contrast, the less abundant ascospores may serve as dispersal agents over longer distances, e.g. for the colonisation of distant forest patches. This hypothesis is supported by the lack of substantial spatial autocorrelation when recurrent genotypes were accounted for in the variogram of gene diversity. Hence, due to their size, ascospores seem to be more suitable for long-distance dispersal than vegetative propagules like thallus fragments, isidia or soredia (Bailey 1976; Galloway & Aptroot 1995).

Effect of disturbances on genetic diversity

Old forests support more rare and endangered lichen species than younger forests (Lesica *et al.* 1991; Rose 1992; Dettki & Esseen 1998; Boudreault *et al.* 2000), and old-forest associated lichens
30 are commonly assumed to be negatively affected by logging mainly because of the loss of suitable habitat, e.g. old trees (Peterson & McCune 2001; Hedenås & Ericson 2003). However, in this study, we found evidence for the contrary: gene diversity and percentage of multilocus genotypes of the epiphytic old-forest lichen *Lobaria pulmonaria* was especially high in demes affected by stand-level timber logging as compared with tree-level logging. Moderate logging of trees, as in the uneven-aged forestry performed throughout the study area, may not reduce lichen populations significantly (Storaunet *et al.* 2000; Rolstad *et al.* 2001), thus still allowing for a high genetic diversity.

The main carrier tree of *L. pulmonaria* in the study area, sycamore maple, profits from temporally enhanced light conditions (Vittoz 1998). Stand-level logging in the western part of the study area may have led to a significant increase in sycamore maple density (Kalwij *et al.* in press). Taking
40 this into consideration, the pattern observed in genetic diversity may be explained by an increase of

available habitat. The genetic diversity of demes seems to reflect whether a subpopulation was reduced in size, i.e. to a high degree in the *burnt* area or a low degree in the other areas. Consecutively, genetic diversity accumulated over time in all areas as new immigrants arrived, but from different starting levels. The tree species which were selectively logged were possibly *Picea abies*, which does not provide suitable habitat of *L. pulmonaria* in the study area, and *Fagus sylvatica*, a tree species which is of secondary importance as carrier tree of *L. pulmonaria* in the study area (Kalwij *et al.* in press). Intensive selective logging of these tree species may have effects similar to tree harvesting with retention of the majority of trees containing *L. pulmonaria*. A recent modelling study indicates that the latter scenario is plausible (Wagner *et al.* submitted). Selective logging of *P. abies* and *F. sylvatica*

10 might be beneficial for *L. pulmonaria*, but probably much less so for lichens dependent on the latter tree species as a carrier tree. Another point is that dense, dark forests are unfavourable for *L. pulmonaria* (Renhorn *et al.* 1997; Hazell & Gustafsson 1999) and, thus, increased light availability may favour *L. pulmonaria* abundance in mixed forests as compared with stands dominated by *P. abies*.

The example of the *burnt* demes affected by stand-replacing disturbances about 130 years ago, which by now have reached large census sizes via multiple immigration events from different sources followed by rapid clonal spread within demes, and which have already reached a certain level of genetic diversity, showed that spatial distribution and the forming of genetic diversity in *L. pulmonaria* are not necessarily impacted by stand-replacing disturbances for many centuries. The reason for this might be the large local propagule pool in the study area. In contrast, in areas where the propagule pool

20 of *L. pulmonaria* is small or where the clearing size or the distance to the closest population of potential founders is large, stand-replacing disturbances may influence the spatial occurrence and genetic diversity of *L. pulmonaria* for very long time periods. This is the case in large areas of Central Europe, where the species is largely restricted to autochthonous forests and where stand-replacing disturbances including clear-cutting have led to local extinction and a lack of recolonisation of secondary stands (Schöller 1997).

In the light of short, human time scales, *L. pulmonaria* may seem dispersal-limited, but relative to the length of its generation time of 35 years or more (Scheidegger & Goward 2002), it is not. Hence, forest dynamics need to be appropriate to the generation time and dispersal ability of *L. pulmonaria* to ensure its persistence in a particular forest stand, comprising sufficient numbers of

30 suitable carrier trees. This seems the case in the mixed forests of our study area, and may apply also in mixed or deciduous old-growth forests in other areas. In our study area, *L. pulmonaria* may indicate the occurrence of disturbance at suitable scales rather than ecological continuity of forests (Kalwij *et al.* in press), which is in accordance with the findings of Snäll *et al.* (2005), but is contrary to what most other studies have suggested (Andersson & Appelqvist 1987; Rose 1992; Gauslaa 1994; Trass *et al.* 1999). In other landscapes, *L. pulmonaria* may still be considered an indicator of the presence of old-growth forests at the whole landscape level, but not necessarily locally, at the level of a particular local forest stand.

Implications for conservation

Our study suggests that retaining remnant trees carrying lichens should be an effective

40 conservation strategy for rare epiphytic lichen species, which has also been suggested by Sillett *et al.* (2000) and Peterson & McCune (2001). Care has to be taken that the forest clearings created by the

logging process are kept small to keep a sufficiently moist microclimate in the stands and that, at the same time, gaps for colonisation of future carrier trees are created (Hedenås & Ericson 2003). Selective cutting could then lead to an increased density of potential carrier tree species. Whether this hypothesised scenario does indeed take place is a question of the successional dynamics of the forest system and the lichen species under consideration. Lichens of primeval forests may well show different types of population dynamics.

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Acknowledgements

Figure Legends

Figure 1. Demes of *Lobaria pulmonaria* in the Swiss study area subjected to molecular-genetic analyses, and reconstructed stand-level disturbances (Kalwij *et al.* in press). Each circle, square and triangle represents the central coordinates of a deme. The disturbance category of demes is shown by

13

squares (*burnt*), circles (*uneven-aged*), and triangles (*logged*). The upper left panel indicates the location of the study area within Switzerland, as indicated by the arrow.

Figure 2. Relationship between disturbance category and number of multilocus genotypes per deme (*G*), percentage of multilocus genotypes per deme (*M*), and unbiased gene diversity (*H*) for *Lobaria pulmonaria* demes from the Swiss Jura. Figures (a) to (c) show means and 95 % confidence intervals.

Figure 3. Relationship between number of multilocus genotypes per deme (*G*), percentage of multilocus genotypes per deme (*M*), unbiased gene diversity (*H*), disturbance category and log-transformed number of colonised trees for *Lobaria pulmonaria* demes from the Swiss Jura. Figures (a) to (c), analysis of covariance of the effect of disturbance categories on *G*, *M* and *H*. East symbol represents a deme. Squares, broken line: *burnt*; circles, black line: *uneven-aged*; triangles, dotted line: *logged*.

Figure 4. Ordination of the first two axes of Principal Coordinate Analysis (PCoA) of demes using Nei's minimum genetic distance (D_m) in *Lobaria pulmonaria*. Each symbol represents a deme, and minimum convex polygons were drawn for each disturbance category to show potential overlap. The variance explained by the first two PCoA-axes was 43.0 %. Squares, broken line: *burnt*; circles, black line: *uneven-aged*; triangles, dotted line: *logged*.

Figure 5. Variogram of genotype diversity (*D*) in *Lobaria pulmonaria* from the Swiss study area. Each symbol indicates the probability of sampling two different multilocus genotypes as a function of their distance in space. Squares, broken line: *burnt*; circles, black line: *uneven-aged*; triangles, dotted line: *logged*. Filled symbols indicate significant spatial autocorrelation.

Figure 6. Variogram of gene diversity (*H*) in *Lobaria pulmonaria* from the Swiss study area. Each symbol indicates the probability of sampling two different alleles of a locus, averaged over six loci, as a function of their distance in space. (a) Not accounting for recurrent genotypes; (b) accounting for recurrent genotypes. Squares, broken line: *burnt*; circles, black line: *uneven-aged*; triangles, dotted line: *logged*. Filled symbols indicate significant spatial autocorrelation.

14

Table 1. The number of sampling plots investigated (number of demes), average deme census size (number of thalli), and thallus area (area) of *Lobaria pulmonaria* in 1-ha plots from the Swiss Jura Mountains, in relationship to disturbance. Four plots had missing data, and were thus not shown.

Disturbance category of deme	Area [dm^2]	Number of thalli	Number of demes
Burnt	572.9	930	11
Logged	405.6	810	9
Uneven-aged	413.8	717	17

Table 2. Analyses of covariance testing the effect of disturbance (factor) and of the log-transformed number of *Lobaria pulmonaria* trees per deme (number of trees = covariate) on the number of multilocus genotypes (*G*) per deme, the percentage of multilocus genotypes (*M*) in demes and Nei's unbiased gene diversity (*H*) in the Swiss study area. SS = sum of squrares, df = degrees of freedom, *F* = F-ratio. Note that the response variable *H* was square-root transformed prior to analysis, while all other response variables remained untransformed.

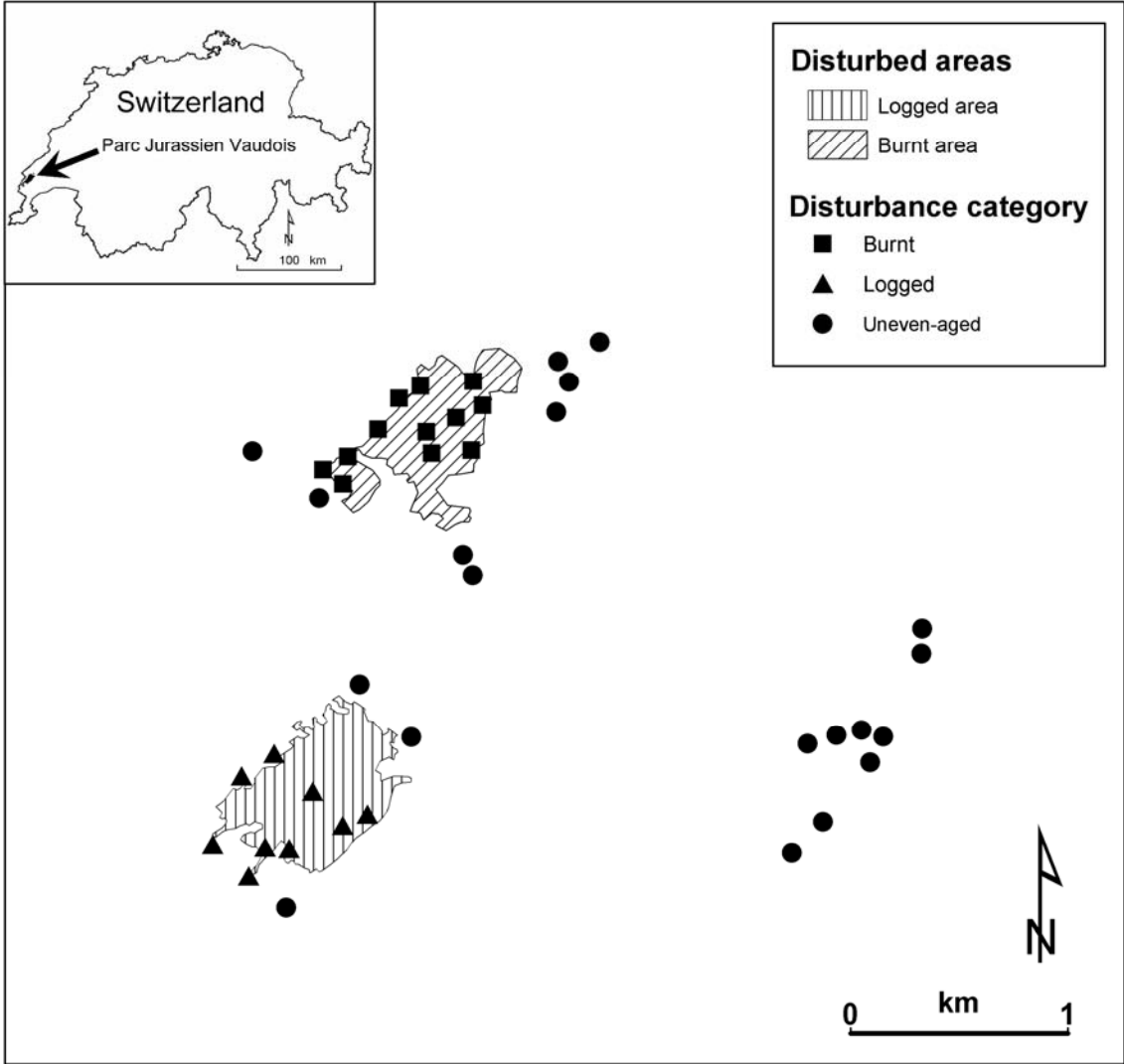| Diversity parameter	Source of variation	SS	df	*F*	*P*
G	Number of trees	220.0	1	49.72	<0.0001
	Disturbance	24.6	2	2.78	0.0759
	Number of trees × Disturbance	36.1	2	4.08	0.0255
	Error	154.9	35		
	Total	435.6	40		
M	Number of trees	0.646	1	12.72	0.0010
	Disturbance	0.621	2	6.11	0.0051
	Error	1.879	37		
	Total	3.145	40		
H	Number of trees	0.601	1	8.05	0.0073
	Disturbance	0.743	2	4.98	0.0121
	Error	2.763	37		
	Total	4.107	40		

10
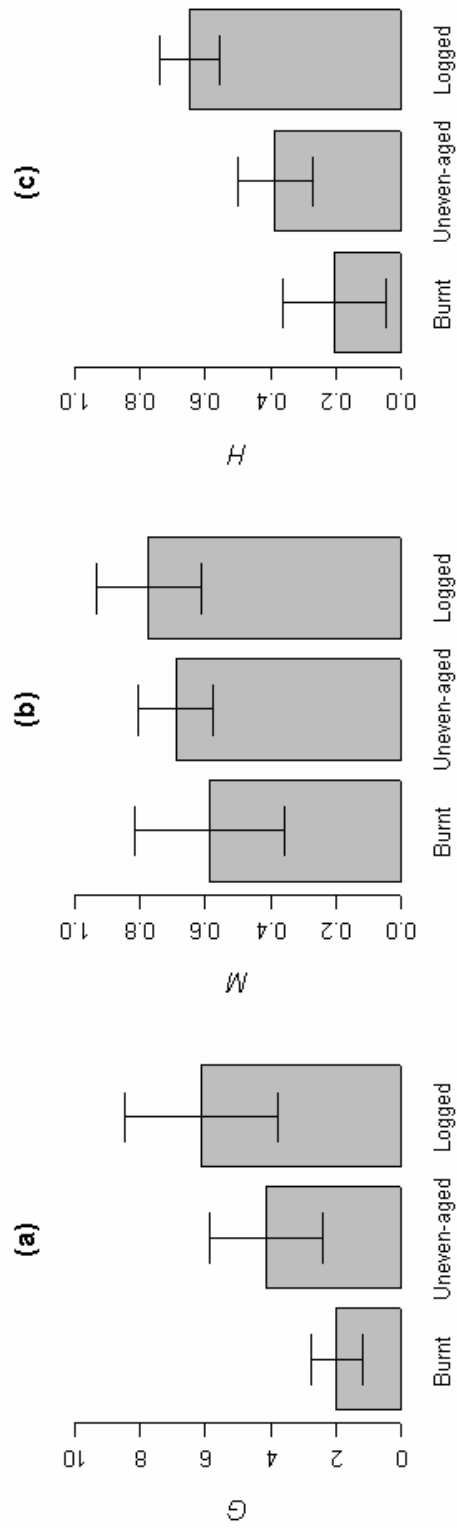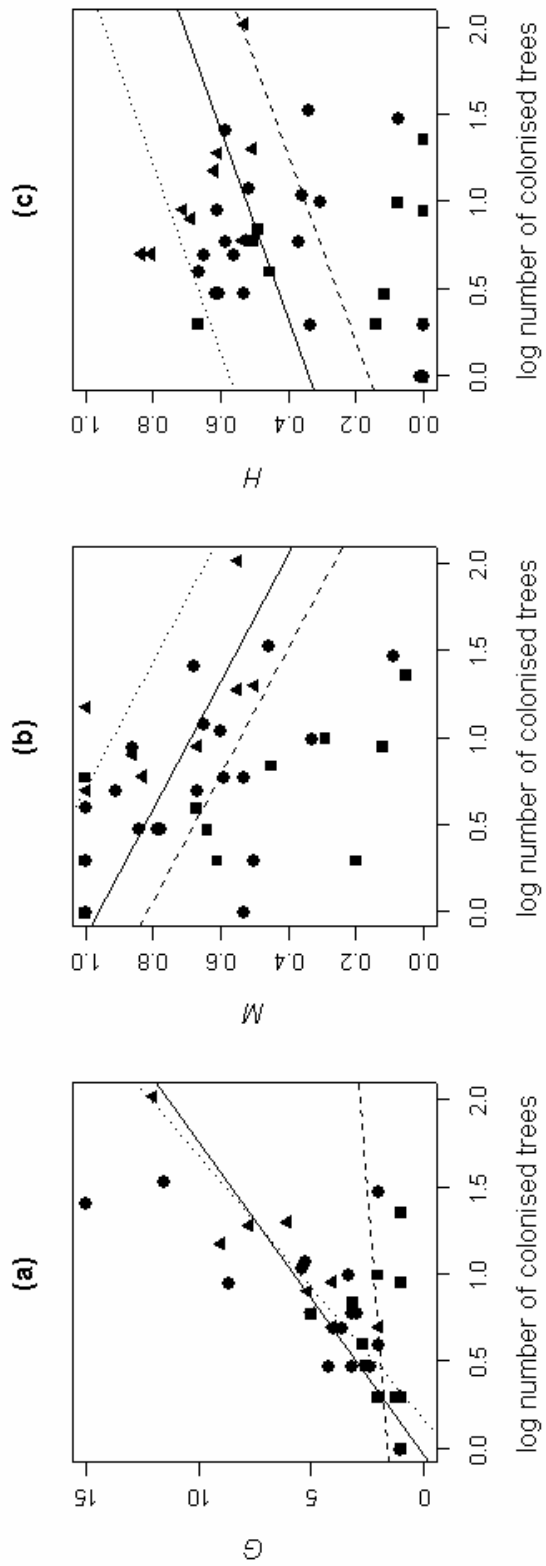
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Figure 2

Figure 3
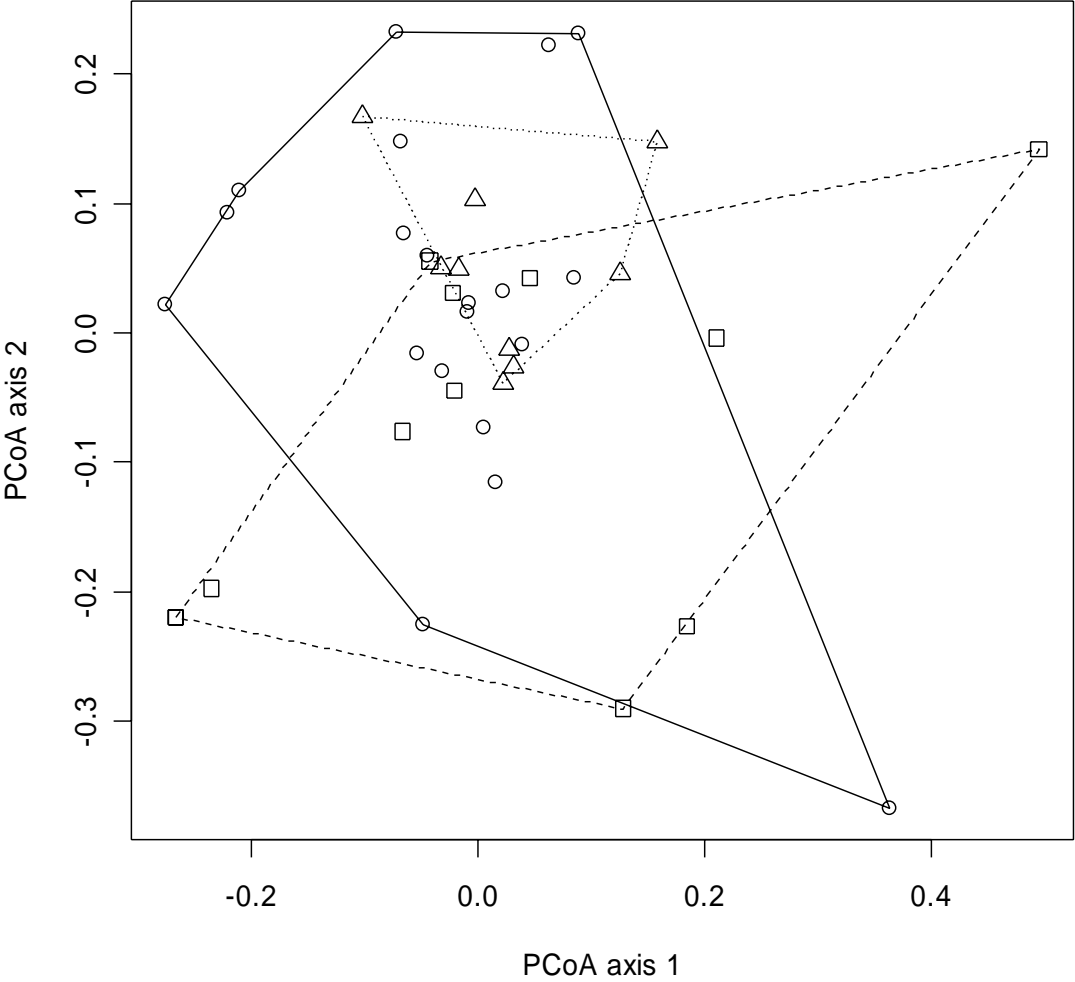
Figure 4

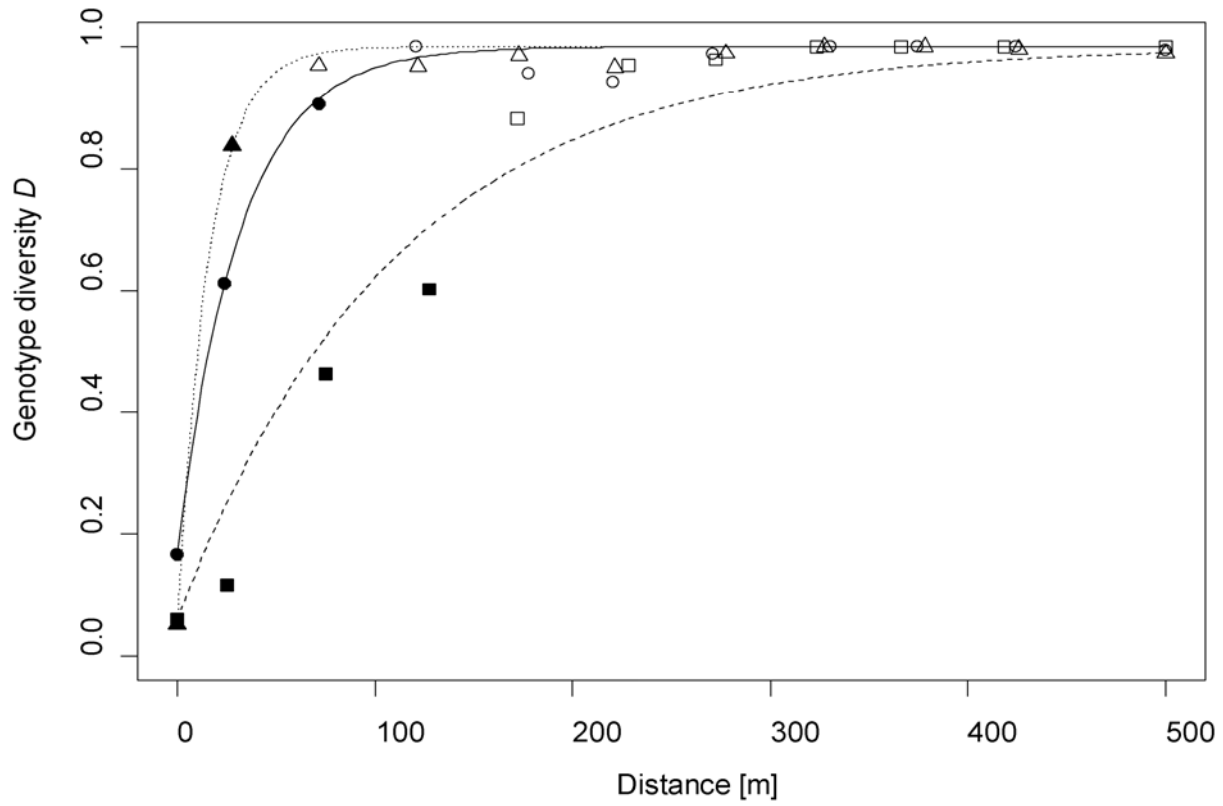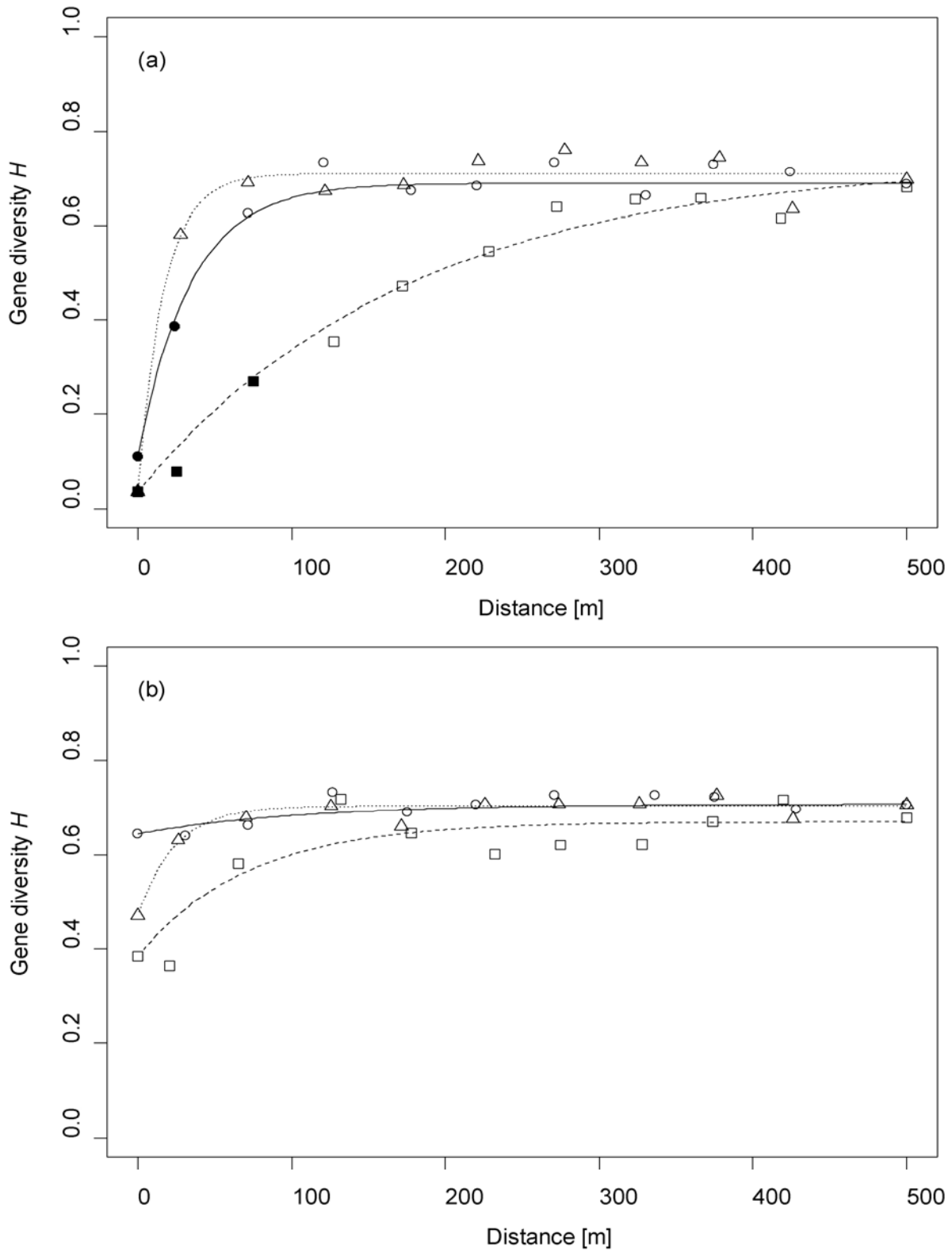Figure 5

Figure 6

Author information box

This work is part of Silke Werth's dissertation research on landscape genetics and dispersal of lichenised fungi. She will continue studying landscape genetics of lichenised fungi at the University of California at Los Angeles. Helene H. Wagner focuses on spatial analysis and modelling as applied in community ecology and landscape genetics. Rolf Holderegger investigates the population and landscape genetics of plants and their application in conservation biology. Jesse M. Kalwij's PhD focused on reconstructing stand-replacing disturbances based on aerial images and on ecological indicators of conservation value. He is currently doing research on the ecology of invasive species at the Centre of Excellence for Invasion Biology (CIB), University of Stellenbosch. Christoph Scheidegger is head of the Division Ecological Genetics at the WSL Swiss Federal Research Institute, and his research interests cover ecophysiology, population genetics, morphology, and conservation biology of lichenised fungi.