

Landscape-level gene flow in *Lobaria pulmonaria*, an epiphytic lichen

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Abstract

Epiphytes are strongly affected by the population dynamics of their host trees. Owing to the spatio-temporal dynamics of host tree populations, substantial dispersal rates – corresponding to high levels of gene flow – are needed for populations to persist in a landscape. However, several epiphytic lichens have been suggested to be dispersal-limited, which leads to the expectation of low gene flow at the landscape scale. Here, we study landscape-level genetic structure and gene flow of a putatively dispersal-limited epiphytic lichen, *Lobaria pulmonaria*. The genetic structure of *L. pulmonaria* was quantified at three hierarchical levels, based on 923 thalli collected from 41 plots situated within a pasture–woodland landscape and genotyped at six fungal microsatellite loci. We found significant isolation by distance, and significant genetic differentiation both among sampling plots and among trees. Landscape configuration, i.e. the effect of a large open area separating two forested regions, did not leave a traceable pattern in genetic structure, as assessed with partial Mantel tests and analysis of molecular variance. Gene pools were spatially intermingled in the pasture–woodland landscape, as determined by Bayesian analysis of population structure. Evidence for local gene flow was found in a disturbed area that was mainly colonized from nearby sources. Our analyses indicated high rates of gene flow of *L. pulmonaria* among forest patches, which may reflect the historical connectedness of the landscape through gene movement. These results support the conclusion that dispersal in *L. pulmonaria* is rather effective, but not spatially unrestricted.

Keywords: barrier, genetic discontinuity, genetic structure, lichenized ascomycetes

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Introduction

Migration among spatially segregated habitat patches is a key process in metapopulations (Levins 1969; Hanski & Gaggiotti 2004). High migration rates are necessary for persistence of organisms that depend on habitat patches prone to rapid turnover in order to maintain metapopulations and to keep up with the spatio-temporal dynamics of the habitat. Epiphytic lichens and bryophytes that colonize mature forest trees generally exhibit migration

characteristics of such patch-tracking metapopulations (Johansson & Ehrlén 2003; Snäll *et al.* 2003, 2004), with the epiphytes' population dynamics being constrained by the dynamics of the host tree population (Snäll *et al.* 2005; Wagner *et al.* 2006).

For epiphytes associated with low-density or patchily distributed host trees, distances among habitat patches (i.e. individuals or groups of host trees) can be long, and the total amount of suitable habitat in a landscape may be low. Host trees need to reach a minimum age before their bark becomes a suitable habitat for epiphytes, but such suitable habitat patches disappear when host trees die or are harvested. As a consequence, there is only a limited time period between patch colonization by an epiphyte and its local extinction. Because diaspores of most epiphytic

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bryophytes, lichens, ferns, and orchids are small and wind-dispersed (Barkman 1958; Bailey 1976; Galloway & Aptroot 1995; Snäll *et al.* 2004), epiphytes would be expected to be good passive dispersers and to exhibit high rates of migration among host trees and/or local populations across a landscape. This should result in high levels of gene flow and, thereby, low levels of genetic differentiation.

In epiphytes specialized on rare or spatially scattered host tree species, long-distance dispersal is required for successful colonization of new habitat patches. Based on this latter reasoning, a limited dispersal ability would be a hindrance for the colonization of, e.g. young forests by old-forest associated epiphytes (Dettki *et al.* 2000; Sillett *et al.* 2000; Hilmo 2002). Experimentally determined dispersal distances of epiphytic lichens are indeed short, in the range of tens of metres (Armstrong 1987, 1994; Walser *et al.* 2001) or a few hundred metres (Werth *et al.* 2006a). Such a limited dispersal ability, coupled with long distances among suitable habitat patches, would imply low rates of gene flow among and substantial genetic isolation of populations of epiphytes. As a consequence, high genetic differentiation is expected among populations for epiphytes with limited dispersal ability.

Population genetic investigations of epiphytes within patchily forested landscapes may reveal which of the above two scenarios holds true: low effective dispersal and gene flow with substantial genetic differentiation, or high dispersal and gene flow paralleled by low genetic differentiation. In the first scenario, isolation-by-distance effects and a spatial aggregation of individuals originating from the same local gene pool are expected, while the second scenario would result in weak isolation by distance, and a random recruitment of individuals from a common gene pool across a landscape. Discriminating between the two scenarios is of interest both as a test of theoretical predictions of metapopulation dynamics as well as for conservation practice since a large number of epiphytic lichens are endangered by habitat fragmentation and change (Tønsberg *et al.* 1996; Thor 1998; Wirth 1999; Scheidegger & Goward 2002; Lidén *et al.* 2004; Radies & Coxson 2004). For instance, if gene flow and dispersal of a given species is restricted to short distances, i.e. among groups of host trees, conservation strategies should aim at increasing the amount of suitable habitat and connecting spatially discrete epiphyte populations (Öckinger *et al.* 2005). But even in continuous forests, natural disturbances or forest management may endanger the long-term persistence of dispersal-limited epiphytes if the host tree population dynamics is faster than the patch-tracking population dynamics of epiphytes.

Our model species was *Lobaria pulmonaria* (L.) Hoffm., which is a foliose epiphytic lichen considered to be endangered in several European countries (Wirth *et al.* 1996; Scheidegger *et al.* 2002; Søchting & Alstrup 2002). It is

associated with old-growth forests and pasture-woodland landscapes (Rolstad *et al.* 2001; Rose 1992), and has been suggested to be dispersal-limited (Öckinger *et al.* 2005). *L. pulmonaria* forms both vegetative and sexual propagules (Yoshimura 1971) at an age of about 25 years (Scheidegger & Walser 1998). Dispersal by vegetative propagules has been determined as the predominant dispersal mode in *L. pulmonaria* (Walser 2004; Wagner *et al.* 2005, 2006; Werth *et al.* 2006b).

In this study, we investigated gene flow in *L. pulmonaria* in a pasture-woodland landscape. The composition of this landscape, i.e. old forests managed with uneven-aged forestry, fire-disturbed young forests, few intensively logged forests and large wooded pastures (Kalwij *et al.* 2005), allowed us to investigate the genetic variation of local *L. pulmonaria* populations differing in age and population history as well as in their degree of spatial isolation. We quantified gene flow among local populations of *L. pulmonaria* within the pasture-woodland landscape. We also assessed whether the spatial arrangement of the different types of landscape elements influenced genetic structure in the lichen. Specifically, we tested the hypothesis that gene flow among populations of the forest-dwelling lichen *L. pulmonaria* is restricted, leading to genetic differentiation as a result of isolation.

Methods

Study area

Our study area was situated in the northern part of the Parc Jurassien Vaudois in the Jura Mountains in western Switzerland with a spatial extent of 46°28'–30'N and 06°10'–16'E and an altitude of 1300–1450 m above sea level. The study landscape represents a typical pasture-woodland landscape composed of Norway spruce forests (*Picea abies*) and wooded pastures on limestone bedrock. Mean annual precipitation and mean July temperature are about 2100 mm and 10 °C, respectively (Kirchhofer 1995). The host trees of *Lobaria pulmonaria* in the study area were sycamore maple (*Acer pseudoplatanus*) and beech (*Fagus sylvatica*), which were both scattered in forested areas (Kalwij *et al.* 2005), but only rarely found on wooded pastures.

In our study area, two large forested areas ('regions') were separated by a large open area about 1 km wide (Fig. 1). While the western and eastern parts of this open area represent large wooded pastures, the central part of the open area, about 200 m wide and 1 km long, is currently covered by herbaceous mire vegetation (see Sjögren 2006, for a map of the mire and adjacent wooded pastures). Peat sediments from this mire have been used to reconstruct vegetation history, and the peat record dates back to 3000 BP (Sjögren 2006).

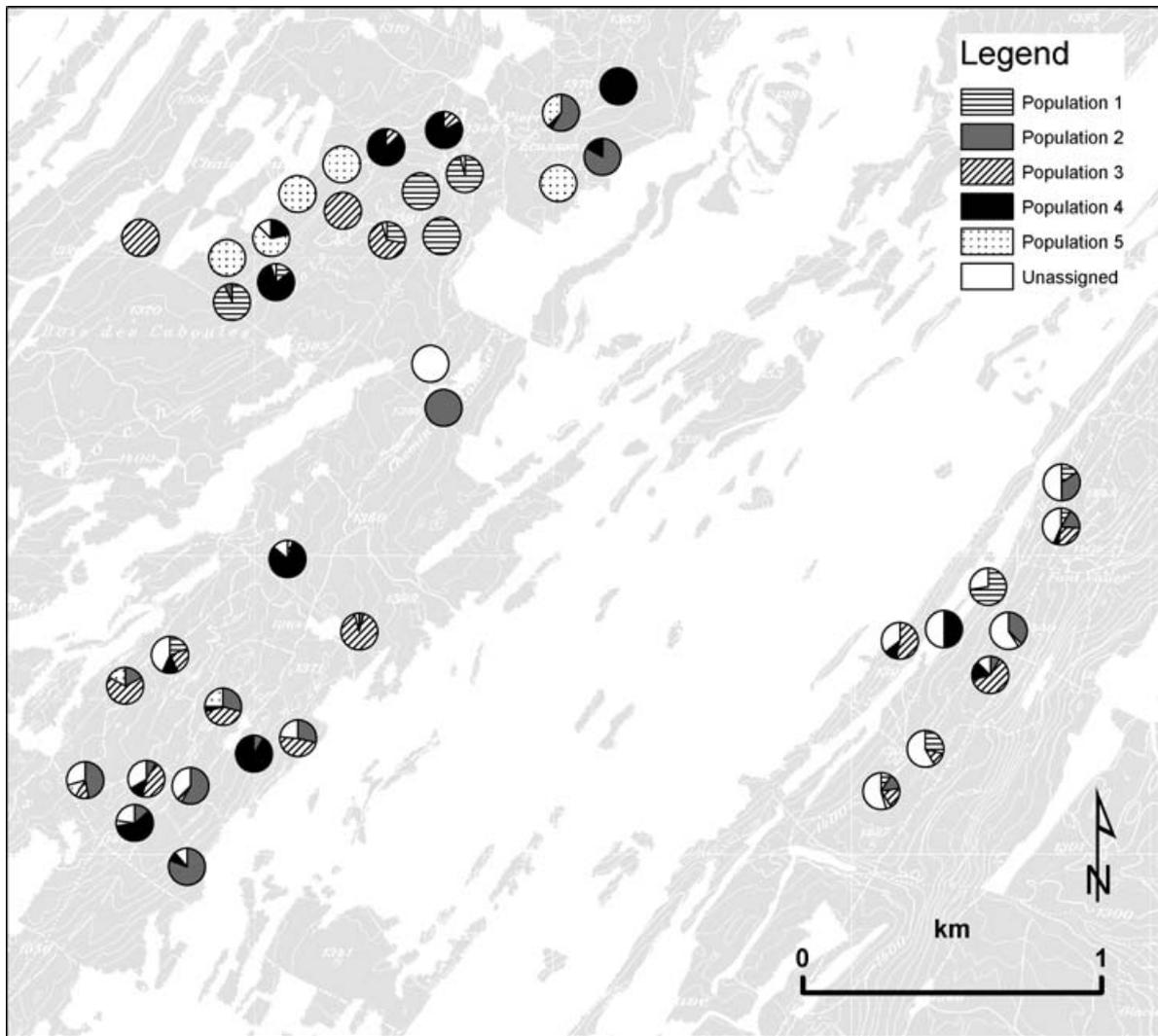


Fig. 1 Map of the study area in a Swiss pasture–woodland landscape showing proportions of ancestry defined from a Bayesian analysis of population structure (Pritchard *et al.* 2000) for *Lobaria pulmonaria*. Each pie chart shows the proportion of assignment of the *L. pulmonaria* samples from respective 1-ha plots to five populations ($K = 5$). Unassigned individuals had a probability ≤ 0.8 of belonging to any of the five populations. Grey, forests; white, wooded pastures. (Digital data from Landeskarte der Schweiz; © Bundesamt für Landestopographie, Berne.)

Sampling

Our study plots are a subsample taken from a total of 251 randomly sampled plots of a previous study (Kalwij *et al.* 2005). Plots containing *L. pulmonaria* were selected in the two forests west and east of the wooded pasture in approximately equal sampling density and ensuring coverage of a range of local population sizes. A hierarchical random sample of 923 thalli was collected from 41 plots of 1 ha. Within each plot, all potential host trees of *L. pulmonaria* were searched for *L. pulmonaria*, and at least 20 samples were collected per plot. If fewer than 20 trees were colonized by *L. pulmonaria*, which was the case in 36 out of

41 plots, multiple thalli were sampled per tree, sampling an equal number of thalli from all colonized trees. If fewer than 20 thalli were present in a plot, every thallus found was included (for a detailed overview of the samples collected in each plot, see Table 1).

Molecular analysis

Total DNA was isolated from cleaned and lyophilized lobe tips of *L. pulmonaria* using the DNeasy 96 plant kit (QIAGEN) according to the manufacturer's protocol. Six unlinked, fungal-specific microsatellite loci, *LPu03*, *LPu09*, *LPu15*, *LPu16*, *LPu20*, and *LPu27* (Walser *et al.* 2004), were

Table 1 Overview of the samples investigated in this study, showing plot ID (ID), the number of *Lobaria pulmonaria* thalli sampled in 1-ha plots (*N*), the number of host trees the lichen was sampled from (Sampled), the number of hosts present in a plot (Hosts), the location of a plot either west or east of the large pasture separating forests of the study area (Area; see also Fig. 1), and the disturbance a plot was subjected to (Disturbance)

ID	Hosts	Sampled	<i>N</i>	Area	Disturbance
W009	9	8	22	West	Burnt
W014	4	4	23	West	Burnt
W015	1	1	4	West	Burnt
W021	2	2	24	West	Burnt
W036	4	4	23	West	Burnt
W037	2	2	15	West	Burnt
W046	7	7	21	West	Burnt
W061	2	1	23	West	Burnt
W071	6	5	22	West	Burnt
W079	10	7	24	West	Burnt
W093	5	5	28	West	Burnt
W098	23	21	41	West	Burnt
W103	5	2	12	West	Logged
W145	19	14	24	West	Logged
W151	104	22	22	West	Logged
W188	5	4	24	West	Logged
W226	15	9	21	West	Logged
W228	8	6	21	West	Logged
W229	20	12	17	West	Logged
W230	6	6	24	West	Logged
W238	9	6	23	West	Logged
W013	10	10	23	East	Uneven-aged
W032	2	2	22	East	Uneven-aged
W035	3	3	28	East	Uneven-aged
W043	5	4	24	East	Uneven-aged
W050	1	1	2	East	Uneven-aged
W082	12	8	24	East	Uneven-aged
W090	6	6	27	East	Uneven-aged
W096	5	5	26	East	Uneven-aged
W202	26	22	22	East	Uneven-aged
W040	4	4	24	West	Uneven-aged
W051	6	6	24	West	Uneven-aged
W052	1	1	3	West	Uneven-aged
W055	2	2	10	West	Uneven-aged
W064	6	5	29	West	Uneven-aged
W075	4	2	24	West	Uneven-aged
W089	2	2	24	West	Uneven-aged
W139	30	23	23	West	Uneven-aged
W185	34	25	26	West	Uneven-aged
W210	10	10	24	West	Uneven-aged
W221	11	9	22	West	Uneven-aged

electrophoresed using an ABI 3100-*avant* automated DNA sequencer (Applied Biosystems) and assigned base-pair sizes using GENOTYPER version 2.1 software based on ROX 500 molecular weight marker (Applied Biosystems). A full description of the methods is given in Werth *et al.* (2006b).

Statistical analysis

We tested the selective neutrality of the set of microsatellite markers using the Ewens-Watterson test for neutrality (Manly 1985) as implemented in POPGENE version 1.32 (Yeh & Boyle 1997).

To test for isolation by distance (Wright 1943) in the haploid fungus, we performed a Mantel permutation test with 1000 permutations (Mantel 1967) of geographic distance between thalli and shared allele distance using the library VEGAN version 1.6–7 (function 'mantel', Dixon 2003; Oksanen 2005) in R (R Development Core Team 2004). Shared allele distances between individuals, a distance measure based on the infinite alleles model, were calculated by using the library APE version 1.4 (function 'dist.gene', Paradis *et al.* 2004) in R. The Mantel statistic, r_M , quantifies the correlation between genetic and geographic distance.

To partition molecular variance at different hierarchical levels, we performed analyses of molecular variance (AMOVA) using 1-ha plots nested within regions and trees nested within plots using the *F*-statistics approach in ARLEQUIN version 3.11 (Excoffier *et al.* 2005). Trees from which only one sample was available were excluded from tree-level AMOVA, excluding 153 of a total of 923 thalli. Four plots from which only a single sample had been collected per tree, were excluded from the plot-level AMOVA (W139, W151, W185, W202; 93 samples removed).

For the purpose of assigning *L. pulmonaria* thalli to populations, identifying recent migrants, and inferring the number of gene pools across the landscape, a Bayesian analysis of population structure was run as implemented in STRUCTURE version 2.1 (Pritchard *et al.* 2000). Here, individual multilocus genotypes are probabilistically assigned to a user-defined number (*K*) of randomly mating demes assumed to be in gametic equilibrium (i.e. gene pools). We ran three replicate simulations for each $K \in \{1, \dots, 40\}$, and, after a burn-in period of 10^5 iterations, 10^6 iterations were run in order to sample the posterior distribution. An admixture model was used in which the fraction of ancestry from each gene pool is estimated for each sampled individual. The prior of individual admixture, alpha, was assumed to be uniform for all gene pools. Panmictic gene pool allele frequencies were assumed independent of each other (Falush *et al.* 2003). Individuals of over 80% probability of ancestry in a given gene pool were regarded as 'assigned' to this gene pool, whereas all other individuals were classified as 'unassigned'. We inferred the number of gene pools using the methods given in Evanno *et al.* (2005). The statistic ΔK was calculated, which is based on the rate of change in the log likelihood of the data between successive *K* values. The true number of gene pools is determined as the modal value of the distribution of ΔK over *K* (Evanno *et al.* 2005).

We attempted to determine whether the spatial configuration of landscape elements influenced population

structure in *L. pulmonaria*, and if genetic discontinuities were found in the landscape which were related to landscape elements potentially acting as barriers to gene flow. First, we performed a partial Mantel permutation test (Oksanen 2005) that allowed us to assess whether gene flow was higher within continuous forests than across a large wooded pasture. In the partial Mantel test, the first matrix was comprised of pairwise allele-sharing distances between thalli ('genetic distance'). The second matrix ('landscape configuration') was a binary matrix with entries of one if *L. pulmonaria* thalli spatially separated by the wooded pasture were compared with each other. All other entries, i.e. thalli separated by continuous forest, were coded with zero. The third matrix consisted of Euclidean geographic distances among thalli ('geographic distance'). The partial mantel statistic, r_M , estimates the correlation between the first two matrices (genetic distance, landscape configuration) while controlling for the effect of the third matrix (geographic distance) (Legendre & Legendre 1998). This procedure allows assessing the effect of landscape configuration disentangled from a mere effect of geographic distance (i.e. isolation by distance). Second, for a posteriori detection of boundaries to gene flow in the study landscape, we used the Monmonier algorithm (Monmonier 1973) implemented in BARRIER version 2.2 (Manni *et al.* 2004). As input coordinates, we used the central coordinates of each 1-ha sampling plot. We did not delete any edges of the original triangulation created by the software. To create barriers, pairwise F_{ST} values, calculated from the coancestry coefficient θ (Reynolds *et al.* 1983) as $-\ln(1 - \theta)$, were calculated with 100 bootstrap matrices in MICROSAT version 1.5 (Minch 2001). We calculated three barriers for each of the 100 bootstrapped matrices.

Results

The six microsatellite loci employed did not deviate significantly from the expectation of selective neutrality, as evidenced by the fact that the observed F value was very similar to the average of the simulated F -statistics and lay within the 95% confidence interval of the simulated values for all loci (Table 2). The set of six microsatellite loci employed in this study is thus appropriate for characterizing neutral genetic variation. We found significant, but weak, isolation by distance in *Lobaria pulmonaria* from the studied Swiss pasture-woodland landscape (Mantel permutation test, $r_M = 0.154$, $P < 0.001$). The analysis of molecular variance (AMOVA) showed that most of the molecular variance was found among plots within regions, and among trees within plots, while molecular variance among regions explained 0% of the total variance (Table 3).

A Bayesian analysis of population structure was performed to assign *L. pulmonaria* thalli to populations, identify recent migrants, and infer the number of gene

Table 2 Ewens-Watterson tests for neutrality for six microsatellite loci of the lichen *Lobaria pulmonaria*, based on 889 samples. The statistics were calculated using 5000 simulated samples. Locus, name of microsatellite locus; n , number of thalli employed in the test; k , number of alleles; Obs.F, observed F -statistics; Mean.F, average value of the simulated F -statistics resulting; SE, standard error of simulated F -statistics; L95, lower 95% confidence interval of simulated F -statistics; U95, upper 95% confidence interval of simulated F -statistics. A superscript of 'NS' indicates that a particular locus does not deviate significantly from selective neutrality

Locus	n	k	Obs.F	Mean.F	SE	L95	U95
LPu03 ^{NS}	889	4	0.701	0.674	0.040	0.339	0.982
LPu09 ^{NS}	889	20	0.272	0.215	0.006	0.114	0.428
LPu15 ^{NS}	889	16	0.236	0.264	0.011	0.135	0.545
LPu16 ^{NS}	889	13	0.223	0.316	0.015	0.157	0.646
LPu20 ^{NS}	889	28	0.101	0.155	0.003	0.087	0.303
LPu27 ^{NS}	889	6	0.511	0.544	0.035	0.266	0.930

Table 3 Results of two analyses of molecular variance (AMOVA) of 37 plots nested within regions (plot level) and of 153 trees nested within plots (tree level) for *Lobaria pulmonaria* in a Swiss pasture-woodland landscape. d.f., degrees of freedom; SS, sum of squares; Var, percentage of molecular variance; Φ -statistics, fixation index. A superscript 'NS' indicates that a respective variance component was not statistically significant ($P > 0.05$). An asterisk indicates that a respective variance component is statistically significant at $\alpha = 0.001\%$. The overall Φ_{ST} from the plot level analysis was 0.462*, and from tree-level analysis was 0.817*

Source of variation	d.f.	SS	Var	Φ -statistics
Plot level				
Among regions	1	5.5	0.0	$\Phi_{CT} = 0.000^{NS}$
Among plots within regions	35	180.8	46.5	$\Phi_{SC} = 0.464^*$
Among thalli within plots	759	201.4	53.5	
Tree level				
Among plots	37	178.3	32.2	$\Phi_{CT} = 0.322^*$
Among host trees within plots	115	131.2	49.5	$\Phi_{SC} = 0.730^*$
Among thalli within host trees	591	53.5	18.3	

pools in the study landscape. The number of gene pools inferred with from the method by Evanno *et al.* (2005) was five, and the five gene pools were spatially intermingled (Fig. 1). Also, for no obvious reason, less individuals were assigned to populations in the eastern part of the study area.

To investigate the effect of landscape configuration on gene flow in *L. pulmonaria*, we performed a partial Mantel test and used the Monmonier algorithm to detect genetic discontinuities in the landscape. We found no correlation between landscape configuration and genetic distance of *L. pulmonaria* (partial Mantel permutation test, $r_M = -0.09946$;

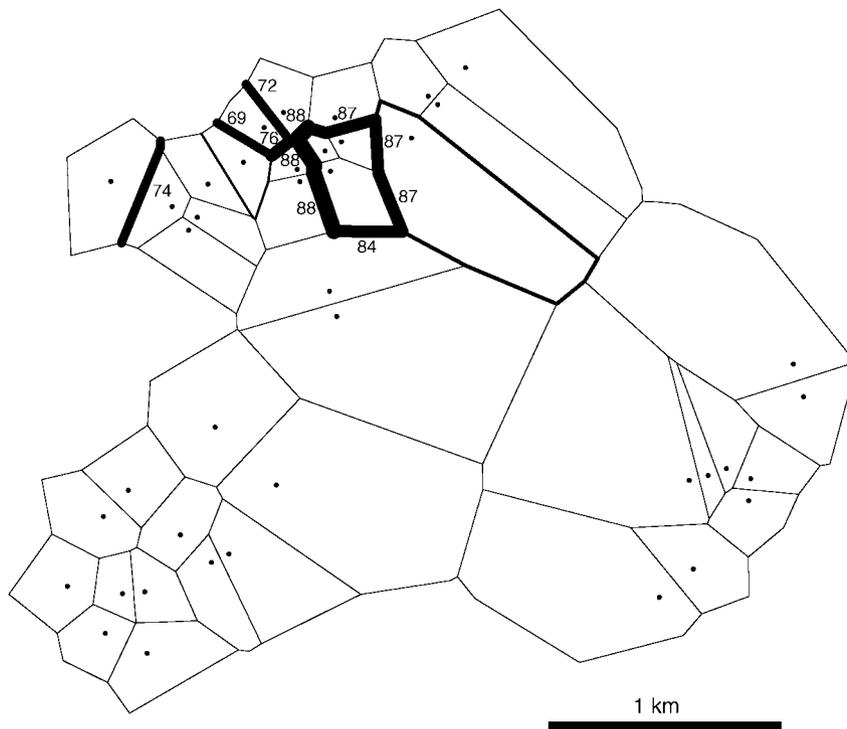


Fig. 2 Genetic discontinuities between plots of *Lobaria pulmonaria* from a Swiss pasture-woodland landscape. Based on 100 bootstrap replicates, barriers were drawn with line sizes proportional to their bootstrap support. For barriers with high bootstrap values (> 50), the respective value is given. Dots show the locations of 1-ha plots, and the lines separating them from each other represent the Voronoi tessellation of the plots' coordinates (Manni *et al.* 2004). In the northwest of the study area, a significant barrier was found for three plots containing the same multilocus genotype.

$P = 1.0$). Thus, genetic distances among *L. pulmonaria* thalli separated by wooded pasture were not significantly different from those among thalli separated by continuous forest, which implies that gene flow across the wooded pasture was as efficient as across continuous forest. Using the Monmonier algorithm (Manni *et al.* 2004), we found genetic discontinuities in the landscape with high bootstrap support (Fig. 2), separating three plots in the north-western part of the study area from neighbouring plots.

Discussion

The aim of our study was to quantify landscape-level gene flow among populations of *Lobaria pulmonaria*. Specifically, we tested the hypothesis of significant genetic differentiation at three hierarchical levels in *L. pulmonaria* – among forested regions, among plots, and among trees. If significant genetic differentiation were found, it would indicate restricted gene flow at a respective spatial scale. Second, we also assessed if landscape configuration influenced the genetic structure of *L. pulmonaria*.

We found significant genetic differentiation in *L. pulmonaria* both among plots and among trees, indicating restricted gene flow at these hierarchical levels (Table 3). The overall measures of genetic differentiation, were highly significant at both hierarchical levels ($\Phi_{ST} = 0.462$ and 0.817 at the plot and tree level, respectively); these are very high values of genetic differentiation. We also found significant differentiation among lichens collected from different

host trees within plots. A few other studies have also found genetic differentiation among lichen populations. For instance, Lindblom & Ekman (2006) investigated populations of the lichen *Xanthoria parietina* in a landscape of a comparable spatial extent as that of our study, with a maximum distance among populations of about 3.7 km. They observed significant overall Φ_{ST} values of 0.199 and 0.203 for DNA sequence data of IGS and ITS, two non-coding regions within the nuclear ribosomal gene cluster. The appropriate scale of comparison with our data is the plot level (see above), and our corresponding Φ_{ST} value was about twice as high as that of *X. parietina*. In contrast, in the terricolous lichen *Cladonia arbuscula*, no significant population subdivision was found ($\Phi_{ST} = 0.019$) when a 2-km range was investigated, which is partly explained by the comparatively low sample size (Robertson & Piercey-Normore 2007). It is striking, however, that the overall genetic differentiation among populations of *Cladonia arbuscula* was 10 and 24 times lower than in *X. parietina* and *L. pulmonaria*, respectively. Similarly, no significant genetic differentiation ($\Phi_{ST} = 0.007$) was found among populations of the epiphytic lichen *Ramalina menziesii* collected across a range of 2 km from an oak-savanna landscape situated in southern California (Werth & Sork submitted). It is hard to come to general conclusions on gene flow in lichens when looking at the above examples. *L. pulmonaria* and *X. parietina* show high genetic differentiation despite of the fact that they exhibit a dispersal syndrome (ascospores in *X. parietina*; ascospores and small

vegetative propagules in *L. pulmonaria*) that should potentially enable them to disperse better than the species for which low differentiation was found, which are both thought to disperse mainly with comparative large and heavy thallus fragments. More studies are needed to understand the relationship between the dispersal syndrome of lichens and its consequences for genetic structure.

Second, we found significant, but weak, isolation by distance in the *L. pulmonaria* population, a result which is consistent with restricted gene flow at the local scale. Isolation by distance is a process by which geographically restricted gene flow creates genetic structure, even in continuous populations (Slatkin 1993; Hardy & Vekemans 1999). The present weak isolation by distance was also in accordance with our analyses of molecular variance (see above) and the Bayesian analysis of population structure. Our finding of weak isolation by distance (Wright 1943) is corroborated by geostatistical methods such as variograms performed on a subset of our data, or the whole data set, respectively (Wagner *et al.* 2005; Werth *et al.* 2006b). It is astonishing that we found isolation by distance despite the fact that the spatial scale observed was comparatively small, i.e. within a landscape of about 9 km². Perhaps less surprisingly, isolation by distance was found on a regional scale in populations of *L. pulmonaria* (= 500 km, Walser *et al.* 2005).

Significant genetic discontinuities were found that separated three genetically almost uniform sampling plots in the northwest of the study area from neighbouring plots (Fig. 2). This pattern is most likely to have arisen from local dispersal and recruitment of *L. pulmonaria* in the northwestern part of the study area that was affected by stand-replacing disturbance 131 years ago (Kalwij *et al.* 2005). Otherwise, the genetic structure of *L. pulmonaria* did not appear to be affected by the spatial arrangement of different types of landscape elements — we found no effects of landscape configuration: (i) There was no significant molecular variance in *L. pulmonaria* attributable to two forested regions separated by a large open area (AMOVA, $\Phi_{CT} = 0.000^{ns}$, see Table 3). (ii) The result of the partial Mantel test indicated that landscape configuration did not account for the observed genetic structure. (iii) Monmonier's (1973) algorithm to detect genetic boundaries did not identify a relevant genetic discontinuity between the forested regions (Fig. 2). (iv) The Bayesian analysis of population structure indicated that five gene pools were present in the landscape we studied, but these gene pools were spatially intermingled and their geographic distribution did not reflect the landscape configuration (Fig. 2). This finding seems to reflect the dynamic nature of *L. pulmonaria* populations and the historical connectedness of the landscape. Indeed, most populations investigated were admixed, with the notable exception of populations that had been disturbed 131 years ago (Kalwij *et al.* 2005;

Werth *et al.* 2006b), situated in the northwestern part of the study area.

Palaeoecological investigations demonstrated that spruce forest with scattered deciduous trees has persisted for 5000 years in our study area in the Swiss Jura Mountains (Mitchell *et al.* 2001); thus, the *L. pulmonaria* population we investigated may be very old. The low genetic differentiation observed among forested regions (Table 3) indicated that the *L. pulmonaria* populations separated by the large open area may have been historically connected by gene flow. From this large open area, currently only one tree colonized by *L. pulmonaria* ('stepping stone') is known, located in the vicinity of the large forest in the west of our study area (S. Werth, unpublished data). Taking into consideration that the studied landscape is a dynamic pasture-woodland, the spatial extent of the open area may have varied considerably over time, giving rise to a varying number of stepping-stone populations which may formerly have connected the two continuously forested regions we investigated. The amount of deciduous trees and thus, the availability of suitable host trees for *L. pulmonaria* has decreased considerably during the last two millennia (Mitchell *et al.* 2001; Sjögren 2006), making the latter scenario very likely. Also, the forest regions may not have been isolated sufficiently long enough to observe an effect of the landscape configuration today.

Overall, we found that gene movement in *L. pulmonaria* was spatially restricted, and one might argue that this reflects spatially restricted dispersal of diaspores in *L. pulmonaria* (i.e. 'dispersal limitation'). However, the hypothesis of high mortality of dispersed diaspores and of juveniles leads to the same expected genetic structure as dispersal limitation. Werth *et al.* (2006a) found a high amount of vegetative and/or sexual diaspores dispersed over distances ≥ 200 m in *L. pulmonaria*. Therefore, dispersal limitation does not seem to be the most important mechanism underlying the high genetic differentiation observed among populations of *L. pulmonaria* in our study area. There are at least two other processes that may lead to the high genetic differentiation we observed: photobiont limitation and establishment limitation.

First, in the case of dispersed ascospores, the absence of appropriate photobiont partners (green algae and cyanobacteria in *L. pulmonaria*) prevents the establishment of thalli from spores. Strong fungal specificity in photobiont choice has indeed been documented in other lichens (Yahr *et al.* 2004). Cyanobacterial photobionts of *L. pulmonaria* do not seem to occur as free-living cyanobacterial strains, for example, indicating that their availability may be limited (Rikkinen *et al.* 2002). This 'photobiont limitation' might well be the mechanism leading to differential survival of dispersed ascospores of *L. pulmonaria* at various spatial scales (Werth *et al.* 2006a), which would be expected to be reflected in the genetic structure of both the lichenized

fungus and its photobionts. Coupled with a study on the photobiont-specificity of *L. pulmonaria*, a detailed community-level investigation of the availability of photobionts associated with *L. pulmonaria* should help to shed light on the importance of photobiont limitation.

Second, transplantation studies have shown that establishment rates of some endangered lichens propagating with vegetative propagules (soredia) are low (Zoller *et al.* 2000); this is also the case in *L. pulmonaria* (Scheidegger 1995; Werth *et al.* 2006a). Werth *et al.* (2006a) observed substantial differences in survival rates of vegetative propagules among the investigated forest stands. Thus, the establishment of *L. pulmonaria* from soredia appears to be limited by unfavourable environmental conditions (Werth *et al.* 2006a). Additionally, diaspores dispersed over long distances might experience reduced establishment if they are maladapted to the environment they have dispersed to. More detailed studies are, however, necessary to fully assess the importance of local adaptation as a factor influencing the establishment of *L. pulmonaria*.

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