Insights into the Ecology and Genetics of Lichens with a Cyanobacterial Photobiont

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Cover: *Lobaria pulmonaria*, *Nephroma bellum,* and fallen bark in an old-growth forest in Finland with *Populus tremula*. Part of the tRNA^{Leu} (UAA) sequence in an alignment. (photos: K. Fedrowitz)

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Abstract

Nature conservation requires an in-depth understanding of the ecological processes that influence species persistence in the different phases of a species life. In lichens, these phases comprise dispersal, establishment, and growth. This thesis aimed at increasing the knowledge on epiphytic cyanolichens by studying different aspects linked to these life stages, including species colonization extinction dynamics, survival and vitality of lichen transplants, and the genetic symbiont diversity in the genus *Nephroma*.

Paper I reveals that local colonizations, stochastic, and deterministic extinctions occur in several epiphytic macrolichens. Species habitat-tracking metapopulation dynamics could partly be explained by habitat quality and size, spatial connectivity, and possibly facilitation by photobiont sharing. Simulations of species future persistence suggest stand-level extinction risk for some infrequent sexually dispersed species, especially when assuming low tree numbers and observed tree fall rates.

Forestry practices influence the natural occurrence of species, and retention of trees at logging is one measure to maintain biodiversity. However, their long-term benefit for biodiversity is still discussed. The results of a 14-year transplantation study with the epiphytic *Lobaria pulmonaria* (paper IV) support the suitability of retention trees for species survival, especially if lichen thalli occur on north-facing sides.

Lichens symbiotic nature requires the dispersal of both mycobiont and photobiont, which often occurs separately. Re-lichenization and symbiont selectivity are hence essential parts for lichen persistence. Using genetic markers, two papers (II & III) indicate that the ‘choice’ of the symbiotic partners in the genus *Nephroma* is not random, and that selectivity patterns vary between tree, local, and global scales. Particular symbiont associations are linked to geographical areas, and several lichen-forming fungi in *Nephroma* share tRNA$^{\mathrm{Leu}}$ (UAA) sequence-identical photobionts over a global scale. Relatively higher selectivity locally compared to globally indicated habitat preferences of particular symbiont combinations, but also a possible founder effect. While it still needs to be identified how and where lichen symbionts acquire their symbiotic partners, papers II and III support the idea that species form photobiont-sharing guilds, which possibly benefits their colonization success.

Keywords: cyanolichen, epiphytes, lichen guild, *Nephroma*, symbiont diversity, aspen, metapopulation dynamics, retention trees, *Lobaria pulmonaria*, boreal forest

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Inblickar i Ekologin och Genetiken hos Lavar med Cyanobakteriell Fotobiont

Sammanfattning

Djupgående kunskap om de ekologiska processer som inverkar på en arts olika levnadsstadier är viktig inom naturvården. Hos lavar omfattar dessa levnadsstadier spridning, etablering och tillväxt. Syftet med denna avhandling var att öka kunskapen om epifytiska cyanolavar genom att studera olika aspekter kopplade till dessa levnadsstadier - processer som artkolonisering och artutdöende, överlevnad och vitalitet hos transplanterade lavar, samt den genetiska mångfalden av symbionter inom släktet *Nephroma*.

Artikel I visar att lokal kolonisering samt lokala stokastiska och deterministiska utdöenden förekommer hos flera epifytiska lavar. Denna så kallade ”habitatföljande” metapopulationsdynamik kunde delvis förklaras av kvaliteten och storleken på habitat, men också av rumslig konnektivitet samt förmodad samverkan mellan arter som använder samma fotobiont. Simuleringar av de undersökta arternas framtida fortlevnad visade på en utrotningsrisk på beståndsnivå för vissa ovanliga och sexuellt spridda arter, särskilt när låga trädantal och observerad trädfallsfrekvens beaktades.

Skogsbruk påverkar den naturliga förekomsten av arter, och en åtgärd för att bevara den biologiska mångfalden är att lämna träd vid avverkning. Åtgärdens långsiktiga nytta för mångfalden diskuteras dock fortfarande. Resultaten från en 14 år lång transplantationsstudie med epifyten lunglav (*Lobaria pulmonaria*; artikel IV) ger stöd för att naturvårdsträden kan hjälpa arter att överleva, särskilt om lavarna växer på nordsidan av stammen.

Eftersom lavar är symbiotiska organismer måste både mykobiont och fotobiont spridas, vilket ofta sker separat. Nybildning av lavar och selektion av ny symbiont är därför viktiga processer för lavars fortlevnad. Genom analys med genetiska markörer, visar resultaten från två artiklar (II & III) på att "valet" av symbiotiskt partner inom släktet *Nephroma* inte sker slumpmässigt och att selektionsmönstret varierar mellan olika träd, och mellan lokal och global nivå. Vissa symbiontföreningar är knutna till specifika geografiska områden, medan flera lavbildande svampar i *Nephroma* delar sekvensidentiska tRNA$^{\mathrm{Leu}}$ (UAA)-fotobionter på en global skala. Relativt sett högre selektivitet på lokal än global nivå visar på livsmiljöpreferenser hos vissa symbiontkombinationer, men också på en möjlig grundareffekt. Även om det återstår att förklara hur och var lavar får sina symbiotiska partners, stödjer artikel II och III idén att arter bildar fotobiontdelande grupper, vilket skulle kunna gynna deras kolonisationsförmåga.

Nyckelord: cyanolav, epifyt, lavgrupp, *Nephroma*, symbiontmångfald, asp, metapopulationsdynamik, hänsynsträd, *Lobaria pulmonaria*, boreal skog

Einblicke in die Ökologie und Genetik von Flechten mit einem Cyanobakteriellen Photobionten

Zusammenfassung

Für den Naturschutz ist ein tiefergehendes Verständnis ökologischer Prozesse, die die Arterhaltung in den verschiedenen Lebensphasen einer Art beeinflussen, notwendig. In Flechten umfassen diese Lebensphasen Verbreitung, Etablierung, und Wachstum. Diese Dissertation hatte zum Ziel, das Wissen über epiphytische Cyanoflechten zu erweitern. Dazu wurden verschiedene Aspekte, die mit oben genannten Lebensphasen verknüpft sind, studiert, einschließlich lokaler Besiedlungs- und Aussterbe-Prozesse, das Überleben und die Vitalität von Flechten-Transplantaten, und die genetische Symbionten-Vielfalt in der Gattung *Nephroma*.

Artikel I zeigt, dass in mehreren epiphytischen Flechten auf lokaler Ebene Neubesiedelung, sowie stochastisch und deterministisch bedingtes lokales Aussterben vorkommen. Diese sogenannten ‚habitat-tracking' Metapopulationsdynamiken konnten teilweise mit Lebensraumqualität und -größe erklärt werden, aber auch mit räumlicher Konnektivität, und möglicherweise durch zwischenartliche Unterstützung aufgrund der Verwendung gleicher Photobionten. Simulationen über das Fortbestehen der untersuchten Arten weist auf ein Aussterben seltener, geschlechtlich verbreiteter Arten in lokalen Beständen hin, besonders wenn von wenigen Bäumen und beobachteten Baumfallraten ausgegangen wird.

Forstwirtschaft beeinflusst das natürliche Vorkommen von Arten, und das Erhalten einzelner Bäumen in Kahlschlägen (Biotopbaum/Überhälter) ist eine Maßnahme, um die Artenvielfalt zu erhalten. Der langfristige Nutzen dieser Überhälter für die Artenvielfalt ist jedoch nicht eindeutig. Die Resultate einer Transplantationsstudie über 14 Jahre mit der epiphytischen Flechte *Lobaria pulmonaria* (paper IV) bestätigen die Eignung von Überhältern für den Erhalt dieser Art, besonders für Flechtenthalli an der Nordseiten der Bäume.

Da Flechten symbiotische Organismen sind, müssen sowohl die Mycobionten als auch die Photobionten der Flechte verbreitet werden, was oft getrennt geschieht. Daher sind die Neubildung der Flechte und die Selektivität der symbiotischen Partner essentiell für das Fortbestehen von Flechten. Die Ergebnisse von zwei Artikeln (II &III), erhalten mit Hilfe von genetischen Markern, deuten darauf hin, dass die Auswahl der symbiotischen Partner in der Gattung *Nephroma* nicht zufällig ist, und das Auswahlmuster maßstabsbedingt (Baum, regional, global) variiert. Einige der symbiotischen Kombinationen sind an geographische Gebiete gekoppelt, und mehrere flechtenbildende Pilze der Gattung *Nephroma* teilen sich weltweit sequenzgleiche tRNA^{Leu} (UAA) Photobionten. Eine relativ höhere Selektivität regional im Vergleich zu global weist auf Lebensraum-Präferenzen bestimmter Symbiont Kombinationen hin, jedoch auch auf einen möglichen Gründereffekt. Es bleibt unklar, wie und wo Flechten ihre symbiotischen Partner herbekommen, jedoch unterstützen die Artikel II und III die Idee photobiont-teilender Flechtengemeinschaften, welche möglicherwiese die Neubesiedlungsfähigkeit einzelner Arten erhöhen.

Stichwörter: Cyanoflechte, Epiphyten, Flechten-Gilde, *Nephroma*, Symbiontendiversität, Zitterpappel, Metapopulationsdynamik, Biotopbaum/Überhälter, *Lobaria pulmonaria*, borealer Wald

Dedication

To the fascinating lichens,
And the wonderful places they are living in.

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Fedrowitz, K., Kuusinen, M., & Snäll, T. Metapopulation dynamics and future persistence of epiphytic cyanolichens in a European boreal forest ecosystem. (*submitted manuscript*)

II Fedrowitz, K., Kaasalainen, U. & Rikkinen, J. (2011). Genotype variability of *Nostoc* symbionts associated with three epiphytic *Nephroma* species in a boreal forest landscape. *The Bryologist* 114(1), 220-230.

III Fedrowitz, K., Kaasalainen, U. & Rikkinen, J. Geographical patterns of symbiont diversity in the lichen genus *Nephroma* (Peltigerales, Ascomycota). (*submitted manuscript*)

IV Gustafsson, L., Fedrowitz, K. & Hazell, P. 14-year old transplantation experiment demonstrates habitat suitability of trees retained at logging for the old-growth forest lichen *Lobaria pulmonaria*. (*manuscript*)

Paper II is reproduced with the permission of the publisher.

The contribution to the papers included in this thesis was as follows:

I KF planned and conducted the fieldwork and analysed the data using generalized (non)linear models. TS had the idea for the study, participated in the study planning, wrote the R code for the spatial analysis, and made the simulation analysis. KF wrote the paper as main author together with TS.

II KF initiated the cooperation and developed the study together with JR. KF planned and conducted field- & major parts of the labwork (labwork taught by UK). KF wrote the paper as main author together with JR.

III KF developed the study together with JR and UK. Samples were collected by several people. KF conducted some fieldwork & substantial parts of the labwork. UK constructed the phylogenetic tree, KF the haplotype net. KF wrote the paper as main author together with JR and UK.

IV KF conducted the statistical analysis and contributed parts of the manuscript. PH and LG designed the study and conducted most field work at the first inventory, and all field work at the re-inventory. LG wrote the paper as main author.

Preface

This thesis aims at increasing the ecological understanding of macrolichens, with a focus on epiphytic cyanolichens in boreal forest landscapes. While a lot is already known about epiphytic cyanolichens, there are still many hypotheses to be tested, and many things to be unravelled. For example, how can epiphytic lichens - being dependent on the dynamics of their habitat patches and limited by the lifespan of the tree - persist in forest landscapes? To which extent is the assumed habitat suitability determined by species occurrence in the habitat, and can intensive forestry maintain cyanolichens in the future? What are the reasons for lichen colonization and extinction events? And, how do the two partners in a lichen symbiosis select each other? Is there a geographical difference in symbiont selectivity, and could a shortage in photobiont availability influence successful establishment rates in lichens?

The list of questions to be asked could be extended, and many answers will depend on species life history characteristics, their habitat, their geographical location, and species studied. In this thesis the focus is on epiphytic lichens in which the fungal partner associates with a cyanobacterium (also referred to as cyanolichens). Biological data from field surveys (papers I & IV) and molecular techniques on collected lichen material (papers II & III) were used to explore ecological questions in different cyanolichens and genetic patterns in the genus *Nephroma* (papers II & III).

Chapter 1 starts with a short introduction to cyanolichens, their ecological requirements, symbiont specificity and selectivity, and some nature conservation aspects concerning my study species. I will define some terms used throughout this thesis, and introduce the reader to some questions connected with above concepts and definitions. The aim of this thesis and the ‘red thread’ will be given in Chapter 2. In Chapter 3, the study areas and biological material will be described, as well as the general methods used. This will be followed by a short summary of the results and a discussion of all papers (Chapter 4). Finally, I will finish with some concluding remarks and perspectives (Chapter 5). Since most of my studies were conducted in Finland (papers I & II) or Sweden (IV), the focal study systems are boreal forests in Fennoscandia.

I have learned that the fascinating world of lichens is endless, but also incessantly amazing, and I hope that this thesis can contribute to a better understanding of these wonderful organisms.

“The cause of failure for any species to flourish in a habitat can be identified by dividing its life cycle into phases, and then examining which of those phases limit population development.”
(Sillett *et al.*, 2000)

1 Introduction

1.1 The main study organisms: epiphytic cyanolichens

1.1.1 Lichens

What are lichens?

Lichens are symbiotic bi- or tripartite organisms, and hence an association of two or three symbiotic partners. Bipartite species consist of a fungus (the mycobiont), and a photosynthesizing alga or cyanobacterium (the photobiont), while tripartite species have an alga as main photobiont partner, and a cyanobacterium in special structures called cephalodia. The majority of lichenized fungi belong to the *Ascomycota*, and the photobiont is either a cyanobacterium (*Cyanophyta*) (cyanobiont henceforth), or a green alga (*Chlorophyta*). However, only few photobionts of lichens have been identified to species or strain level (e.g. Rikkinen, 2002; Nash III, 2008), and the phylogenetic number of lichen-forming fungi is probably underestimated when using morphological and chemical characters alone (Crespo & Lumbsch, 2010). This calls for more molecular research on the identity of both symbiotic partners, in order to advance in questions regarding co-evolution, specificity, selectivity, and re-lichenization (Beck, 2002; Beck *et al.*, 2002).

Due to their symbiotic nature involving heterotrophic and autotrophic components, which in addition can be parasitized by lichenicolous fungi (Lawrey & Diederich, 2003) or colonized by bacteria (Mushegian *et al.*, 2011), lichens are often referred to as small ecosystems instead of individual organisms or populations (e.g. Rikkinen, 1995; Yoshimura *et al.*, 2002). However, for simplicity I refer to a lichen as an individual organism throughout this thesis, and several lichen thalli from the same species found on a single tree are referred to as one local population (papers I & II). When conducting genetic analysis on lichen thalli (papers II & III), one thallus was defined by being spatially apart from other thalli if several thalli occurred on the same tree.

Lichen species are named after their fungal biont and therefore belong taxonomically to the kingdom of fungi. They can be found worldwide and in most habitats (Galloway, 2008) and are consequently fascinating study objects.

The lichen symbiosis – mutualistic or parasitic?

There are different views on the symbiotic nature of lichens. ‘Symbiosis’ was first described by de Bary in 1879 as ‘the living together of differently named organisms’, which includes mutualistic as well as parasitic associations (Smith & Douglas, 1987).

Many researchers and general text books see the lichen symbiosis as being mutualistic, assuming that not only the mycobiont is gaining from the association by obtaining carbohydrates and nitrogen (in the case of cyanobionts), but that also the photobiont may benefit from being less exposed to high light and temperature conditions (Nash III, 2008). Other lichenologists view lichens as a form of controlled parasitism in which the mycobiont ‘imprisons’ the photobiont (Ahmadjian, 1993). A relatively recent view is the comparison of the lichen symbiosis with agricultural systems in which gardeners (the mycobionts) cultivate crops (the photobionts) (e.g. Sanders, 2001).

Personally, I find it more intriguing how (or how long) lichen-bionts survive when not being in symbiosis, to which extent they occur free-living, and how both symbiotic partners recognize and find each other, and I therefore refer to lichens simply as symbiotic organisms.

Can lichen-bionts live without their symbiotic partner?

If and to which extent the bionts in a lichen association occur free-living is still relatively poorly understood. For the continuous occurrence of lichens within a habitat it is, however, of importance if and how long both bionts can survive without each other, given that biont availability could be a limiting factor for the successful establishment of lichens (paper I).

The mycobiont is often referred to as being an obligate biont, meaning that it cannot occur free-living. This assumption is made since mycobionts grow very slowly in isolation, and would probably not be able to compete with other fungi in a free-living state (Nash III, 2008). However, Wedin et al. (2004) could show that lichenized and non-lichenized fungal species of the genera *Conotrema* and *Stictis*, respectively, did not form separate phylogenetic species. They concluded that the same fungal species is able to live with or without a symbiotic partner, probably depending on the availability of compatible photobionts. In other studies, fungi have been found to first live as parasites on lichens before associating with photobionts themselves (e.g. Friedl, 1987), or to form a pre-thallus with a suboptimal photobiont until the correct photobiont is found (e.g. Ott, 1987; Beck *et al.*, 1998).

Some photobiont genera occur commonly free-living (e.g. *Gleocapsa*, *Nostoc*, *Scytonema*, and *Trentepohlia*) while others have not often been observed in a free-living state (*Trebouxia*) (Nash III, 2008). However, since few lichen photobionts have been identified to species or strain level, it is difficult to really know if and how often the same photobiont species or strain is present both free-living and lichenized (Beck, 2002; Rikkinen, 2002; Nash III, 2008).

Dispersal propagules in lichens

Several lichen-forming fungi belonging to the *Ascomycota* produce sexual fruiting bodies (mainly ‘apothecia’ or ‘perithecia’) which contain fungal spores. Due to its symbiotic existence, a juvenile lichen thallus only appears after the fungal spore has associated with a photobiotic partner, a process that is referred to as lichenization (or ‘re-lichenization’ to emphasize that a new generation is ‘born’). I refer to this form of reproduction as ‘sexual’, and to lichens that produce sexual fruiting bodies as ‘sexually dispersing’ or ‘dispersing by fungal spores’ (papers I, II & III).

All lichens can potentially disperse also symbiotically by thallus fragments that have broken off, though these may not necessarily regenerate a thallus (Büdel & Scheidegger, 2008). However, several lichens produce special diaspores, often as thallus outgrowth, for the combined dispersal of both symbionts. These symbiotic diaspores differ in structure, and the most important ones are soredia and isidia (Büdel & Scheidegger, 2008). Lichens that produce symbiotic diaspores are in this thesis referred to as ‘symbiotically dispersing’ and their reproductive strategy as being ‘symbiotic’, ‘asexual’, or ‘vegetative’, since no recombination of the genome occurs (papers I, II & III).

1.1.2 Cyanolichens

About 12% of all lichens are cyanolichens, i.e. lichens in which the mycobiont associates with cyanobacteria, such as *Nostoc* (Rikkinen, 2002). The heterocystous cyanobacterium *Nostoc* can – in addition to carbohydrate production - fix nitrogen from the atmosphere (N_2). In tripartite cyanolichens, such as *Lobaria pulmonaria* (1.1.8), the main carbohydrate-producing biont is a green alga, and the cyanobacterium is located externally or internally in specialized structures called cephalodia. In this thesis, all lichens with cyanobacterial symbionts, i.e. bi- and tripartite species, are referred to as cyanolichens (as in e.g. Rai & Bergman, 2002; Rikkinen, 2002; Richardson & Cameron, 2004).

Cyanolichens are very sensitive to human impacts such as air pollution, acid rain, and habitat disturbance (Richardson & Cameron, 2004), and many epiphytic cyanolichen genera are constrained to or most abundant in old-growth forests (McCune, 1993; Kuusinen, 1996a; Richardson & Cameron, 2004). Several cyanolichens, including many of my study species from the *Lobarion* community (1.1.6), are used as indicator species for air quality, continuously high humidity, long forest continuity, and forests of high value for nature conservation (e.g. Kuusinen, 1996a; Nitare, 2000; Geiser & Neitlich, 2007).

1.1.3 *Nostoc* in the lichen symbiosis

Nitrogen-fixing cyanobacteria of the genus *Nostoc* (*Bacteria, Cyanobacteria, Nostocales, Nostocaceae*) occur free-living as well as in symbiotic association with several different organisms, including bryophytes, pteridophytes, gymnosperms (cycads), angiosperms (*Gunnera*), and lichen-forming fungi (Paulsrud, 2001).

Within the lichen thallus, *Nostoc* cells are either confined to a layer between the upper cortex and medulla (as e.g. in bipartite *Nephroma* species), or *Nostoc* colonies are penetrated by fungal hyphae (e.g. in *Collema* species). In tripartite lichens, *Nostoc* cells are restricted to specialized structures, cephalodia. Internal cephalodia (e.g. in *Nephroma arcticum*) are located in the medulla, while external cephalodia (e.g. in *Peltigera aphtosa*) are situated on the thallus surface (Purvis, 2000; Rai & Bergman, 2002).

Lichen-forming *Nostoc* strains show considerable genetic variation, which has been studied using nucleotide sequences of e.g. the tRNA $^{\mathrm{Leu}}$ (UAA) intron, 16S rDNA sequences, or the *rbc*LX gene region as genetic markers (e.g. Paulsrud *et al.*, 1998, 2000; Costa *et al.*, 2002; Rikkinen *et al.*, 2002; Summerfield *et al.*, 2002; Wirtz *et al.*, 2003; O'Brien *et al.*, 2005; Elvebakk *et al.*, 2008). While some morphologically different lichen-forming *Nostoc* strains have been given botanical names, such as *Nostoc punctiforme* or *Nostoc commune*, these are in general not used among lichenologists since the taxonomy of lichen-forming cyanobacterial is still discussed (Rikkinen, 1995, 2002; Nash III, 2008).

Symbiotic and free-living *Nostoc* strains form a monophyletic group among the nostocalean cyanobacteria (Rikkinen, 2002), but genetically identical *Nostoc* strains (based on the tRNA$^{\mathrm{Leu}}$ sequence) have to my knowledge only once been reported for free-living and symbiotic *Nostoc*, in Antarctica (Wirtz *et al.*, 2003). Thus, it still needs to be researched whether lichen-forming *Nostoc* commonly also occurs as free-living organism.

1.1.4 Epiphytes

Epiphytes are vascular angiosperms (e.g. orchids or bromeliads) or cryptogams (e.g. bryophytes or lichens) that grow on other living plants. They obtain water from the air or from rainwater, which likely includes nutrients collected from the bark when running down the tree stem (Barkman, 1958). Some epiphytes capture additional nutrients with aerial roots. Lichen epiphytes are not thought to be parasitic as they produce carbohydrates by photosynthesis, and they use their host plant merely to attach themselves to. However, some epiphytic lichens may be more closely connected with their host plant as e.g. lichen-fungal hyphae of *Evernia prunastri* have been found to penetrate into the xylem of its host tree (Ascaso *et al.*, 1980). Epiphytes potentially also benefit from a rough bark structure since diapores may easier ‘strand’ in rough surfaces (but see Sillett *et al.*, 2000).

In northern Europe, the epiphytic community consists typically of bryophytes and lichens. All obligate epiphytes have in common that they occur on clearly defined habitat patches (trees), which are separated from each other by an unsuitable matrix, and they can thus be studied using the metapopulation framework (Hanski, 1999). A local population in these species is defined as all single thalli of the same species on a tree, and the metapopulation as consisting of all local populations within a certain forest landscape (paper I). Ecological processes at the local scale include species growth, interactions with other species such as competition, facilitation, or predation, and local colonizations and extinctions (1.2). At the metapopulation scale, epiphyte species disperse and colonize new habitats, but the metapopulation as a whole will also be effected by natural or anthropogenic disturbances, including changes in habitat size, quality, or distance to surrounding habitat patches (1.2).

1.1.5 Epiphytic lichens in forests

Lichens are major components of boreal forest biodiversity (Esseen *et al.*, 1997). Large epiphytic species provide shelter and food for invertebrates, as well as nesting material for birds. Nitrogen-fixing cyanolichens contribute to the nitrogen budget of forest ecosystems (Cameron & Richardson, 2006), which could be especially important in nitrogen-poor habitats such as late-seral temperate forests (Campbell *et al.*, 2010). Nitrogen is not only released after the lichen has died and disintegrates, but also leaks from the living thallus (Crittenden, 1983; Knowles *et al.*, 2006).

In Europe, epiphytic cyanolichens are mostly found on deciduous trees, but it has been suggested that they previously also occurred more commonly on (the already more acidic) coniferous trees in humid areas of the northern hemisphere and declined as a result of acid rain (Goward & Arsenault, 2000b). In northern Europe, several epiphytic cyanolichens are confined to aspen (*Populus tremula*, 1.4.2) and Goat Willow (*Salix caprea*). Both tree species are pioneer species that colonize forest gaps. Aspen and Goat Willow are of minor economic value, but they are important for a number of red-listed species, especially as large old trees in old-growth forests (Kuusinen, 1994a; b). According to the Finnish Red List, the most important threats to lichens in boreal forests today are the loss of old large trees, the disappearance of old-growth forests, and changes in tree species composition (Rassi *et al.*, 2010). From an ecological and conservation perspective it is therefore important to have a better understanding of epiphytic cyanolichens, and to contribute to their continuous persistence in our forest ecosystems.

1.1.6 The *Lobarion* community

The *Lobarion* is an oceanic-montane community consisting predominantly of epiphytic lichen species and being associated with climax woodland (James *et al.*, 1977; Rose, 1988; Gauslaa, 1995). It is named after the lichen genus *Lobaria* (Schreber) Hoffm., but includes also other foliose species of the genera *Sticta* (Schreber) Ach., *Pseudocyphellaria* Vainio, *Parmeliella* Müll. Arg., *Pannaria* Delise, *Nephroma* Ach., *Peltigera* Willd. and *Parmelia* Ach., as well as several crustose lichens, and a number of bryophytes (Rose, 1988). Most of my study species are included as major species in the European *Lobarion* (Rose, 1988), and some of them are described in more detail below (1.1.7 *Nephroma*, and 1.1.8 *Lobaria pulmonaria*).

The community is usually described as epiphytic but can locally also be found on rocky shores, cliffs, and screes (Rose, 1988). Favourite habitats of species in this community include broad-leaved tree species with less acidic bark but also *Abies alba* growing on richer soils (Gauslaa, 1985; Rose, 1988). Old forests with long ecological continuity and trees with relatively high bark pH seem especially important for the occurrence of species from the *Lobarion* (Gauslaa, 1985).

It is assumed that the *Lobarion* community has in the past occurred in most European woodlands, and that its species, especially the cyanolichens, have started to decline since the beginning/mid-twentieth century (Rose, 1988). This decline is attributed to changes in bark pH due to acid rain, replacing the *Lobarion* with lichen communities that tolerate lower pH (e.g. *Pseudevernion*), but also sulphur dioxide pollution alone and changes in forest management are mentioned as possible causes (Rose, 1988; Gauslaa, 1995).

1.1.7 The lichen genus *Nephroma*

The lichen genus *Nephroma* (*Peltigerales, Ascomycota*) Ach. (papers I, II & III) with about 35 species occurs in oceanic to boreal-montane areas. It is almost cosmopolitan in its distribution but most diverse in temperate areas (James & White, 1987; White & James, 1988; Smith *et al.*, 2009). Eight species of this genus occur in Fennoscandia (Vitikainen, 2007), but some of them are red-listed or extinct (see below). The species belong to the foliose macrolichens and are called 'kidney-lichens' in English, which refers to the shape of their apothecia (Dobson, 2005). However, apothecia are not common in all *Nephroma* species (Table 1, page 43ff.), and some, e.g. *N. parile*, *N. isidiosum*, or *N. occultum*, disperse mainly symbiotically.

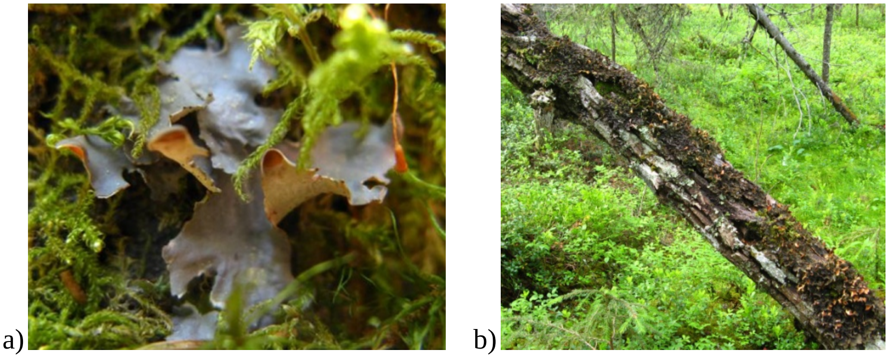


Figure 1. Apothecia in *Nephroma bellum (a) and Nephroma* species on *Salix caprea* (b) in Teeri-Lososuo, Finland

Most *Nephroma* species are bipartite cyanolichens associated with cyanobacteria of the genus *Nostoc* as photobionts. Some species in this genus (e.g. *N. arcticum*) are tripartite, and in these the mycobiont associates with a photosynthetic green alga (*Coccomyxa*) and houses a nitrogen-fixing cyanobacterium (*Nostoc*) in cephalodia.

Nephroma species vary in their habitat requirements, but many are found as epiphytes on tree bases, especially on deciduous trees such as *Salix caprea* or *Populus tremula* (Fig. 1) (papers I & II). Other habitats of this genus include mossy boulders or soil in heaths, grasslands, and forests (e.g. *N. expallidum*), and many species occur in shady places (Vitikainen, 2007; Smith *et al.*, 2009).

The genus is sensitive to SO_2 pollution and species are often characteristic for old woodlands (Smith *et al.*, 2009). Many *Nephroma* species are used as indicator species for areas of high conservation values, including old-growth forest habitats (Kuusinen, 1996a; Nitare, 2000). Several *Nephroma* species are red-listed or extinct in (parts of) Europe (Wirth, 1995; Vitikainen, 2007). In the current red-list of Finland, *N. bellum* and *N. resupinatum* are classified as near-threatened, and *N. helveticum* and *N. laevigatum* as critically endangered (Rassi *et al.*, 2010). *N. laevigatum* is also classified as near threatened in Sweden (Gärdenfors, 2010), and presence of *N. helveticum* has in Fennoscandia only been reported for Finland (Vitikainen, 2007). At the time of my field collections in Finland for paper II, *N. bellum* and *N. resupinatum* were not red-listed (Rassi *et al.*, 2010), and only minute thallus fragments were collected for genetic analysis.

1.1.8 *Lobaria pulmonaria*

Lobaria pulmonaria (L.) Hoffm. (papers I-IV) is a relatively large foliose macrolichen that can be found on the bark of broad-leaved trees, in the boreal forest especially on *Populus tremula*, *Salix caprea* and *Sorbus* species, but also on low scrubs and mossy rocks. Sometimes it is found on *Picea* species in old-growth forests, and rarely on old walls (Smith *et al.*, 2009; Jørgensen & Tønsberg, 2007). In my study areas in Finland and Sweden (papers I-IV) *L. pulmonaria* has an epiphytic growth-form (Fig. 2).

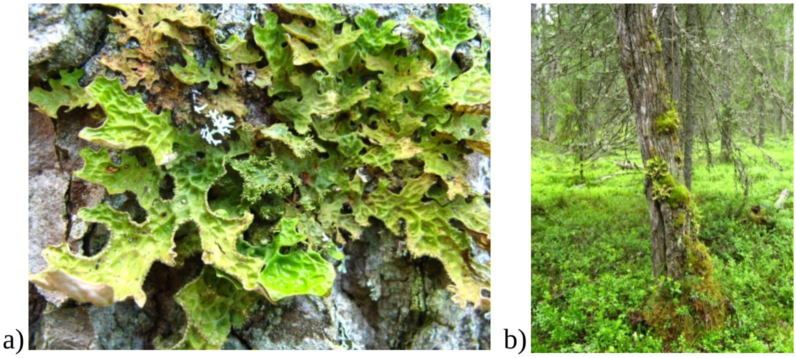


Figure 2. Lobaria pulmonaria (a) on *Salix caprea* (b) in Teeri-Lososuo, Finland

The species distribution area comprises oceanic and montane Europe, Macaronesia, North America, Asia, and Africa (Smith *et al.*, 2009), but due to forest management and air pollution it has declined or disappeared in many areas during the last decades (e.g. Scheidegger *et al.*, 1998; Yoshimura, 1998; Jüriado & Liira, 2010). *L. pulmonaria* is currently classified as near-threatened in Finland and Sweden (Rassi *et al.*, 2010; Gärdenfors, 2010).

L. pulmonaria is called ‘lungwort’ in English since it resembles lung tissue, and has been used by herbalists to treat lung diseases (Brodo *et al.*, 2001). The genus name *Lobaria* refers to the ‘lobed’ thallus shape (Dobson, 2005).

The species is tripartite, having a green alga (*Dictyochloropsis*) as main photobiont, and *Nostoc* in internal cephalodia. It produces isidia and soredia, but apothecia are not common (Smith *et al.*, 2009).

L. pulmonaria is a widely used model species in ecological and physiological (transplantation) experiments (e.g. Scheidegger *et al.*, 1995; Hazell & Gustafsson, 1999; Gauslaa, 2006; Johansson *et al.*, 2011; Gaio-Oliveira *et al.*, 2004), in experiments of lichen endozoochory (Boch *et al.*, 2011), in molecular studies (e.g. Werth *et al.*, 2007; Widmer *et al.*, 2010) and in metapopulation studies, including species dispersal ability, colonization, and extinction (e.g. Gu *et al.*, 2001; Walser *et al.*, 2001; Öckinger *et al.*, 2005; Snäll *et al.*, 2005b; Wagner *et al.*, 2006; Werth *et al.*, 2006; Öckinger & Nilsson, 2010). The species is used as an indicator for the ecological continuity of old-growth forest conditions (Kuusinen, 1996a; Nitare, 2000) and considered a flagship or umbrella species for nature conservation (Scheidegger & Werth, 2009).

1.2 Lichen-ecological aspects

The occurrence of epiphytic cyanolichens is influenced by many factors, including habitat quality (1.2.1), habitat availability and quantity (1.2.2), species traits (e.g. morphology, photobiont type, reproductive strategy, and dispersal ability), competition with and facilitation by other species, as well as predation (1.2.3). In general, species occurrence patterns need to be differentiated from processes that lead to the observed occurrences, since patterns are snapshots in time while processes are marked by changes happening over time. For example, habitats may become fragmented or their quality may change over time, and changes in species composition including species extinctions may be delayed (”extinction debt”; Tilman *et al.*, 1994), as for example suggested for lichens and fungi in northern Swedish boreal forests (Berglund & Jonsson, 2005) and for epiphytic lichens in Scotland (Ellis & Coppins, 2007). Local colonization and extinction events reflect species occurrence changes over time, and are important for inferences on species long-term persistence (paper I).

1.2.1 Habitat quality

Habitat suitability or quality is usually judged by observations of species occurrences. However, realized and fundamental niche can differ, i.e. species may have a larger fundamental niche but could be limited due to predation by or competition with other species (Hutchinson, 1957; cited in Begon *et al.*, 2006), as well as by their ability to successfully colonize new habitats

(”regeneration niche”, Grubb, 1977). For example, when transplanted to different heights on a tree, some epiphytic lichens have been shown to grow better at canopy heights where they are naturally absent (Antoine & McCune, 2004).

Habitat quality is determined by several ecological factors. The complexity of these factors interacting with each other makes it sometimes difficult to identify the main cause of species habitat preference. Furthermore, a single tree contains different microhabitats due to e.g. tree inclination, bark cracks, and twisting of the tree (e.g. *Salix caprea*) (Kuusinen, 1994a). However, several attributes have been identified to influence habitat quality for epiphytic lichens, including tree habitat features (e.g. tree diameter, tree age, bark structure, bark chemistry and bark pH), microclimatic attributes (e.g. humidity and light conditions), geological and geographic aspects (e.g. soil moisture and soil chemistry), and regional climatic conditions (oceanic vs. continental climate) (e.g. Barkman, 1958; Gauslaa, 1985; Gustafsson & Eriksson, 1995; Kuusinen, 1996b; Ellis & Coppins, 2006). Habitat may be suitable but of less quality if other species, such as bryophytes, are strong competitors (Scheidegger *et al.*, 1995). Also pollutants may change habitat quality and result in a shift from sensitive to tolerant communities (Wolseley *et al.*, 2010). On the other hand, bryophytes may also increase habitat quality for some species due to their water-holding capacity (Sillett & McCune, 1998), and could potentially provide a better adhesive substrate for diaspore attachment than pure bark (but see Sillett *et al.*, 2000).

Cyanolichens do not thrive on too acidic bark (Gauslaa, 1985), but bark pH differs not only among tree species but also on the same tree (Gauslaa, 1995 and personal observation). Bark pH may be locally altered by other trees close by, as shown by Goward and Arsenault (2000a) where the bark of spruce trees was less acidic due to nutrient-rich leakages of nearby aspen trees (‘dripzone effect’). Furthermore, lichen epiphytes and bark wounds have been suggested to modify bark chemistry and pH (Gauslaa & Holien, 1998; Fritz & Heilmann-Clausen, 2010). Also, differences in soil mineral status can influence bark chemistry (Gauslaa, 1985; Gustafsson & Eriksson, 1995). Not only bark chemistry but also its structure (thickness, roughness and porosity) can possibly explain habitat quality for epiphytic lichens (Barkman, 1958; Gauslaa, 1995; Gustafsson & Eriksson, 1995). For example, bark microtopography influenced establishment and survival of soredia in *Hypogymnia physodes* (Armstrong, 1990).

Many cyanolichen species depend on large, old, deciduous, broad-leaved trees, which could reflect bark structure and chemistry (Gustafsson *et al.*, 1992; Mikhailova *et al.*, 2005). Old-growth forests have been suggested as optimal habitat for many cyanolichens (e.g. Kuusinen, 1996a), possibly because they provide a good balance between humidity and light availability (Gauslaa *et al.*, 2006). Humidity is of importance since lichens are poikilohydric organisms, i.e. unable to store water, and cyanolichens depend on liquid water from outside (Nash III, 2008). Light is needed for species photosynthetic activity, but too high levels of light and temperature can increase photoinhibition or be detrimental (e.g. Gauslaa & Solhaug, 1996, 1999).

1.2.2 Habitat availability & quantity

Habitat availability describes whether suitable habitat exists, while habitat quantity refers to the amount or size of suitable habitat. Habitat loss is a great threat to biodiversity, not only because of diminishing habitat size but also due to fragmentation and increasing edge effects (Hanski, 2011). A decrease in large old trees and old-growth forests (i.e. forests with long ecological continuity and heterogeneous structure) in Fennoscandia has lead to reduced availability and quantity of habitat for several old-growth dependent species, including epiphytic cyanolichens (Esseen *et al.*, 1997; Hanski, 2000; Rassi *et al.*, 2010). Since new habitats need to be reached by dispersed diaspores, habitat availability alone is insufficient for species persistence, but the spatial distance to the next occupied patch is also important for the colonization of new habitats. It is doubtful if the size and connectedness of the current nature conservation approaches in Fennoscandia (1.4) suffice to support epiphytic cyanolichens and other (red-listed) species in the long-term (Larsson, 2011) (paper I).

1.2.3 Colonizations, extinctions, and habitat patch dynamics

Successful dispersal and establishment result in colonization, i.e. a species being present in a previously unoccupied patch, while extinction can be defined as the absence of a species in a patch that earlier was occupied. The observation of colonizations and extinctions therefore needs two survey occasions (paper I). In comparison, occurrences accounting for species presence or absence can be estimated at a single time snapshot. Local population turnover, i.e. appearance and disappearance of single thalli within a larger population on the same tree, should not be confused with metapopulation turnover, i.e. colonizations and extinctions of entire populations on single trees within the larger metapopulation (Fig. 3; paper I).

Data on metapopulation dynamics, i.e. on colonization and extinction events of local populations, make it possible to project species future persistence (Akçakaya & Sjögren-Gulve, 2000). Furthermore, an understanding of how environmental conditions affect colonizations and extinctions can contribute to a deeper understanding of these processes, and ultimately to more reliable predictions of species dynamics.

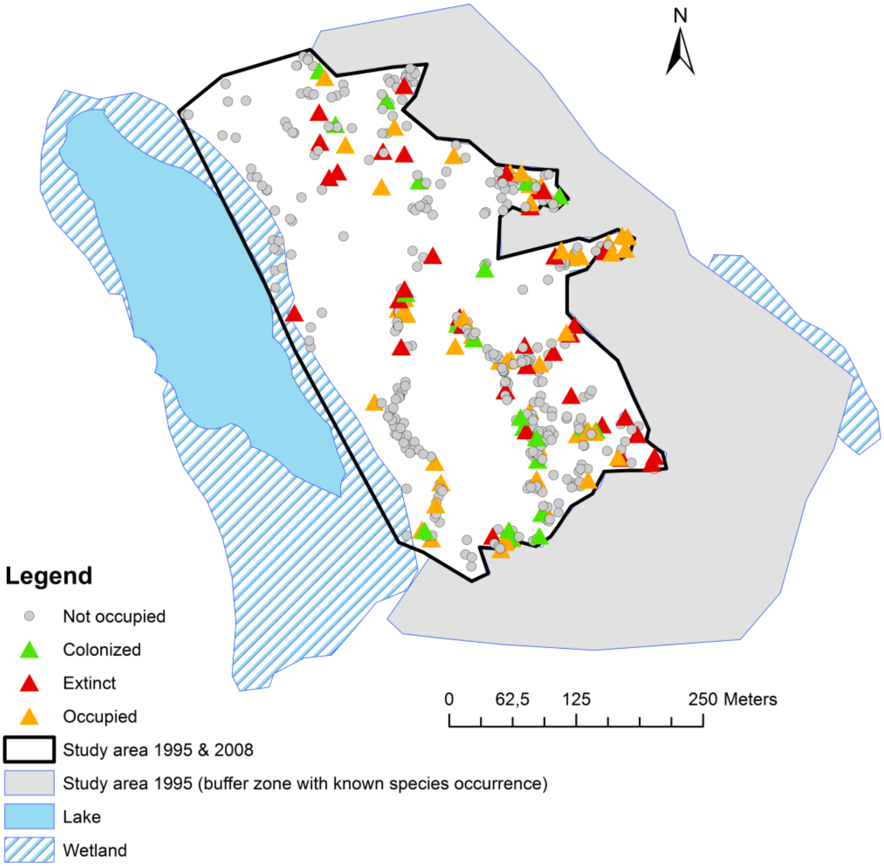


Figure 3. Metapopulation dynamics of *N. parile* on *P. tremula* in Kotinen, Finland (paper I). The entire area lies within a larger forested area. In 2008, species were surveyed in part of an area that was surveyed in 1995 (black line – 2008; black line and grey area – 1995).

Colonizations of unoccupied habitat patches has been explained by habitat patch distance to adjacent occupied patches, as well as by local habitat conditions, such as habitat patch preference, quality, and size (e.g. Hanski, 1998; Fleishman *et al.*, 2002; Snäll *et al.*, 2005a). Larger metapopulations can be assumed to have higher colonization rates than smaller metapopulations since the former can produce a larger amount of dispersal propagules. Cryptogamic epiphytes are thought to have a steep dispersal kernel, i.e. high amounts of diaspores fall close to the dispersal source but the amounts decline quickly with distance (Walser *et al.*, 2001; Dettki & Esseen, 2003; Snäll *et al.*, 2004a, 2005a). Dispersal of propagules may occur by wind, water, or by attachment to insects or birds, but also through the gut passage of lichen feeders such as snails or mites (Fröberg *et al.*, 2001; Meier *et al.*, 2002).

Lichen establishment may be influenced by competition, facilitation, photobiont availability, and predation, in addition to the above mentioned variables for habitat quality. Competition includes here the replacement of species during succession, overgrowth by other species, and a structuring of communities due to space and light availability (Lawrey, 1991; Armstrong & Welch, 2007). Herbivore predation of diaspores or young thalli fragments has been suggested to stop or reduce establishment success (Scheidegger *et al.*, 1995; Asplund & Gauslaa, 2008). Facilitation could include the provision of adhesive or moist substrates by mosses or by certain bark microtopography, capturing dispersal fragments and possibly providing a suitable habitat for juvenile thallus growth (but see Sillett *et al.*, 2000). In addition, spore-dispersed cyanolichens need to relichenize and could hence be facilitated by species providing them with suitable photobiont partners – which could at the same time be regarded as competition for photobiont partners.

According to the classic metapopulation theory, extinctions of local populations are thought to occur as a result of demographic or environmental stochasticity (Hanski, 1998). The extinction risk decreases with increasing patch area, which is often positively correlated with population size (Hanski, 1999). However, in epiphytic species, also the dynamics of their habitat patches, i.e. growth and fall of trees, need to be accounted for to predict their metapopulation dynamics, since species will disappear after tree fall (”habitat tracking metapopulations”; Thomas, 1994; Thomas & Hanski, 1997). Figure 4 illustrates a possible division into stochastic and deterministic extinctions as used for paper I. Stochastic extinctions can be further divided into extinctions from living and dead trees, since tree death is likely to affect species occurrences (paper I). If local extinctions are found to occur almost exclusively due to tree fall, stochastic extinctions can be ignored when predicting metapopulation dynamics (‘patch-tracking metapopulations’; Snäll *et al.*, 2003). It is necessary to account for the relative significance of stochastic and deterministic extinctions in order to correctly predict the metapopulations dynamics and persistence of a species.

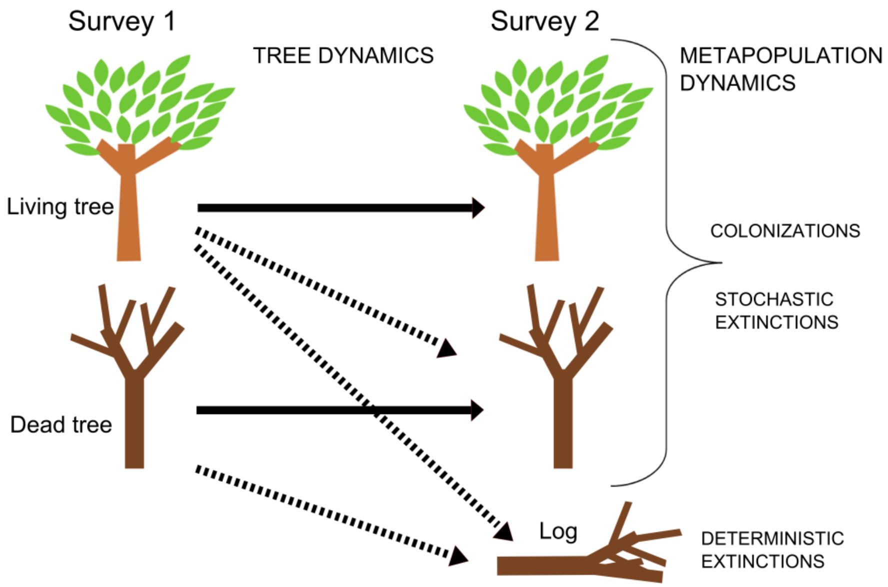


Figure 4. Metapopulation dynamics of the epiphyte linked to the dynamics of its host tree species. Arrows indicate time between the two survey years. Black lines denote that tree condition had stayed the same (living or dead) while dotted lines symbolize changes from living to dead tree, or from tree to log.

1.3 Selectivity and specificity in lichens

1.3.1 Definition

Selectivity and specificity in lichens refer to species symbiont association patterns, based on taxonomic or genetic diversity of myco- and photobionts. The terms are differently defined throughout the literature, which can lead to some confusion. I define specificity according to Smith and Douglas (1987) as the "degree of taxonomic difference between partners with which an organism associates", and selectivity as the preferential association between symbiotic partners (Galun & Bubrick, 1984). Specificity and selectivity are in this thesis both referred to from the fungal perspective, unless otherwise stated. This is done for simplicity and since mycobionts are thought to be more selective in choosing their symbiotic partner than photobionts (specialist vs. generalist pattern; Otálora *et al.*, 2010), but it does not reflect any belief in the amount of activity of either symbiont in selecting a partner. High selectivity in both mycobionts and photobionts has to my knowledge only been reported once (Otálora *et al.*, 2010).

The difference between specificity and selectivity is illustrated in Figure 5, showing that high specificity restricts the mycobiont to a certain group of genetically compatible photobionts (Fig. 5a), while selectivity is the further choice from this group of potential partners (Fig. 5b) (papers II & III).

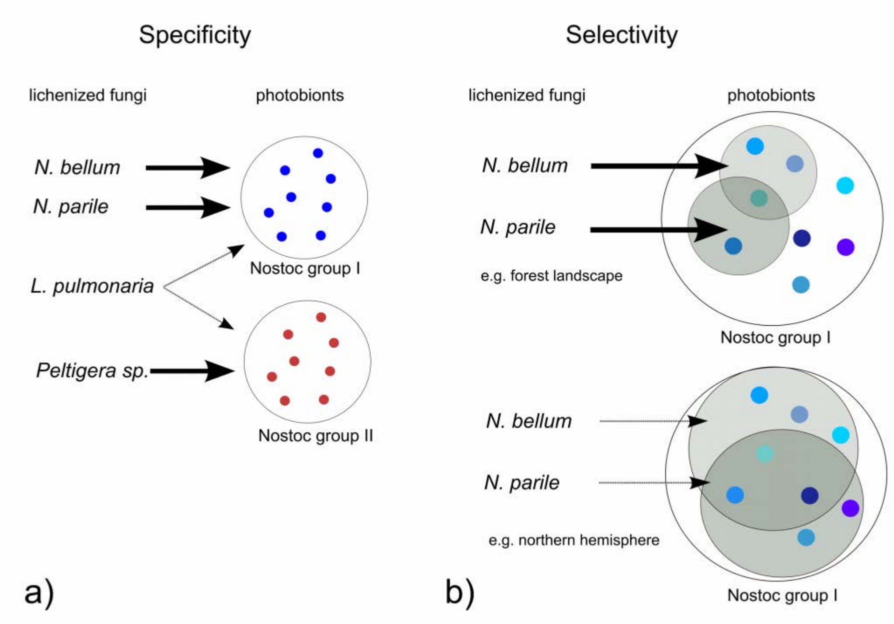


Figure 5. Schematic picture for the concepts of specificity (a) and selectivity (b) in lichens. Thick and thin arrows indicate high and low specificity or selectivity, respectively. Many *Nephroma* or *Peltigera* species are highly specific since they only associate with a particular group of *Nostoc* photobionts, indicated by blue or red dots (a). Selectivity, i.e. the choice of photobiont genotypes indicated by different colors of blue (b) may be scale-dependent and higher locally (upper part) compared to globally (lower part) (papers II & III).

1.3.2 Lichen guilds

Several lichenized fungi are known to associate with photobionts from the same genus, e.g. most lichenized fungi in cyanolichens house the genus *Nostoc* (Nash III, 2008). The further observation that certain lichen species, which associate with similar or sequence-identical *Nostoc* genotypes, commonly form assemblages in similar habitats, has lead to the hypothesis that species may facilitate each other by photobiont sharing (Rikkinen *et al.*, 2002; Rikkinen, 2003). Within one such photobiont sharing guild, symbiotically dispersing core species are thought to be the predominant dispersers of the photobiont, while sexually dispersed fringe species gain from the photobiont distribution of the core species (Fig. 6). One such ecological assemblage in which lichen-forming fungi associate with photobionts from a common pool of *Nostoc* cyanobionts is termed the *Nephroma* guild and includes many epiphytic cyanolichens (papers II & III) (Rikkinen *et al.*, 2002; Rikkinen, 2003). Lichen guilds are thought to exist not only among cyanolichens but also in green algae lichens (Peksa & Škaloud, 2011; Piercey-Normore & Deduke, 2011), and several guilds are likely to co-occur in the same ecological environment (Rikkinen, 2003). In tripartite species, which associate with two different photobionts, the mycobiont could even belong to two different guilds (Rikkinen, 2003). There are several mechanisms for how spore-dispersed species can obtain (and hence 'share') photobionts within a lichen-guild (e.g. Rikkinen, 2003). A large amount of symbiotically dispersed propagules will land on suboptimal habitat and disintegrate ('symbiotic breakdown'), and thus free the associated photobionts. Similarly, photobionts will be released after the death of a lichen species. Some species may even be able to acquire photobionts from pre-thalli or juvenile thalli of other species. Many mechanisms involved in photobiont sharing are still poorly understood, but molecular methods have in recent years advanced the knowledge on lichen-guild communities, e.g. by identifying species that associate with sequence-identical photobiont genotypes (e.g. Rikkinen *et al.*, 2002) (papers II & III).

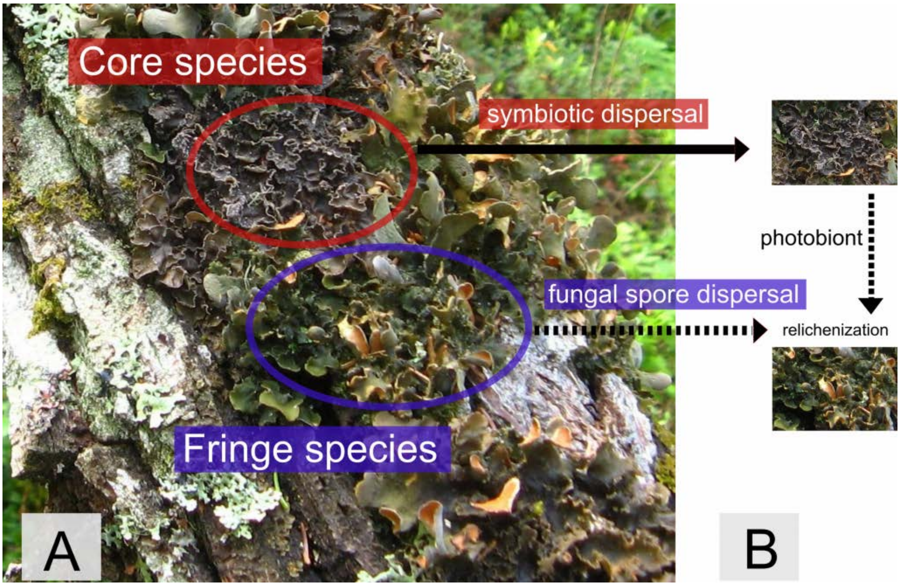
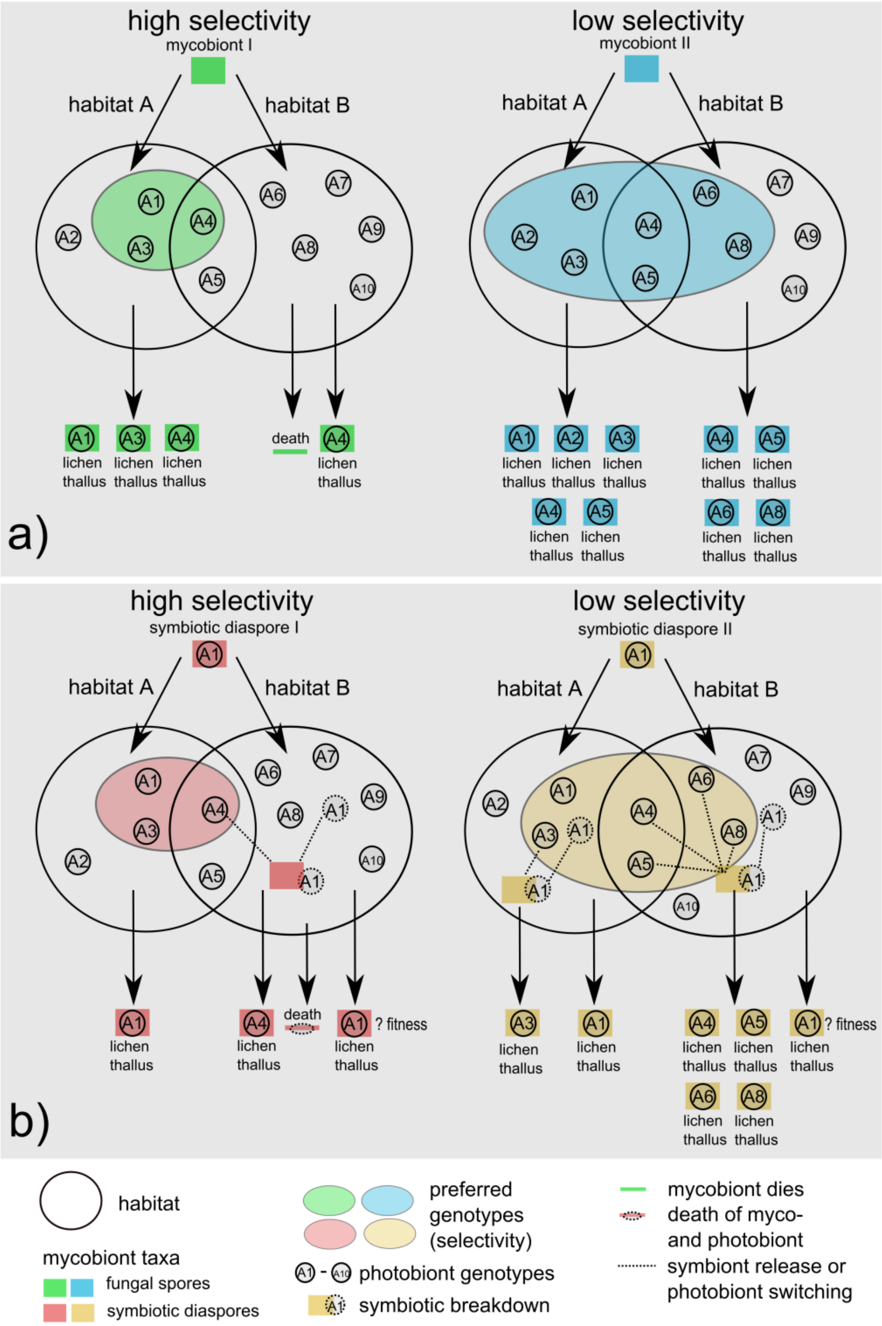


Figure 6. Simplified figure of the lichen guild hypothesis (Rikkinen *et al.*, 2002; Rikkinen, 2003). Core and fringe species (coloured circles) within the same lichen guild occur together in a habitat (A). The mycobiont of the sexually dispersed fringe species (e.g. *N. bellum* or *N. resupinatum*) acquires its photobiont from the symbiotically dispersing core species (e.g. *N. parile*) after dispersal (B).

1.3.3 Symbiont diversity in lichens – why (not) being selective?

Identifying specificity and selectivity can potentially help to unravel lichen ecological processes by linking symbiont patterns to species geographical location, their life history traits, the environment they are living in, and last, but not least, their connection to other community members (1.3.2) (e.g. Beck *et al.*, 2002; Rikkinen *et al.*, 2002; Yahr *et al.*, 2006; Piercey-Normore & Deduke, 2011). For example, selection pressure in the extreme Antarctic environment has possibly caused low cyanobiont selectivity (Wirtz *et al.*, 2003). Reproductive strategy could influence selectivity, since relichenization may be more successful for sexually dispersing species if they exhibit low selectivity (e.g. Beck *et al.*, 2002). Symbiotically dispersing species, on the other hand, are likely to exhibit high selectivity due to the joint dispersal of both bionts. However, photobiont switching may also occur in symbiotically dispersed species (e.g. Wornik & Grube, 2010), maybe as a result of varying fitness of certain symbiont associations in different environments (e.g. Yahr *et al.*, 2006). Figure 7 shows a conceptual model on how selectivity patterns can result from a combination of symbiont availability, environmental habitat conditions, and fitness of the total lichen (holobiont) (Rikkinen, 1995, 2003; e.g. Beck *et al.*, 2002; Yahr *et al.*, 2006; Otálora *et al.*, 2010).

Figure 7. (next page) High and low selectivity in sexually (a) and symbiotically (b) dispersed species. The pool of genetically compatible photobionts differs between habitat A and B as a result of environmental factors and dispersal success. High selectivity decreases the chance of finding a suitable photobiont but certain symbiont combinations may exhibit higher fitness in certain habitats. In symbiotically dispersing species, high selectivity could lead to good colonization ability of new habitats since relichenization is not necessary, or to reduced holobiont fitness and symbiotic breakdown. Low selectivity implies that many of the available photobionts from the pool can be selected. Selectivity patterns therefore differ between mycobiont taxa, the environment they encounter, species reproductive strategy, and photobiont availability.


high selectivity
mycobiont I
habitat A
habitat B
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
death
lichen thallus
low selectivity
mycobiont II
a)
high selectivity
symbiotic diaspore I
? fitness
low selectivity
symbiotic diaspore II
b)
habitat
mycobiont taxa
fungal spores
symbiotic diaspores
preferred genotypes (selectivity)
A1 - A10 photobiont genotypes
A1 symbiotic breakdown
mycobiont dies
death of myco- and photobiont
symbiont release or photobiont switching

1.4 Forest nature conservation in Fennoscandia

1.4.1 Forestry in Finland and Sweden

Intensive forestry has modified the vast forest landscapes of Finland and Sweden during the last century. Finland was the sixth and Sweden the second largest exporter of pulp, paper, and sawn timber in 2009 (Swedish Forest Industries Federation, 2011). The shift from selection felling towards clearcut practice in the mid-20thcentury changed the tree species composition from mixed to more single-species stands, and the heterogeneous old-growth forests to even-aged, young forest stands, with reduced natural disturbances and less amount of dead-wood (Östlund *et al.*, 1997; Esseen *et al.*, 1997; Löfman & Kouki, 2001; Gustafsson & Perhans, 2010). The changes in forestry practices and intensity during the last century have lead to an increase in threatened species, including lichens (e.g. Esseen *et al.*, 1997; Rassi *et al.*, 2010). After the United Nations Conference on Environment and Development (UNCED) in Rio de Janerio, Brazil, in 1992 there has been growing interest in sustainable forest management and the development of different nature conservation approaches (1.4.3), but it is controversial if these measures are sufficient to sustain biodiversity (e.g. Hanski, 2000; European Commission, 2003; Gustafsson & Perhans, 2010; Larsson, 2011).

1.4.2 European aspen *Populus tremula*

European aspen *Populus tremula* L. is a common deciduous broad-leaved tree with a wide distribution in temperate and boreal forests of Eurasia (MacKenzie, 2010; Myking *et al.*, 2011). It is a typical pioneer species that grows rapidly after disturbances such as forest fires, storms, or smaller gap dynamics in the forest, but can also persist for a long time in mature forest stands if no large-scale disturbances occur (Kouki *et al.*, 2004; Latva-Karjanmaa *et al.*, 2007; Myking *et al.*, 2011). The species can live for approximately 100 – 200 years, and reproduces vegetatively by clonal distribution or sexually with seeds (Latva-Karjanmaa *et al.*, 2007; Myking *et al.*, 2011).

The contribution of *Populus tremula* to biodiversity in boreal forests is high since it is the habitat for hundreds of invertebrates, fungi, and epiphytes, with over 100 of these species being specialized to aspen as host (Kouki *et al.*, 2004). Furthermore, a disproportional number of red-listed species occurs on aspen (Tikkanen *et al.*, 2006). Also several epiphytic lichen species live on aspen due to its relatively high bark pH (Kuusinen, 1994b) (paper I, II, & IV).

Aspen has previously been intensively eliminated from managed forests since it has low economic value, can hinder the regeneration of other species by the formation of dense root suckers, and because it is the intermediate host of the pine rust fungus *Melampsora pinitorqua* (Latva-Karjanmaa *et al.*, 2007). While the value of aspen for biodiversity is now being recognized and supported by e.g. retention trees (1.4.3) (paper IV), it is still unclear whether the present regeneration of young aspen cohorts is sufficient for a continuous aspen abundance, especially of old aspen trees, in the future (Kouki *et al.*, 2004; Edenius *et al.*, 2011).

1.4.3 Nature conservation approaches

Approaches for the conservation of forest biodiversity include the protection of forested areas by setting-aside land as e.g. national parks or nature reserves, and the integration of conservation practices in production forests (Parviainen & Frank, 2003; Lindenmayer *et al.*, 2006).

Two measures currently applied in Fennoscandia that aim to support biodiversity in production forests are the retention of trees on clearcut sites (paper IV), and the (voluntary) protection of woodland key habitats (WKH) from intensive forestry (Gustafsson *et al.*, 2010; Timonen *et al.*, 2010). The tree retention approach comprises the protection of single trees, tree groups, buffer zones adjacent to lakes, watercourses, and mires, and the creation and saving of dead wood at logging. This measure has the purpose to life-boat and support species, increase structural variation in the otherwise plane clearfell site, improve connectivity to other forest patches, and to sustain ecosystem functions (Gustafsson *et al.*, 2010). WKHs are small patches on productive forest land that are of key importance for maintaining biodiversity at the landscape-level, mainly because of their habitat structure and species composition (Timonen *et al.*, 2010). They are legally protected in Finland, and on a voluntary basis or by forest certification in Sweden (Timonen *et al.*, 2010).

2 Thesis aim and red thread

2.1 Thesis aim

The aim of this thesis is twofold. First, I want to increase the knowledge on epiphytic lichen persistence in dynamic boreal forest landscapes. Many studies have reported patterns (i.e. species occurrences), but processes (i.e. changes over time) are less often studied. Examples of these processes are local colonization and extinction events of epiphyte populations from single trees that lead to dynamics in the species metapopulation (1.2). Habitat quality and size are in general important for epiphytic species to survive (1.2), but the relative importance of stochastic versus deterministic extinctions of local populations and the colonization rate of epiphytic cyanolichens is largely unknown. We were therefore interested to find out to which extent local colonizations and extinctions occur in epiphytic cyanolichens, and which environmental variables explain these events (paper I). The observed metapopulation turnover rates were further used to project species future persistence for different numbers of host trees and tree fall rates (paper I). Epiphytic lichen persistence is closely connected to forestry, and it is hence important to know how forestry practices can simultaneously maintain wood production and biodiversity. To retain trees is a measure to life-boat species in order to mitigate negative effects of forestry on species persistence (1.4.3). However, can retention trees maintain old-growth forests species? We compared the survival and vitality of lichen transplants on retention trees in clearcut sites with forest trees to get further insights into species ecology and to improve the knowledge base for conservation measures for forest practitioners (paper IV).

The second aim of this thesis is to increase the knowledge on genetic symbiont diversity in the genus *Nephroma* (papers II & III), a predominantly epiphytic genus of cyanolichens. Symbiont diversity mirrors the ‘choice’ in mycobiont photobiont association and probably depends on the availability of symbiont partners as well as ecological optimal conditions of the symbiosis (1.3). In addition, reproductive strategy is thought to play a role, especially for lichen-forming fungi that disperse by fungal spores and need to re-establish the symbiotic association after dispersal (1.1.1). Phylogenetic information on the lichen-forming fungus in *Nephroma* has been obtained in several studies with molecular methods (Lohtander *et al.*, 2002, 2003; Piercey-Normore *et al.*, 2006; Sérusiaux *et al.*, 2011), and also data on the diversity of *Nostoc* photobionts are available for some *Nephroma* species (e.g. Paulsrud *et al.*, 1998, 2000; Rikkinen *et al.*, 2002; Myllys *et al.*, 2007). We therefore know that lichen-forming fungi in the genus *Nephroma* are specific in their photobiont choice, i.e. they associate with a specific group of *Nostoc* photobionts. However, the degree of selectivity in lichen-forming fungi within this genus is not fully understood. Further, more knowledge is needed to reveal if there is a difference globally and locally, and if symbiont diversity is influenced by species reproductive strategy. Also, an extensive study on symbiont diversity patterns in *Nephroma*, obtaining both bionts from the same lichen thalli and using samples from a wide geographical range, has not been conducted. Local and global symbiont patterns in the genus *Nephroma* were hence examined in papers II and III.

2.2 The red thread

While the two aims in this thesis may at first sight seem disconnected from each other, they are both linked to the life cycle of epiphytic cyanolichens, with each paper contributing to a small part of the bigger picture (Fig. 8). This simplified life cycle consists of three different stages: dispersal, establishment, and thallus growth (including reproduction) (Sillett *et al.*, 2000).

Several factors influence the success or failure in every stage, and these factors are likely to differ between species (Fig. 8). For example, reproductive strategy determines not only the size of the dispersal propagules, which influences dispersal distance, but also the need for photobiont acquisition (papers I, II, and III). Establishment in macrolichens happens often in two stages, the formation of a pre-thallus, and the morphogenesis into a juvenile thallus (Honegger, 2008). Exceptions are certain symbiotic diaspores, for example thallus fragments, which can directly grow into a juvenile thallus. The pre-thallus stage is “an inconspicuous, nonstratified crust” which can also be formed by incompatible bionts (Beck *et al.*, 1998; Honegger, 2008). Such a formation between unsuitable symbiont partners may help the mycobiont to survive until it can get hold of a compatible photobiont (e.g. Beck *et al.*, 1998).

However, morphogenesis into a juvenile thallus will only take place in association with a compatible photobiont (Beck *et al.*, 1998; Honegger, 2008). Environmental conditions, photobiont availability, and the effects of competition or predation will influence establishment success (papers I, II, and III) (Fig. 8). The final stage of the life cycle includes thallus growth and reproduction. Vitality and survival of mature thalli (a prerequisite for their growth and reproduction) will depend on habitat quality, competition with and predation by other species, but also on tree death and fall (papers I and IV) (Fig. 8).

In order to efficiently and successfully conserve species, information regarding the species ecology in all life stages is useful. Likewise, species metapopulation dynamics (paper I) will be influenced by success or failure in each stage.

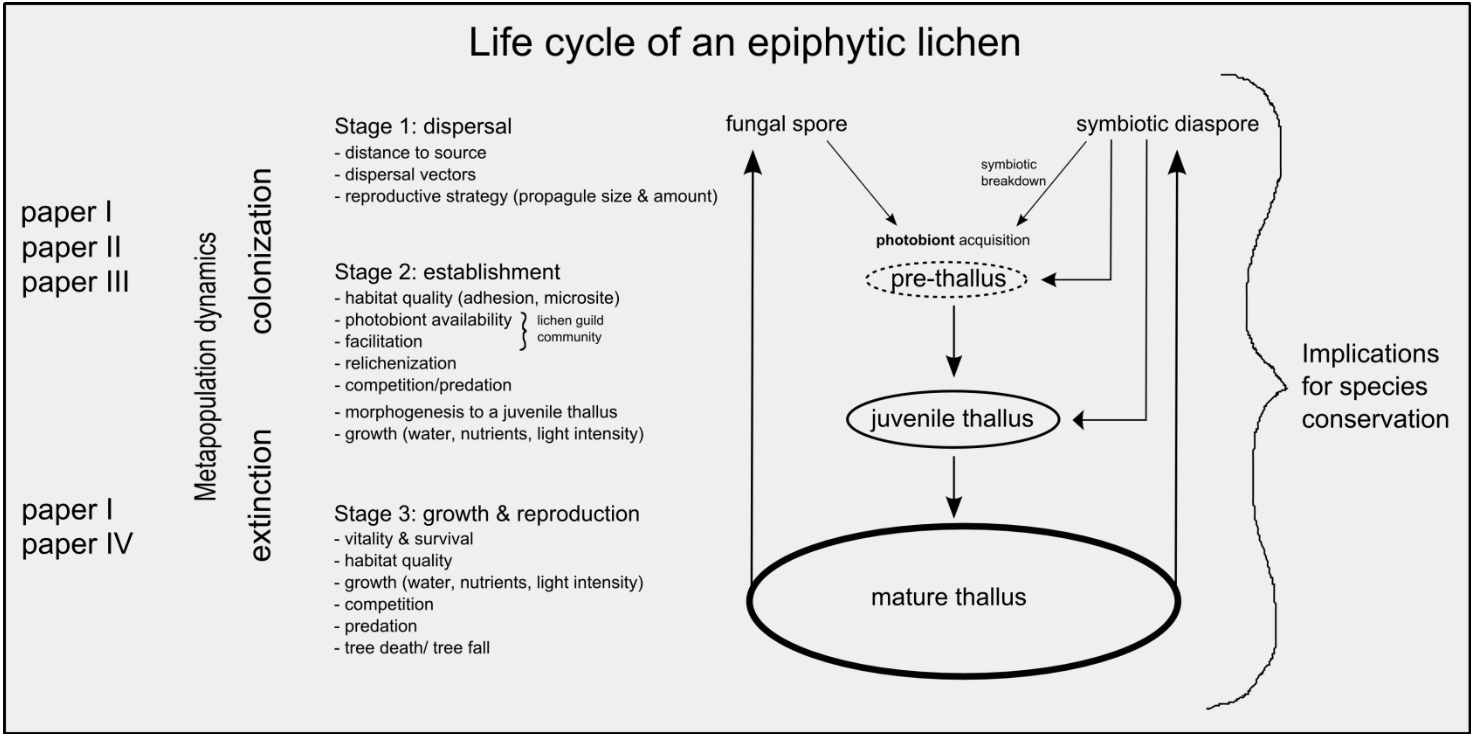


Figure 8. Schematic figure showing the three different stages in the life cycle of an (epiphytic) lichen and some ecological factors that may influence success in the respective stage and lead to local colonization and extinction events. Knowledge on the different stages of a species lifecycle can improve implications for conservation. The connection to the papers in this thesis is given to the left (figure inspired by Beck *et al.*, 1998; Sillett *et al.*, 2000; Scheidegger & Werth, 2009).

3 Materials and methods

3.1 Biological material

I studied epiphytic lichens with a cyanobacterial photobiont belonging to the *Lobarion* community (1.1.6). My focus was on species in the genus *Nephroma* (1.1.7) (papers I-III) and *Lobaria pulmonaria* (1.1.8) (papers I-IV) (Table 1). Other study species included were *Parmeliella triptophylla* (papers I-II) and *Protopannaria pezizoides* (papers I-II) (Table 1). Several species of the genus *Peltigera* were used for genetic comparison in paper II, while *Lobaria retigera* (Bory) Trevisan and *Sticta limbata* (Sm.) Ach were used as phylogenetic outgroups in paper III. Several of my study species are currently red-listed in Finland or Sweden (Gärdenfors, 2010; Rassi *et al.*, 2010) (Table 1).

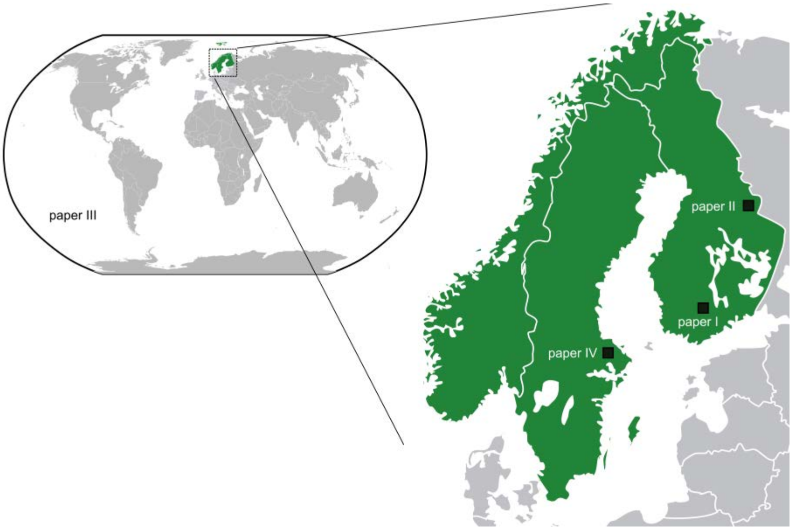


Figure 9. Location of the study sites in Fennoscandia (papers I, II, and IV). Samples for paper III were collected by several people in different parts of the world and obtained from GenBank.

3.2 Study sites

The 25 ha study site in paper I is located in the nature reserve Kotinen (61°1514′N, 25°04′E) in the southern boreal zone, Finland (Ahti *et al.*, 1968) (Fig. 9). The old-growth forest is dominated by 80-150 years old *Picea abies* (L.) H. Karst (Norway spruce) with interspersed *Betula* L. (birch) species, *Populus tremula* L. (aspen) and *Pinus sylvestris* L. (Scots pine) (Finnish Environment Institute, 2009) (Fig. 10). This study site was chosen since an extensive data set from a previous lichen survey including tree coordinates was available (Kuusinen pers. comm.; Riiali *et al.*, 2001), which was a prerequisite for the study of colonization extinction events.

Figure 10. Kotinen nature reserve, Finland

The second study site (paper II) is a 900 ha area within the Teeri-Lososuo nature reserve, situated in eastern Finland (64°07′N, 29°33′E) (Fig. 9). The forest landscape is dominated by differently sized old-growth coniferous forest stands with interspersed deciduous trees within a matrix of more open boreal peat lands (Fig. 11). Also in this area lichens had previously been surveyed (Konttinen, 1998), but exact tree coordinates were not available. However, these earlier observations on lichen occurrences were used to locate sample sites for the three epiphytic *Nephroma* species.

Figure 11. Teeri-Lososuo forest landscape, Finland

Lichen samples for paper III were collected by several people in a number of different geographical locations, including Asia (China, Japan), Europe (Finland, Norway, Scotland, Sweden, Russia, France, Canary Islands), North America (USA incl. Hawaii and Canada), and South America (Argentina). Additional samples were obtained from GenBank.

The study sites in paper IV are located in the hemi-boreal zone (Ahti *et al.*, 1968) in east-central Sweden (approx. 60°02′N, 18°22′E) (Fig. 9). The sites are located within an area of 7 500 km^2 dominated by coniferous forests. The prevailing trees were *P. abies* and *P. sylvestris* with some broad-leaved trees, mostly *Betula* species (Hazell & Gustafsson, 1999).

Table 1. *Main study species, their reproductive strategy (predominantly vegetative – v, or sexual – s), geographic distribution, and habitat preference, with a focus on Fennoscandia. Red-list status was checked for Finland, Sweden, and Norway, with some additional remarks from below cited literature. Roman numbers behind the species indicate the papers of this thesis in which they appear. ** denotes tripartite species. Species naturally occurring in Finland or Sweden are marked in bold (Brodo* et al.*, 2001; Ahti* et al.*, 2007; Smith* et al.*, 2009; Gärdenfors, 2010; Rassi* et al.*, 2010). (Table continues on the next page)*

Lichen species	Reproductive strategy		Geographic distribution	Habitat	Red-list
Nephroma arcticum** $^{(III)}$ (L.) Torss.	s	apothecia frequent	circumpolar, arctic to boreal, Europe, N America, Asia	terrestrial (among mosses)	rare in central Europe & Scotland
N. bellum $^{(I, II, III)}$ (Sprengel) Tuck.	s	apothecia frequent	circumpolar, continental, Europe, Asia, N America	epiphytic, saxicolous (mossy rocks)	NT $^{\mathrm{Fin}}$
N. expallidum** $^{(III)}$ (Nyl.) Nyl.		apothecia rare; lobules	circumpolar, Europe, Asia, N America	terrestrial (among mosses)	very rare in central Europe
N. helveticum $^{(III)}$ Ach.	s & v	apothecia common; phyllidia and isidia	± cosmopolitan (if conspecific with *N. tropicum*)	saxicolous (mossy rocks), epiphytic	CR $^{\mathrm{Fin}}$; extinct in British Isles & Scandinavia, rare elsewhere in Europe
N. isidiosum $^{(III)}$ (Nyl.) Gyeln.	v	isidia; apothecia unknown	circumpolar, Europe, Asia, N America, Africa	saxicolous; epiphytic	very rare

Lichen species	Reproductive strategy		Geographic distribution	Habitat	Red-list
N. laevigatum $^{(I, III)}$ Ach.	s	apothecia common	circumpolar, (sub)oceanic; Europe, N America, Asia, Africa	epiphytic, saxicolous	NT ^{Sw}; CR ^{Fin}; largely extinct in European north central lowlands
N. occultum $^{(III)}$ Wetm.	v	soredia	endemic in NW America	epiphytic	endemic in NW America
N. parile $^{(I, II, III)}$ (Ach.) Ach.	v	soredia; apothecia rare	circumpolar, temperate – arctic; Europe, Asia, America, Africa	epiphytic, saxicolous (mossy rocks)	
N. resupinatum $^{(I, II, III)}$ (L.) Ach.	s	apothecia frequent; phyllidia	circumpolar, hemiboreal - arctic, Europe, Asia, N America, S Africa	epiphytic, saxicolous (mossy rocks)	NT ^{Fin}; extinct in British Isles & Denmark
N. tangeriense (Maheu & A. Gillet) Zahlbr.$^{(III)}$	v	folioles; apothecia rare	SW England, Channel Islands, Isles of Scilly, Wales, W Mediterranean, Macaronesia, Asia, Africa	terrestrial, saxicolous (mossy rocks), epiphytic	
Lobaria pulmonaria (L.) Hoffm.** $^{(I-IV)}$	v	soralia, isidia; apothecia infrequent	oceanic & montane Europe, Macaronesia, N America, Asia, Africa	epiphytic, saxicolous (mossy rocks)	NT $^{Swe; Fin}$
Parmeliella triptophylla (Ach.) Müll. Arg.$^{(I, II)}$	v	isidia; apothecia infrequent	Europe, Iceland, Greenland, Macaronesia, N & C America, Asia, Africa	epiphytic, occasionally on rocks	NT ^{Fin}
Protopannaria pezizoides $^{(I, II)}$ (Weber) P.M. Jørg. & S. Ekman	s	apothecia often abundant	Europe, Macaronesia, N & S America, Asia, Africa	epiphytic, saxicolous, terrestrial	NT ^{Fin}

3.3 Methodological approaches

Paper I: Two surveys of cyanolichen communities were conducted on the same trees in 1994-1996 and 2008, and several additional environmental variables were measured in 2008. Generalized (non-) linear models were used to investigate which variables for local environmental conditions, variables assumed to reflect species interactions, and spatial connectivity explained colonization and extinction dynamics of our study species. Simulations on species persistence were based on the observed metapopulation turnover and tree fall rates.

Paper II: Samples of three *Nephroma* species were collected from a relatively large forest landscape in eastern Finland. If possible, all three species were collected from the same tree, and sometimes several thalli of the same species were taken. Molecular analysis yielded sequences for the cyanobacterial tRNA^{Leu} (UAA) intron. Sequences were compared and related to species reproductive strategy. The most variable P6b-region of the intron were illustrated using secondary folding structures (Mathews *et al.*, 1999; Zuker, 2003).

Paper III: Samples of *Nephroma* species were collected with a focus on the northern hemisphere. Sequences for the fungal ITS and the cyanobacterial tRNA^{Leu} (UAA) intron were generated from the same thalli, and additional sequences for both genetic markers were added from GenBank. We studied the diversity of *Nephroma* mycobionts within a global, phylogenetic framework and investigated the genetic diversity of their associated *Nostoc* photobionts using a haplotype network.

Paper IV: Transplants of *L. pulmonaria* were used to assess if retention trees cold serve as suitable habitats for this species. Survival, i.e. how much of the thallus had remained, and vitality of the transplants was recorded two and thirteen years after transplantation. The data were analyzed using generalized linear mixed models.

4 Results and discussion

In this Chapter, I present some of the main results of papers I-IV under the heading of the two main study aims. Papers will not be discussed consecutively but I will start with results on survival and vitality of species transplants on retention trees (‘species occurrence’ in a wider sense, paper IV) and species metapopulation dynamics (paper I) (4.1), followed by symbiont diversity patterns of *Nephroma* on a local and global scale (papers II and III) (4.2).

4.1 Epiphyte persistence in dynamic forest landscapes

Forestry is one of the major industries in Sweden and Finland (Finnish Forest Industries Federation, 2009; Swedish Forest Industries Federation, 2011). At the same time, both countries have agreed on the conservation and sustainable use of biological diversity by signing the UN Convention on Biological Diversity (CBD) in 1992. One approach to mitigate effects of forestry on biodiversity is tree retention, but the extent to which lichens benefit from this measure is not yet clear. Using transplants of the epiphyte *Lobaria pulmonaria* we could show that this species was able to survive over a decade on retained aspen trees on clearcuts, and that survival was highest on the northern sides of these trees (paper IV) (Fig. 12a). These results were at the same time surprising and reassuring, but they also open some new questions. The surprise was certainly that adjacent forest habitats were not as successful for transplant survival as the retention trees on clearcut sites, even though vitality of the transplants did not differ between forest and clearcut trees (Fig. 12b). Does this mean that the previous assumption of *L. pulmonaria* as flagship and umbrella species for communities in old-growth forests (1.1.8) needs to be abandoned? Other studies have presented similar results (the reassuring moment), for example transplanted *L. pumonaria* grew similarly well or better in clearcuts than in old forests in western Oregon, USA (Sillett *et al.*, 2000), and Norway

(Gauslaa *et al.*, 2006), and faster in open *Quercus* forests in Portugal than in their original *Picea abies* forest habitats in Sweden (Gaio-Oliveira *et al.*, 2004). What we observe is probably the difference in species fundamental and realized niche, i.e. that old-growth forest habitats are the realized niche of *L. pulmonaria*, but that habitats with higher light intensity can also be suitable for its existence if transplanted there (fundamental niche). It is encouraging that retention trees can host vulnerable species for over a decade, especially on their north side, and this supports tree-retention as a conservation tool for forest management.

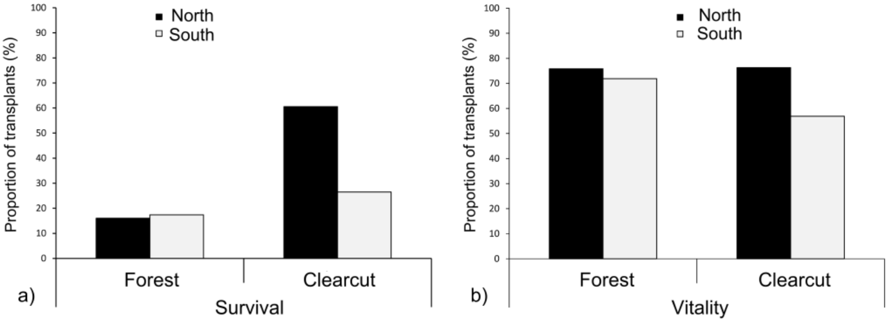


Figure 12. Survival (a) and vitality (> 50% vital) (b) of *L. pulmonaria* transplants after 14 years.

However, the entire story is probably not as simple as to just switch the optimal habitat for *L. pulmonaria* to be that on a clearcut retention tree, especially since vitality of the transplants did not differ between trees in the forest or on the clearcut (Fig. 12b) (paper IV). I want to consider three points here. Firstly, our study was based on transplants, not on real occurrences of *L. pulmonaria*. This has the beauty that we were able to study the species under ‘experimental conditions’, i.e. the same sizes of thalli etc., but it omits that natural occurrences are the result of a species successful colonization process, including dispersal and establishment. The results of studies involving mature thalli need to be interpreted with care since habitats suitable for mature lichen thalli could potentially be unsuitable during species establishment phase (the ”regeneration niche”, Grubb, 1977). Some indications for this phenomenon in *L. pulmonaria* have been described by Scheidegger (1995) who recorded lower survival of transplanted diaspores compared to mature thalli. They attributed this to differences in the ecological microclimate of the study sites and broader ecological amplitude of mature thalli, but further investigations are needed. Our study was designed to evaluate the life-boating of mature lichen thalli, and for this the regeneration niche can be neglected. Still, if we are concerned about species long-term survival, including reproduction and further establishment, we should also consider possible differences in the environmental requirements for diaspores, juvenile growth, and mature thalli. Secondly, the different response of survival and vitality in trees in the forest or clearcut suggests that some factor in the forest affected survival but not vitality of the transplanted species. Invertebrate grazing is one possible cause for the disappearance of thalli (e.g. Asplund & Gauslaa, 2008). A recent study observed a generally lower abundance and species density of land-snail fauna on clearcuts compared to forests (Hylander, 2011), which supports the hypothesis that snail grazing may affect survival in forests more than on clearcuts. The third point concerns the habitat quality of current old-growth forest habitats: have they changed to the worse for some species? *L. pulmonaria* has the ability to acclimate to sudden changes in light conditions, given that hydration is regular, but growth and natural occurrence are assumed to be restrained by a constant balance between light availability and the risk of desiccation (Gauslaa *et al.*, 2006). The heterogeneous structure and function of natural boreal forests is the result of spatial and temporal variable disturbance and successional processes such as fires, insect-outbreaks, wind throw, and tree dynamics, with a variation of light, space, and nutrients occurring throughout the forest (Esseen *et al.*, 1997; Kuuluvainen, 2002). It has been suggested that forests today are denser and therefore too dark for lichen growth, possibly as a result of decreased disturbance processes, an increase in nutrient input, and climate change (Gauslaa *et al.*, 2007). It could hence also be that, while species seem vital, their long-term survival in old-growth forest habitats is hindered by too much shade. Conservation measures to restore structural heterogeneity include the artificial creation of small and large-scale gaps, but more research on habitat changes of old-growth forests as well as on the optimal niche for *L. pulmonaria* could also improve the understanding of survival and vitality of this species in different habitats.

While habitat quality certainly plays a role for species survival in general, another important factor for the long-term survival specifically of epiphytes in old-growth forests is the continuous availability of their host trees. Using empirical data on several epiphytic macrolichens and their host trees we projected species future persistence in four scenarios that differed in initial number of host trees and whether trees fell or not (paper I). We could show that a low number of host trees increased extinction risk for some infrequent sexually dispersed species, especially when tree fall was included. This implies that set-asides of small habitats with low tree numbers may not be efficient in preserving species in the long term. All species facing extinction risk were characterized by low colonization rates, emphasizing that conservation strategies need to ensure that species colonization rates remain large enough to counteract local extinctions. Increased host tree numbers could possibly increase species persistence, but this is difficult to achieve within the next years since the aspen trees need to have a certain age and diameter to be suitable host trees. Nevertheless, growth of future host trees should be promoted close to current cyanolichen populations, in the hope that species will persist until the next generation of trees has reached an appropriate size for lichen colonization.

Metapopulation turnover, i.e. local colonizations and extinctions, has not been studied widely in epiphytic lichens (but see Snäll *et al.*, 2005a; b; Löbel *et al.*, 2006a; b) and it has been suggested (Snäll *et al.*, 2003) and shown (Johansson *et al.*, in press; Snäll *et al.*, 2005a) that a local population persists on its habitat patch until the tree falls ('patch tracking metapopulation') (1.2.3). However, our empirical data in paper I show that epiphytic macrolichens had not only colonized new habitat patches, but that some local populations also had disappeared from standing trees after a time span of 13 years. The observed local extinctions were not exclusively the result of tree fall (deterministic) or tree death, but stochastic extinctions also occurred from living trees. The studied macrolichens hence follow the habitat tracking model (Thomas, 1994; Thomas & Hanski, 1997), such as previously suggested for *L. pulmonaria* (Öckinger & Nilsson, 2010) and for epixylic lichens (Caruso *et al.*, 2010). Still, causes for local extinctions from living trees have not been investigated widely, and the result highlight the need for further studies in this field. Snails and arthropods (e.g. insects and mites) have been shown to feed on lichens, as already mentioned above (e.g. Asplund & Gauslaa, 2008), and it has been suggested that snail grazing could cause local extinctions in certain areas (Gauslaa, 2008). In general, small populations are more likely to disappear than large populations. We found that populations on larger trees were less likely to go extinct, probably because larger habitat patches increase species abundance in epiphytes (Snäll *et al.*, 2004b; Öckinger & Nilsson, 2010).

In order to explain species colonizations we need to 'zoom out' from a local population at a single tree to the entire metapopulation. We could e.g. show (paper 1) that a larger amount of occupied habitat patches in the first survey year resulted in higher colonization rates. This is not surprising, since more propagules can be produced if a species is frequent, compared to less abundant species. However, distance between occupied trees and the closest (unoccupied) habitat patches is also important for species colonization success, since it can be expected that most propagules will land relatively close to their dispersal source (Walser *et al.*, 2001; e.g. Snäll *et al.*, 2005a), and we found indications that this is also the case for *N. parile* (paper I). If we now 'zoom back in' to a single tree there are also at least two features that may be important for colonization success: one is tree size, and the other the presence of species from the *Nephroma* guild in the first survey year (paper I). Larger trees increased colonization success for *P. triptophylla*, which can be explained by attributes interlinked with tree size, such as age and bark microtopography (1.2.1). For example, large trees have a bigger surface than small trees, and usually more heterogeneous and rough bark, and are therefore more likely to capture and hold diaspores. The second feature, the occurrence of species from the *Nephroma* guild, increased colonization of *N. bellum*. This could be the result of photobiont sharing among species (1.3.2), if photobiont availability is a limiting factor, but it may also simply be the result of habitat similarity between species (Myllys *et al.*, 2007).

In conclusion, our papers indicate that the persistence of epiphytic macrolichens with a cyanobacterial photobiont in boreal forest landscapes depends on habitat quality, quantity, and connectivity. We could show that local colonization and extinction events shape the epiphyte metacommunity, and that tree fall also plays a role for species persistence. While retention trees, and especially their north-facing sites, can be valuable to life-boat species, the continuous availability of species host trees in a heterogeneous environment close to present occurrences is probably crucial for the species' long-term persistence (papers I & IV).

4.2 Symbiont diversity in the genus *Nephroma*

Successful dispersal and establishment are prerequisites for a lichen to colonize new habitat patches, with establishment often involving the process of re-lichenization. Symbiont diversity will be shaped by the 'choice' of the two bionts at re-lichenization, the fitness of a symbiotically dispersing holobiont with possible symbiotic breakdown after dispersal, and the general availability of photobionts at any given place (1.3).

Based on the cyanobacterial marker tRNA$^{\mathrm{Leu}}$ (UAA) we could show that several bipartite lichen-forming fungi of the genus *Nephroma* associate with sequence-identical *Nostoc* photobionts on a local and global scale (papers II & III) (Fig. 13). Previous studies on the *Nostoc* photobiont in *Nephroma* have already shown that species are highly specific in their photobiont choice, e.g. associate bipartite species only with a specific group of *Nostoc* photobionts (Rikkinen *et al.*, 2002). Moreover, also sequence-identical *Nostoc* strains were previously found among several species of the *Nephroma* guild and in few occasions also from different parts of the world (Paulsrud *et al.*, 1998, 2000; Rikkinen *et al.*, 2002; Myllys *et al.*, 2007). However, we are the first to show

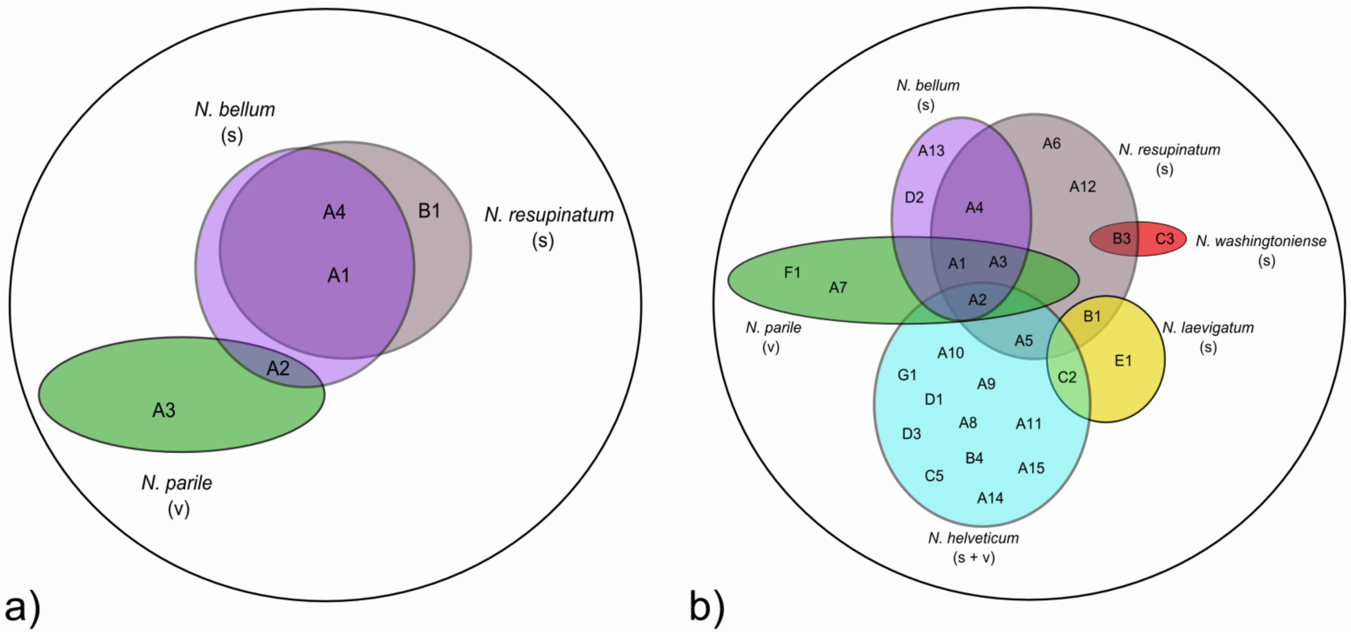


Figure 13. Local (a) and global (b) *Nostoc* symbiont selectivity in the genus *Nephroma* (papers II & III) with *Nostoc* genotype names (small letter and number) as used in paper III. Coloured circles indicate selection of *Nostoc* genotypes by lichen-forming fungi, and main reproductive strategy is indicated by s=sexual (dispersal by fungal spores) or v=vegetative (symbiotic dispersal). On a local scale (a), the symbiotically dispersing species *N. parile* has its 'own' *Nostoc* genotypes, which are usually not shared with *N. bellum* and *N. resupinatum* (exception: one tree on which *N. bellum* associates with A2), while *N. bellum* and *N. resupinatum* frequently share the same genotypes A1 & A4 (paper II). On a global scale (b), species are less selective, i.e. they associate with a wider range of photobionts from the pool, and e.g. genotypes A2 & A3 are here shared between several species. Note that not all study species were included and that some species (e.g. *N. helveticum*) may in reality consist of several taxa.

from extensive sampling of several *Nephroma* species and different geographical locations that high specificity is maintained in the genus *Nephroma* on a global scale (Fig. 13). These results also support the idea of lichens occurring in specific guilds, which are built around a restricted pool of closely related cyanobionts and shared by species within this guild (Rikkinen *et al.*, 2002; Rikkinen, 2003) (1.3.2). One of the core species is *N. parile*. However, also other species belonging to the *Nephroma* guild, e.g. *Parmeliella triptophylla*, are likely to act as photobiont dispersing core species, since *N. parile* has so far only been found to associate with some of the detected *Nostoc* genotypes and did not share its genotypes widely on a local scale (paper II) (Fig. 13). Tripartite *Nephroma* species, on the other hand, belong to a different photobiont sharing guild since they all associate with another group of *Nostoc* photobionts (Lohtander *et al.*, 2003; paper III). At the same time, the lichen-forming fungi of tripartite *Nephroma* species are not monophyletic (Lohtander *et al.*, 2002; paper III). This strengthens the hypothesis that an evolutionary transition between symbiosis types must have occurred several times (Lohtander *et al.*, 2002), and that such transition cannot simply have occurred by an additional association with (or loss of) a second photobiont (Lohtander *et al.*, 2003). Our papers indicate that photobiont sharing in *Nephroma* is locally connected with species reproductive strategy (paper II), while globally there is no such trend (paper III). While the overall diversity of *Nostoc* symbionts in *N. parile* does not differ markedly from the diversity observed in sexually dispersed *Nephroma* species, the fungal symbiont in *Nephroma parile* s.lat. appears to be genetically more uniform than the mycobionts of some other *Nephroma* taxa, with individual ITS genotypes found from widely distant localities and sometimes from different continents. This relative homogeneity might be partly related to the fact that *N. parile* mainly disperses by symbiotic propagules, and sexual recombination of the gene pool in this fungus might thus be relatively infrequent. Vertical transmission of the photobiont due to symbiotic dispersal in *N. parile* is more likely to result in high selectivity, but symbiotic breakdown after dispersal could lead to better establishment success in ecological diverse habitats – assuming that certain symbiont combinations have a better fitness in particular habitats than others (e.g. Rikkinen, 1995, 2003; Beck *et al.*, 2002; Yahr *et al.*, 2006; Otálora *et al.*, 2010). On the other hand, lichen species dispersing with fungal spores, such as *N. bellum*, are likely to be less selective in their photobiont choice and may in a given locality associate with any genetically compatible photobionts they encounter. The photobiont pool of genetically compatible photobionts may differ between regions as a result of environmental preference (e.g. Piercey-Normore & Deduke, 2011). Consequently, both reproductive strategies could lead to geographic mosaic patterns (Thompson, 2005) in which lichenized fungi from the same taxon associate with different groups of photobionts in different parts of the world (paper III) (Fig. 14).

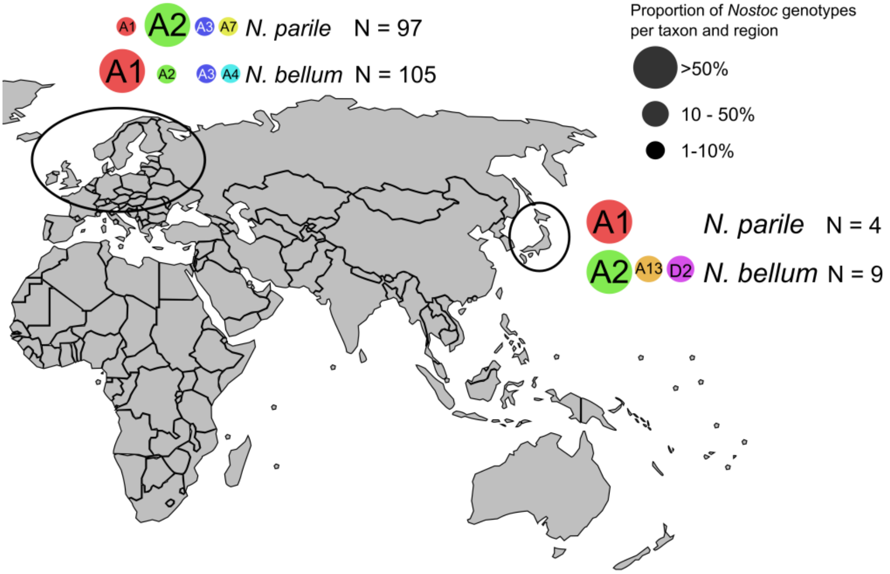


Figure 14. The observed tRNA^{Leu} (UAA) *Nostoc* genotype distribution in *N. bellum* s. lat. and *N. parile* s. lat. in Europe and Japan.

This observed photobiont diversity pattern of higher selectivity locally than globally (paper III) has previously also been shown for the lichen *Cladonia subtenius* (Yahr *et al.*, 2006). Interestingly, the preference in our study species is reverse, with *N. parile* associating with *Nostoc* genotype A1 in Japan and with genotypes A2 and A3 in Europe and North America, while *N. bellum* prefers *Nostoc* genotype A1 in Europe and A2 in Japan (Fig. 14). In *N. parile*, this pattern was even found for a single mycobiont ITS genotype, while in *N. bellum*, no mycobiont ITS genotype was found to occur in both Europe and Japan. Nevertheless, such mosaics suggest that once a certain combination of symbionts has been successfully established in a certain area, this particular combination has a tendency to become regionally dominant. Similarly we found that certain symbiont combinations were dominant on single tree trunks (paper II). These patterns can be attributed to a founder effect, usually explained by low colonization. However, probably also other mechanisms are involved, such as improved fitness of certain myco-photobiont combinations in particular habitats, as described above (e.g. Rikkinen, 1995, 2003; Beck *et al.*, 2002; Yahr *et al.*, 2006; Otálora *et al.*, 2010). One limitation of our findings in paper III is that sampling effort differed between countries, with only few samples being obtained from Japan and many from Europe, and also, most European samples came from a single location in Finland. It would hence be interesting to investigate these results further with a more even sampling.

We conclude that species of the genus *Nephroma* are specific and selective in their association with symbiotic partners, but that their selectivity is scale-dependent. Particular symbiont associations are linked to geographical areas, which could indicate a difference in fitness of specific mycobiont-photobiont combination in different areas. Several lichen-forming fungi share sequence-identical photobionts over a global scale, which supports the idea of photobiont-sharing lichen guild communities.

5 Conclusions and perspectives

The persistence of lichens depends on success or failure in the different phases of their life, i.e. dispersal, establishment, and growth, but also on their environment, which can cause single thalli or entire populations to disappear. Since lichens are symbiotic organisms, their dispersal and establishment involves at least two different partners, which adds to the complexity in their life cycle and distribution.

Our results on symbiont patterns in the genus *Nephroma* indicate that the ‘choice’ of the symbiotic partners is not random, and that selectivity patterns vary between tree, local, and global scales (papers II & III). The next step would be to extend these studies to a community level, and to combine genetics with environmental variables. For example, photobiont sharing in species belonging to the *Nephroma* guild could be compared in different ecological habitats on a global scale to test if certain symbiont combinations are linked with environmental variables. This could give further insights, not only to specificity and selectivity patterns on a community level, but also to the colonization process of different species. The opportunity and ability of lichen-bionts to switch symbiont partners may be beneficial since species could become more flexible in colonizing different habitats.

The epiphytic macrolichens studied formed habitat tracking metapopulations in which local colonizations, stochastic, and deterministic extinctions occurred. These dynamics could to some extent be explained by metapopulation related variables, but more research is needed to explain the observed local species extinctions from living trees (paper I), which may have similar causes as the lower survival of transplanted species of *L. pulmonari*a in forests compared to retention trees on clearcuts (paper IV). It will be difficult to witness extinctions directly, but if time and money were abundant it would be interesting to film several lichen thalli in different habitats, and to follow their fate directly. More realistic studies include snail trapping experiments in which the amount of lichen-feeding snails is recorded in the same habitats where the extinctions were observed. Also, further feeding experiments with organisms that may eat lichen thalli could increase our knowledge on possible causes for extinction.

Implications for species conservation include the importance of maintaining a continuous availability of old *Populus tremula* trees in the boreal forest landscapes of Fennoscandia. These trees need to be in the vicinity of already occupied trees to ensure that dispersal is not a limiting factor. Tree retention should be encouraged, and especially those trees that contain lichens on their north sides should be retained (paper IV). These measures are not only important for lichens with cyanobacterial photobionts, but will also benefit other species depending on old aspen.

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