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Estimating the Dispersal Capacity of the Rare Lichen

Cliostomum corrugatum

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Estimating the Dispersal Capacity of the Rare Lichen

Cliostomum corrugatum

Abstract

The objective of this study was to estimate the dispersal rate in an organism assumed to be confined to tree stands with unbroken continuity. We used the lichen-forming ascomycete *Cliostomum corrugatum*, which is largely confined to old oak stands. Five populations, with pairwise distances ranging from 6.5 to 83 km, were sampled in Östergötland, south-eastern Sweden. DNA sequence data from an intron in the small subunit nuclear ribosomal RNA gene was obtained from 85 samples. Nearly all molecular variance (99.6 %) was found within populations and there were no signs of isolation-by-distance. The absolute number of immigrants per population per generation (estimated to 30 years), inferred by Bayesian MCMC, was found to be between one and five. Altogether, evidence suggests abundant gene flow in the history of our sample. A simulation procedure demonstrated that we cannot know whether effective dispersal is ongoing or if it ceased at the time when oaks started to decrease dramatically around 400 y BP. However, a scenario where effective dispersal ceased already at the time when the postglacial reinvasion of oak had reached the region around 6000 y BP is unlikely. Vegetation history suggests that the habitat of *C. corrugatum* was patchily distributed in the landscape since the early Holocene. Combined with the high dispersal rate estimate, this suggests that the species has been successful at frequently crossing distances of at least several kilometres and possibly that it has primarily been limited by the availability of habitat rather than by dispersal.

Keywords: dispersal, establishment, ecological continuity, old-growth forests, *Quercus*,
ascomycete

Introduction

Many organisms, belonging to a variety of taxonomic groups like wood-decay fungi, lichens, bryophytes, vascular plants, and insects, seem to be confined to habitat patches that have persisted presumably unchanged over an extended period of time (Berg et al. 1994; see Nordén and Appelqvist 2001: 781 for references to specific organism groups). The concept ‘ecological continuity’ (EC), coined by Rose (1974), has been used to refer to the temporally unbroken continuity of such habitat, often assumed to be primeval or old-growth forests. It has also been proposed that certain species can be used as indicators of EC (e.g., Rose 1974; Tibell 1992; Selva 1994; Kuusinen 1996; Økland 1996; Lindblad 1998; Selva 2003) when historical data are difficult to obtain. The EC concept has been criticized for often being vaguely defined on spatial and temporal scales (Gauslaa and Ohlson 1997; Nordén and Appelqvist 2001; Sverdrup-Thygeson and Lindenmayer 2003). In most real applications, EC implicitly refers to the forest stand level. Using indicator species to assess EC is also problematic, for several reasons: the group of species claimed to indicate EC probably includes species with poor dispersal capacity as well as species with particular microhabitat requirements. Their dispersal capacity and habitat requirements are often poorly or not at all understood (Nordén and Appelqvist 2001; Rolstad et al. 2002). Forest history and dynamics is poorly known and often judged from anecdotal evidence (Rolstad et al. 2002). The spatial scale at which indicators are assumed to work is usually undefined, the spatial precision of the indicator species being dependent on dispersal capacity (Rolstad et al. 2002; Sverdrup-Thygeson and Lindenmayer 2003; Kalwij et al. 2005). Finally, it cannot be uncritically assumed that species richness or the number and

abundance of rare species is strictly positively correlated with temporal continuity (Ohlson et al. 1997; Fenton and Bergeron 2008; Löhmus and Löhmus 2008). However, there is ample evidence that some species are indeed confined to EC habitats and that red-listed species or certain taxonomic groups are represented by more species in older-than-average and unmanaged forests compared to younger and managed ones (e.g., Gustafsson and Hallingbäck 1988; Goward 1994; Fritz and Larsson 1996; Spence et al. 1996; Nilsson and Baranowski 1997; Martikainen et al. 1999; Uliczka and Angelstam 1999; Hedenås and Ericson 2000; Martikainen et al. 2000; Cameron 2002; Penttilä et al. 2004; Tikkanen et al. 2006; Rivas Plata et al. 2007; Fritz et al. 2008).

A central question is *why* some organisms are confined to sites with long temporal continuity, or at least prefer them. There are two main, not mutually exclusive, explanations for this phenomenon: (1) limitation by dispersal, dispersal primarily taking place only at very short distances and being virtually absent at larger distances, and (2) limitation by habitat availability. If dispersal is the primary limiting factor, occurrence in EC habitats is likely to be of a relictual nature, whereas this is not necessarily the case when habitat availability is the primary limiting factor. Limitation by habitat availability is indeed a realistic phenomenon, as the structural complexity and consequently the number of microhabitats in a forest has been shown to be higher under old-growth conditions (Zenner 2004). In both cases, preserving currently occupied patches and creating new habitat may be necessary for the long-term preservation of an organism restricted to EC habitats. However, the distances that can be allowed in this network of currently and potentially occupied patches depend crucially on the dispersal capabilities of the organism in question. Unfortunately, a scientifically based knowledge of effective dispersal (i.e. dispersal

followed by establishment) at the landscape level is currently missing in most species. This includes also species restricted to EC habitats, many of which are also red-listed and in need of proper management for long-term persistence.

The objective of this study was to estimate the rate of dispersal at the landscape level in an organism restricted to forests with long temporal continuity. We selected the lichen-forming ascomycete *Cliostomum corrugatum* (Ach.) Fr. as a study species. *Cliostomum corrugatum* is a rare lichen that is largely confined to very old stands of *Quercus robur* (Berg et al. 2002) and has been suggested to be an indicator of EC (Arup 1997). We used DNA sequence data from a nuclear marker, combined with a population genetics approach, to address the question at hand. We are not aware of any previous investigations of genetic variation at small spatial scales in a crustose (crust-forming) lichen.

Materials and methods

Study species

The epiphytic crustose lichen *Cliostomum corrugatum* (Ach.) Fr. (Ascomycota, Lecanoromycetes, Lecanorales, Ramalinaceae) possesses a greyish thallus containing a green alga as its photosynthesizing symbiont. The thallus bears conspicuous, light yellow to light brown, 0.5-1.2 mm wide apothecia (fruiting bodies producing putatively meiotic ascospores) as well as black, 0.2-0.5 mm wide pycnidia (producing mitotic conidia that may function as spermatia, fungal diaspores, or both) (Thor and Arvidsson 1999). In Sweden, its geographical distribution largely follows that of *Quercus*. Its primary habitat is coarse bark of old trunks of *Quercus robur* in relatively dry and semi-open forests or

parklands (Thor and Arvidsson 1999), mainly on the flat terminal parts of the bark structure and not on either side of the cracks in the bark (pers. obs.). *C. corrugatum* has occasionally been encountered on the coarse bark of other deciduous trees as well as on wood of decorticated stumps, old wood structures, and twigs of *Picea abies* (Thor and Arvidsson 1999). No vegetative diaspores (soredia or isidia) containing tissue from both symbionts are produced (Thor and Arvidsson 1999). *Cliostomum corrugatum*, like all ascomycetes except some yeasts, is presumed to have a dominantly haploid life cycle. Dikaryotic and diploid stages appear only as very small amounts of hyphae confined to the apothecia. *Cliostomum corrugatum* is rare in northern Europe and red-listed in, e.g., Sweden (Near Threatened; Gärdenfors 2005), Norway (Critically Endangered; Kålås et al. 2006), Denmark ('Vulnerable' but not evaluated according to recent IUCN criteria; Stoltze and Pihl 1998), Finland (Near Threatened; Rassi et al. 2001), Germany ('Critically Endangered' but not evaluated according to recent IUCN criteria; Ludwig and Schnittler 1996), and the United Kingdom (Vulnerable; Woods and Coppins 2003). The lichen is small but its distinctive morphology and habitat makes it relatively easy to detect in the field.

Sample area and sites

Samples of *C. corrugatum* were collected on tree trunks of *Q. robur* up to two meters above the ground between January 5 and February 4 2005 at five sites in central Östergötland, south-eastern Sweden (Fig. 1; Table 1). *Quercus* colonized this area approximately 7000 years BP (Brewer et al. 2002). Central Östergötland supports one of the highest densities of old oaks in Sweden. Altitudinal differences in this region are small and the soil is fertile and consists of sedimentary silt and clay particles deposited during or

Fig. 1 – The five sample sites of *Cliostomum corrugatum* in the province of Östergötland, southern Sweden, indicated with black dots.

after the end of the last glaciation. The ice retreated from this region 10000 years ago (Lundqvist 1998), but due to land depression, all sites were initially below sea level.

Pairwise distances between sites were evenly distributed and ranged from 6.5 to 83 km. The smallest tree trunk inhabited by *C. corrugatum*, out of all investigated, was 0.65 m in diameter at breast height (dbh). Johansson et al. (in press) demonstrated a positive correlation between the probability of occurrence of *C. corrugatum* and tree trunk size of *Q. robur*. Owing to difficulties determining the boundaries between adjacent lichen thalli, only one sample was taken per tree to avoid the risk of sampling the same individual twice. The minimum number of samples per site was set to 15.

*Table 1 – Geographical location and short description of the five sites where *Cliostomum corrugatum* was sampled in Östergötland, south eastern Sweden.*

Site	Latitude/Longitude	Description
Bjärka-Säby	58°16'29.9" N, 15°44'24.0" E	Parkland mixed with arable land
Orräng	58°17'32.2" N, 15°51'19.9" E	Grassland with patches of open oak stands
Solberga	58°20'19.2" N, 15°11'49.8" E	Forest adjacent to river
Bråborg	58°36'56.5" N, 16°22'00.4" E	Mixed forest in NE facing slope to Baltic sea
Stegeborg	58°26'17.2" N, 16°35'58.0" E	Small patchy tree stands in agricultural landscape

Laboratory methods and sequence editing

Methods for DNA extraction, PCR amplification, and sequencing followed Lindblom and Ekman (2006), except that we mainly used apothecial tissue (or tissue from the thallus or pycnidia, when apothecia were not available). We first targeted the internal transcribed spacer region (ITS) and the intergenic spacer (IGS) of the nuclear ribosomal DNA. Because of low variability, we turned our attention to the group 1 intron situated between positions 1516 and 1517 at the end of the small subunit (SSU) of the nuclear ribosomal RNA gene (Gargas et al. 1995). This region was amplified using the forward primer ITS1F (White et al. 1990), situated at the end of the SSU but upstream of the intron site, and the newly designed reverse primer ITS1-Cc1-R, situated in the first part of ITS1. The new primer was designed because the widely used combination of ITS1F and ITS4 (Gardes and Bruns 1993) to amplify the entire ITS region in many cases either failed or resulted in multiple PCR products. The sequence of the new primer is 5'-ATG GTA AGG TAA TCA CAG GGT GTA-3'. The amplification, PCR clean-up, and sequencing procedures were identical to the ones used by Lindblom and Ekman (2006) for the ITS region. Sequencing was performed using both PCR primers. The technique used here, particularly when using overlapping forward and reverse reads, has by far the lowest error rate of any currently used sequencing procedure (Johnson and Slatkin 2007). Only sequences from reads with a low level of noise relative to the signal were processed for further analysis. Resulting sequences were manually edited and aligned using BioEdit version 7.0.5.3 (Hall 1999). The identity of the sequences obtained with the new primer pair was confirmed by comparing with sequences initially generated with ITS1F and ITS4. The ITS part of these sequences were

in turn subjected to a BLAST (megablast) search against the nr/nt database at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) on 22 October 2008. The top 69 hits against the amplified ITS were taxa in the Ramalinaceae (expectation scores $\leq 3 \times 10^{-137}$ and query coverage ≥ 72 %). Each haplotype sequence was submitted to GenBank and given accession numbers EU218541-EU218551.

Statistical analyses

In order to create a simple overview of haplotype relationships and frequencies present in the sample, we constructed a haplotype network using the 95 % statistical parsimony criterion as implemented in the software TCS version 1.13 (Clement et al. 2000). A likelihood model was fitted to the data using a hierarchical likelihood ratio test as implemented in MODELTEST version 3.7 (Posada and Crandall 1998). The best model reported was HKY85 without rate heterogeneity. Using Arlequin version 3.1 (Schneider et al. 2000), we first conducted a Ewens-Watterson-Slatkin exact test of selective neutrality in the entire sample (Slatkin 1994; Slatkin 1996). The null distribution under neutrality was obtained by simulating the null hypothesis 10000 times (Stewart 1977). We conducted an analysis of molecular variance (AMOVA) (Excoffier et al. 1992), assessing significance by 10000 permutations. A Mantel matrix correlation test between pair-wise values of $F_{ST}/(1 - F_{ST})$ (Slatkin's linearized F_{ST}) and pair-wise values of the natural logarithms of geographic distance between populations (Rousset 1997) was used to check for the presence of isolation-by-distance (Wright 1943), i.e. a spatial aggregation of genetically similar individuals. Significance was assessed by 10000 permutations. Wherever applicable, Tamura distances were used, as they most closely corresponded to HKY85. In all

calculation involving Arlequin, indels were given weight 1 (i.e., they were counted as the ‘fifth character state’).

The main part of our analysis, however, consisted of a direct estimation of population parameters, including dispersal, using Bayesian Markov chain Monte Carlo (MCMC), as implemented in the software LAMARC 2.0.2 (Kuhner et al. 2005; Kuhner 2006; Beerli 2006; Kuhner and Smith 2007). In its official terminology, LAMARC measures ‘migration’. This word is usually interpreted as one or more individuals contributing to N_e (the effective population size) in one population leaving that population and entering a new population. In our case, individuals are sessile and dispersal is expected to occur via ascospores or perhaps conidia, without any individuals contributing to N_e ever moving between populations. However, migration in the true sense is not a prerequisite for population parameter estimates by LAMARC to be valid for dispersing sessile organisms (Peter Beerli and Lucian Smith pers. comm. 2007). This is because the model in LAMARC decouples migration from size fluctuations, and anyway restores population size to its original size when individuals migrate. Bayesian MCMC has the advantage of explicitly handling uncertainty in parameter estimates, which is all the more important when the amount of data, as in this case, is small and uncertainty about estimates can consequently be expected to be large. In the Bayesian MCMC, the F84 model was used, because this is the model implemented in LAMARC that most closely corresponds to HKY85. The transition to transversion ratio, which is treated as fixed by LAMARC, was calculated under maximum likelihood using PAUP* 4.0b10 (Swofford 2003) on a collapsed version of our dataset. For this estimate (ratio = 7.01), empirical nucleotide frequencies were used under the F84 likelihood model. An empty population was added to account for ‘ghost

populations' (Slatkin 2005) and unsampled populations, following the recommendation by Kuhner (2003) and Beerli (2004). Prior distributions were set to uniform in linear space on the interval $[10^{-3}, 10^4]$ for migration and uniform in logarithmic space on the interval $[10^{-8}, 10]$ for the population mutation rate θ (corresponding to the extreme lower and upper boundaries allowed by the software). These priors essentially meant assuming that small and large values of migration are equally likely a priori, whereas small values of θ are more likely than large values a priori. The prior distribution of population size fluctuations, when applicable, was uniform on the interval $[-500, 1000]$ in linear space (LAMARC allows only linear priors for size fluctuations). The proposal rate for population parameters was set to ten times the proposal rate for genealogy rearrangements. This was necessary to alleviate problems with poor effective sample sizes of population parameter estimates, particularly θ . Preliminary runs with adaptive heating indicated that Metropolis coupling, the use of heated chains, was unnecessary. However, we discovered that population parameter estimates were more precisely repeatable when using heating. Consequently, all subsequent runs were conducted with one heated chain at a temperature of 1.1, allowing information from the heated chain to be swapped into the cold chain every 10 generations. LAMARC by default treats population parameters as unconstrained. This means that when θ , migration, and size fluctuation are estimated jointly, there is one θ and one size fluctuation parameter for each population as well as one migration parameter in each direction between each pair of populations (migration is treated as asymmetric). With five sampled populations and one void population, as in our case, this amounts to a large total number of parameters that may not be supported by the data. Therefore, we performed tests of model adequacy, which involved the use of Bayes factor (Kass and Raftery 1995) to compare

models based on the harmonic mean estimator (Newton et al. 1994). Starting with simple models, we added parameters only if there was ‘strong’ support (Kass and Raftery 1995: 777) for a more complex model, i.e. if twice the difference in harmonic mean $\ln$ likelihood exceeded 6. For θ , we tested a model where all values are identical against an unconstrained model. Size fluctuation was either set to zero, treated as equal across all populations, or unconstrained. Migration was set to zero, treated as equal across all populations, as different between population pairs but symmetric, or unconstrained (asymmetric between population pairs). In all cases, the void population was treated as unconstrained, because we do not know how many real-world populations it represents. Because LAMARC 2.0.2 only reports the data $\ln$ likelihood for the last sample of the MCMC chain, we created a workaround by splitting the analysis into several consecutive ‘initial chains’, the likelihood being reported at the end of each such chain. We allowed 42500 initial chains, each 200 generations long, i.e. a total of 8.5 million generations. Software was written in RealBasic to extract data $\ln$ likelihoods from the output (‘outsumfile’). Likelihoods were subsequently imported in a Microsoft Excel spreadsheet, likelihoods plotted, and the harmonic mean $\ln$ likelihood calculated across the stationary phase of the run. Plotting $\ln$ likelihood against generation indicated that the true burn-in was in the order of 100000-150000 generations, but we anyway discarded the first 500000 generations. Using this scheme, we arrived at a model treating size fluctuations as absent (set to zero), and θ and migration as equal across all populations. This does not mean that the true scenario was this simple, only that the current data contained no information to support a more complex model. Final estimates of θ and migration were obtained by summing results across three identical runs, each 8.5 million generations in length and

discarding the initial 500000 generations as burn-in. In the haploid case, $\theta = 2N_e\mu$, where μ is the per site mutation rate per generation. Migration is measured as $M = m/\mu$, where m is the proportion of immigrants into a population per generation. Software was written in RealBasic in order to extract the posterior distribution of $N_em = \theta M/2$, the absolute number of immigrants into a population per generation, from the joint distribution of θ and M . The unimodal posterior probability distributions were finally transformed into 95 % equal-tail credible intervals by removing 2.5 % of the total probability at each end of the posterior probability distribution.

Finally, we performed a simulation study using SimCoal 2.1.2 (Laval and Excoffier 2004), with the purpose of evaluating the temporal information contained in the migration rate estimates obtained by LAMARC under different demographic histories. LAMARC assumes migration rates to be constant over time, from the present to coalescence of the sample, but this is rarely the case in real populations. Therefore, estimated migration rates might be averages over recent evolutionary time, with limited information about ongoing migration. We wanted to answer two specific questions: can we separate between a model with and a model without migration after the start of the dramatic decrease of oaks a few hundred years ago? Similarly, can we separate between a model with and a model without migration after the immigration of oaks to Östergötland 6000 years ago? We assumed a generation time of 30 years for *C. corrugatum*, based on a combination of the demography and phenology of the lichen as well as the growth rate of the inhabited oak trees (Lättman et al., unpublished results). The demographic history was divided into three phases (in backward time): (1) the first 13 generations, corresponding to the time during which old oaks decreased dramatically in the region (Eliasson and Nilsson 2002); (2) generations 14

to 200, corresponding to the period limited by the immigration of oak to the region; (3) generations 201 until coalescence, corresponding to the history of the sample during which oaks had not yet immigrated into the region. We assumed the most probable estimates of θ and M obtained from the LAMARC analysis and translated them into N_e and m using an estimate of μ . The (short-term) pedigree rate of mutation, which should not be confused with the (long-term) phylogenetic substitution rate (Howell et al. 2003; Ho et al. 2005, 2007), has been found to be approximately $1-2 \times 10^{-8}$ for several organisms (Drake et al. 1998; Nachman and Crowell 2000; Denver et al. 2004). However, Lutzoni and Pagel (1997) reported up to 10-fold higher mutation rates in lichen-forming fungi compared to non-mutualistic relatives that could not be ascribed to significantly relaxed negative selection. Therefore, we settled for $\mu = 10^{-7}$ per site per generation. The following scenarios were simulated (subscripts of m refer to the three phases described above): (A) $m_1 = m_2 = m_3 = 0.001$ (migration has remained constant and is ongoing), (B) $m_1 = 0$, $m_2 = m_3 = 0.001$ (migration ceased 13 generations ago in connection with a decrease in available oak habitat), and (C) $m_1 = m_2 = 0$, $m_3 = 0.002$ (migration ceased once the postglacial expansion of oak reached the region). In scenario C, we doubled the migration rate in phase 3 in order to maintain an approximate average migration rate of 0.001 over the entire time span (assuming that coalescence occurred during the bottleneck caused by the latest glaciation). In all three scenarios, we simulated a 20-fold increase in population size from phase 1 to phase 2 (corresponding to a 95% population reduction in forward time). 200 data sets were simulated per scenario. The transition to transversion rate used in LAMARC was maintained in SimCoal. Each data set simulated by SimCoal was analyzed using Arlequin

3.1, and three types of summary statistics were collected in order to compare them with observed values: (1) the proportion of within-population variation inferred by an AMOVA, (2) Tajima's D (Tajima 1989) averaged over populations, and (3) the average number of polymorphic (segregating) sites per population. 95% ranges were constructed by removing the five most extreme values at each end of the distributions.

Results

We found three IGS haplotypes, two of which were represented by a single individual each and the third by 79 individuals. ITS proved to be difficult to amplify and sequence. Eight samples, which were successfully sequenced, displayed no variation at all. We found the variation in IGS and ITS to be insufficient and discarded this data in subsequent statistical analyses. The SSU intron, on the other hand, was represented by eleven haplotypes (Table 2). Out of the 96 samples, 85 were successfully extracted, the SSU intron amplified, sequenced, and consequently included in the statistical analyses. The SSU intron length varied from 612 to 613 nucleotides, and the resulting alignment was 614 positions including gaps. Ten positions were variable. A haplotype network is presented in Fig. 2. This shows that the eleven haplotypes are separated by single mutational steps and that the two common haplotypes (represented by 30 and 46 thalli, respectively) are internal and hence presumably older than the infrequent terminal haplotypes. The neutrality test indicated no deviation from neutral conditions (all populations $p = 1.00$, Bjärka-Säby $p = 0.79$, Orräng $p = 0.68$, Solberga $p = 0.84$, Bråborg $p = 0.38$, and Stegeborg $p = 0.40$). The AMOVA indicated that 0.4 % of the variance is between populations and 99.6 % within populations. The reported fixation index ($\Phi_{ST} = 0.004$) was not significant ($p = 0.35$).

Table 2 – Variable nucleotide sites in the alignment of eleven haplotypes of the position 1516 SSU intron. The alignment was 614 sites in length. GenBank accession numbers for each haplotype are indicated.

Haplo- type	Sequence and base number										Origin					GenBank Accession nos
	0	0	1	1	1	2	2	4	5	5	Bjärka- Säby	Orräng	Solberga	Bråborg	Stegeborg	
	2	7	0	4	5	6	9	7	1	2						
	6	9	3	1	1	4	5	1	6	3						
1	G	A	C	C	C	–	A	A	A	–	8	6	5	7	4	EU218541
2	•	•	•	•	•	–	C	•	•	–	7	9	10	7	13	EU218542
3	•	G	•	•	•	–	•	•	G	C	1					EU218543
4	•	•	T	•	•	–	C	•	•	–	1					EU218544
5	•	•	•	T	•	–	•	•	•	–	1					EU218545
6	A	•	T	•	•	–	C	•	•	–		1				EU218546
7	•	•	•	•	T	–	C	•	•	–		1				EU218547
8	•	•	•	•	•	C	•	•	•	–			1			EU218548
9	•	G	•	•	•	–	•	•	•	C			1			EU218549
10	•	•	•	•	•	–	•	G	•	–			1			EU218550
11	•	G	•	•	•	–	•	•	•	–				1		EU218551
Total											18	17	18	15	17

Fig. 2 – Unrooted haplotype network for Cliostomum corrugatum with the two common haplotypes 1 (n=30) and 2 (n=46) in the centre. Remaining terminal haplotypes are represented by one thallus each. One mutational step between haplotypes is represented by a line.

Consequently, the AMOVA provided no evidence of significant neutral differentiation among populations. The Mantel test revealed no indication of significant isolation-by-distance, the correlation between Slatkin's linearized F_{ST} and the logarithm of geographic

distance being non-significant ($p = 0.70$). Estimates of θ , M , and $N_e m$ obtained via LAMARC, including 95 % equal-tail credible intervals, are reported in Table 3. The absolute number of successful establishments per generation per population was estimated to be between one and five, with the median of the posterior probability being two. With four other sampled populations, from which dispersal and establishment into a population can take place, the total number of successful establishments from the sampled populations is four times higher, i.e. between four and 20 with the median at eight successful establishments per generation from the other four populations. Dispersal from unsampled populations, the number of which is unknown but presumably rather large, comes in addition. Migration (M) may have been underestimated, or at least truncated, because much of the posterior probability accumulated right below the highest upper limit allowed by LAMARC for that parameter. Table 4 accounts for the simulation of three different demographic scenarios. This simulation shows that the observed values of the proportion of within-population variation, the average Tajima's D across populations, and the average number of polymorphic sites across population is compatible with both a model of ongoing migration as well as a model where migration ceased 13 generations ago. The observed values are, however, incompatible with a model where migration ceased 200 generations ago, because of the higher expected proportion of within-population variation.

Discussion

Dispersal in *Cliostomum corrugatum*

Knowledge about effective dispersal rates and dispersal distances are paramount to any scientifically-based conservation measure. Yet, such knowledge is unavailable for most organisms. We used DNA sequence data from an intron near the terminal end of the nuclear small subunit ribosomal DNA to infer effective dispersal rates between populations of *C. corrugatum*. Other markers, ITS and IGS, failed to produce useful amounts of variation. The limited amount of data available to us, a single gene, made it imperative to apply analytical methods that reveal the uncertainty in parameter estimates (in addition to methods that calculate point estimates). We handled uncertainty in our estimates by use of a Bayesian as well as a simulation approach.

Indirect estimates of dispersal rates and dispersal distances through a point estimate of population differentiation (AMOVA), a point estimate of the correlation between interpopulational genetic and physical distances (Mantel test), as well as a Bayesian direct measure of dispersal (Table 3) all indicate that effective dispersal between populations at this spatial scale has been substantial and without measurable restrictions. The Bayesian approach indicates that the most likely number of successful establishments per generation between the sampled populations is between four and twenty. The number of immigrants needed to prevent neutral divergence of populations has been suggested to be ca 5 (Lacy 1987), 1–10 (Mills and Allendorf 1996), or more than 10 (Vucetich and Waite 2000) per generation. The very wide Bayesian posterior distribution of dispersal rate also demonstrates that our estimate is indeed associated with considerable uncertainty.

Table 3 – Population parameter estimates obtained using the coalescent in a Bayesian MCMC framework, as implemented in LAMARC 2.0.2. $\theta = 2N_e\mu$, $M = m/\mu$, and $N_em = \theta M/2$, where N_e is the effective population size, μ the per site mutation rate per generation, and m the per generation proportion of immigrants into a population from another population. MPE = most probable estimate, corresponding to the mode of the posterior probability distribution. 95 % CI = 95 % equal-tail credible interval.

Parameter	MPE	95 % CI
θ	$6.1 \cdot 10^{-4}$	$3.2 \cdot 10^{-4} - 1.2 \cdot 10^{-3}$
M	9541	4225 – 9988
N_em	1.9	1.1 – 4.8

However, although it remains unknown exactly how high the rate of dispersal is at this spatial scale, the credible interval clearly excludes low dispersal rates even though we used a uniform prior distribution ranging from no dispersal at all to very high dispersal rates. A possible explanation for the high rates of successful dispersal is that dispersal is not as passive as one might think. Perhaps dispersal in *C. corrugatum* is facilitated by winged insects carrying ascospores, conidia, or pieces of lichen thallus. Ascospores and algal cells have been shown to be viable after having passed through the gut of mites (Meier et al. 2002). Mites, in turn, could be carried over large distances with the help of mammals or birds. A number of insects have been shown to be faithful to the kind of oak trees that *C. corrugatum* inhabits (Niklasson and Nilsson 2005).

An assumption of the Bayesian coalescent analysis was that dispersal rates have remained approximately constant from the present to coalescence in backward time, otherwise inferred rates will reduce to averages over time. The simulation study, although a simplistic picture of the real events, efficiently demonstrates that we cannot separate

between a model with constant and ongoing dispersal from a model where migration ceased at the time when oak habitat started to decrease dramatically around four centuries ago (Table 4, scenario A and B). In other words, we cannot know whether effective dispersal is ongoing or whether recent fragmentation, owing to human influence on landscape characteristics, has caused connectivity between populations to decrease. On the other hand, a scenario where effective dispersal ceased already at the time when the postglacial reinvasion of oak had reached the region is implausible (Table 4, scenario C).

Table 4 – Median and 95 % ranges of the proportion of within-population variation, average value of Tajima’s D per population, and the average number of polymorphic sites per population obtained when simulating three different demographic scenarios: A (constant and ongoing migration), B (migration ceased around the time when the oak habitat started to decrease dramatically around four centuries ago), and C (migration ceased once the postglacial reinvasion of oak had reached the region). Details of the simulation parameters are found in the text. The observed parameter values are included for comparison.

	Proportion of within-population variation	Tajima’s D (average per population)	No. of polymorphic sites (average per population)
Observed	0.004	-0.312	3.6
Scenario A	0.086 (-0.010 – 0.230)	0.358 (-0.690 – 1.717)	4.6 (1.0 – 12.2)
Scenario B	0.086 (-0.001 – 0.274)	0.363 (-0.692 – 1.441)	4.7 (1.4 – 12.2)
Scenario C	0.144 (0.014 – 0.307)	0.410 (-0.615 – 1.306)	4.0 (1.0 – 12.6)

Could the high inferred dispersal rates be a consequence of high connectivity in large and effectively continuous populations of *C. corrugatum* from the time of oak reinvasion until around 1600 AD? Current knowledge of the vegetation history of southern Sweden allows some inferences about the dispersal capabilities of *C. corrugatum*, although we can

say nothing about ongoing dispersal. In the historic agricultural landscape of Sweden, we know that from 1558 until 1830 oaks were considered state property to meet the needs of timber for the navy. This royal decree was increasingly being disregarded by peasants, and the 1825 reinventory of oaks made some 30 years earlier disclosed an 80 % reduction of timber oaks during this short period of time (Eliasson and Nilsson 2002). Another inventory of oaks in Östergötland in 1813 demonstrated that more than 80 % of the oaks were found in the enclosed meadows and fields surrounding the villages (Eliasson and Nilsson 2002). The remainder of the oaks were found outside the village enclosures, grazing intensity (and thereby the amount of sun-lit oaks) progressively decreasing with increasing distance from the villages. Before and after the 1558-1830 period, oaks were probably uncommon inside village enclosures. The Swedish system of a clear division between areas inside and outside village enclosures has a tradition that goes back at least 1000 years but probably as much as 2000 years (Ekstam et al. 1988; Niklasson and Nilsson 2005). During this period, *C. corrugatum* habitat was probably patchily distributed, oaks occurring under semi-open and sun-lit conditions almost exclusively being found in or near villages. Further back in time, prior to the advent of agricultural landscape, human influence was primarily by cultivation of temporary clearings in the forest (Niklasson and Nilsson 2005). Recent developments in paleoecology (Mitchell 2005; Birks 2005) indicate that in the early and mid-Holocene, much of lowland Europe was covered by closed-canopy forests, contrary to earlier suggestions involving wood-pastures kept open by megaherbivores (Vera 2000). In closed-canopy forests, *C. corrugatum* would have been restricted to steep, south- or west-facing slopes (Ek et al. 1995) and lake and river edges. This type of habitat is likely to have been highly patchily distributed in the landscape. In

conclusion, dispersal between patches suitable for *C. corrugatum* during the last 6000 years in Östergötland must commonly have involved crossing distances of at least several kilometres, even if oaks were notably more common than in the present-day landscape of southern Sweden. Indeed, inferred dispersal rates are high enough to suspect that the postglacial occurrence of *C. corrugatum* was primarily limited by the availability of habitat and not by dispersal. Limitation by habitat availability has been suggested also for lichens in stands of aspen (*Populus tremula*), inferred from a combination of occupancy patterns, successional history, and stand characteristics (Hedenås and Ericson 2004).

Dispersal in lichens – the current state of knowledge

There is considerable disagreement in the literature concerning the ability of lichens to disperse and establish. Like in most other organisms, effective dispersal rates seem to be scale-dependent, but this explains only part of the disagreement. Vegetative diaspore dispersal at short distances, up to a few hundred meters, has been suggested to be effective, although several studies did not investigate the success rate of establishment (Armstrong 1987, 1990; Tapper 1976; Heinken 1999; Lorentsson and Mattsson 1999). Dispersal limitation has been reported for tree-living lichens within tree stands, between tree stands in close proximity, or up to a few kilometres apart (Dettki et al. 2000; Sillett et al. 2000; Hilmo and Såstad 2001; Johansson and Ehrlén 2003; Walser 2004; Öckinger et al. 2005), as well as between populations of an asexual terrestrial lichen at a distance of up to a few kilometres (Cassie and Piercey-Normore 2008). However, genetic studies of *Xanthoria parietina* and *Lobaria pulmonaria* suggest otherwise: effective dispersal at this scale shows no sign of being restricted, although ascospores have been found to disperse, on average, at

longer distances than heavier vegetative diaspores (Lindblom and Ekman 2006, 2007; Wagner et al. 2006; Werth et al. 2006a, 2006b). At large spatial scales, populations being separated by hundreds of kilometres or more, genetic studies of lichen populations revealed severe dispersal restrictions (Printzen et al. 2003; Palice and Printzen 2004; Walser et al. 2005), whereas studies relying on biogeographic patterns (Munoz et al. 2004), trapping of lichen fragments in the atmosphere (Harmata and Olech 1991), or observations of lichen fragments on bird feet (Coppins and James 1979) implicitly proposed effective dispersal to be frequent. Finally, the small size and weight of the ascospores has been taken as indirect evidence in favour of lichens being able to disperse “widely” (Nordén and Appelqvist 2001).

What is the conservation message contained in our results? We have inferred high rates of dispersal at landscape level in the history of a set of populations of a red-listed crustose lichen confined to EC habitats. The often-repeated claim that lichens confined to EC habitats are poor dispersers at more than very local scales may be a severe underestimate of their capabilities. As mentioned above, there are indications that some rare taxa restricted to EC forests are indeed poor dispersers at the landscape level, but that conclusion might not apply universally. Furthermore, there is a non-negligible risk that the species so far investigated are not representative among the lichens; the majority of EC species being crustose like *C. corrugatum*. Unfortunately, life-history traits might not help us to accurately predict dispersal ability (Johansson and Ehrlén 2003; Duminil et al. 2007). Our knowledge of the dispersal capabilities of lichenized fungi therefore remains in its infancy.

Methodological issues

There are two methodological issues that need to be discussed briefly. Firstly, lateral transfer of group I introns between positions (Bhattacharya et al. 2002) and even interspecific horizontal transfer (Martin et al. 2003; Simon et al. 2005) in the nuclear SSU rRNA gene has been implicated in a phylogenetic perspective. However our haplotype network (Fig. 2), which is typically star-shaped and separates the 11 haplotypes by single mutational steps, strongly indicates that horizontal transfer did not affect our study.

Secondly, we primarily used fruiting bodies (apothecia) for DNA extraction, because extractions from the vegetative thallus were more likely to be troubled with contamination by other lichenized or non-lichenized fungi present in the habitat. Apothecia contain very small amounts of dikaryotic tissue as well as meiotic ascospores that could potentially contain genetic material from another, presumably nearby, individual, the ‘father’.

However, we did not experience problems with multiple mixed PCR products as evidenced by chromatograms with superimposed base calls. We cannot say whether this means that *C. corrugatum* is homothallic (the haploid equivalent of self-fertilizing) or just that the amount of ‘father DNA’ was too small to be detected among the dominant ‘mother DNA’ in the vegetative hyphae making up the vast majority of the apothecial tissue.

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