Should I Stay or Should I Go?

Reproductive and Dispersal Strategies of Site-specific Liverwort Species

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ACADEMIC DISSERTATION

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Cover: Gemmae of *Anastrophyllum hellerianum.*

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals:

I   Pohjamo, M. and Laaka-Lindberg, S. (2004) Demographic population structure of the leafy hepatic *Anastrophyllum hellerianum.* Plant Ecology 173, 73-81.

II   Pohjamo, M. and Laaka-Lindberg, S. (2004) Reproductive modes in a leafy hepatic *Anastrophyllum hellerianum.* Perspectives in Plant Ecology, Evolution and Systematics 6, 159-168.

III   Pohjamo M., Laaka-Lindberg S., Ovaskainen, O. and Korpelainen H. (2006) Dispersal potential of spores and asexual gemmae in a epixylic hepatic *Anastrophyllum hellerianum.* Evolutionary Ecology 20, 415-430.

IV   Korpelainen, H., Laitinen, R. and Pohjamo, M. (2004) Lack of intraspecific variation in cpDNA in *Trichocolea tomentella* (Ehrh.) Dumort. Journal of Bryology 26, 221-223.

V   Pohjamo, M., Korpelainen, H. and Kalinauskaitė, N. (2008) Restricted gene flow in the clonal hepatic *Trichocolea tomentella* in fragmented landscape. Biological Conservation, in press, doi:10.1016/j.biocon.2008.02.016

CONTRIBUTIONS

The following table shows the major contributions of authors to the original articles.

	I	II	III	IV	V
Original idea	SL-L, MP	MP, SL-L	MP, SL-L	MP, HK	MP, NK
Field work	MP	MP	MP, SL-L	MP	MP, NK
Ecological data	MP	MP	MP	-	MP, NK
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Data analyses	MP	MP	MP, OO	HK	MP, HK
Manuscript preparation	MP, SL-L	MP, SL-L	MP, OO, HK	HK, MP	MP, HK

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CONTENTS

1 Introduction

In Scandinavia, the extensive forestry and other utilisation of habitats have resulted in a tremendous decrease in the area of pristine forest during the last century (Östlund et al., 1997). The area of intensively managed forests, in which the conditions for old-growth-forest-dwelling species are seldom found, has expanded over the forest landscape (e.g., Esseen et al., 1997; Frisvoll and Prestø, 1997; Niemelä, 1999; Hanski, 2000; Horne et al., 2006; Yrjölä, 2002; Hilden et al., 2005). Consequently, numerous epixylic and saproxylic organisms and other substrate-specific forest species have become internationally, nationally or regionally threatened or extinct (Gärdenfors, 2005; Rassi et al., 2001; Ulvinen et al., 2002).

To decrease the conflict between biodiversity and forest management, recommendations have recently been presented for managing commercial forests in a more sustainable manner (e.g., Heinonen et al., 2004; Hyvän metsänhoidon…2001; summarised in Horne et al., 2006). Based on these recommendations, "woodland key habitats", i.e., the habitats important for forest biodiversity, where red-listed species are likely to occur, should be preserved (e.g., Meriluoto and Soininen, 1998; Heinonen et al., 2004). The conservation value of woodland key habitats is presumably significant as it provides a vital contribution to reserve networks for biodiversity in highly fragmented landscapes (Götmark and Thorell, 2003). However, there has been criticism of the role of the small-sized woodland key habitats and especially Forest Act habitats (mean size 0.62 ha in privately owned forests in Finland, Yrjönen, 2004) as a refuge for specialised species dependent on the microhabitat characteristics of pristine forests (Hanski, 2000, 2007; Horne et al., 2006; Pykälä et al., 2006).

1.1 Ecological and genetic effects of habitat destruction

Habitat destruction, including the loss of habitat, deterioration of habitat quality and fragmentation of the remaining habitat, has contributed towards the decline in species diversity (Hanski, 1999). Even moderate habitat destruction is predicted to cause extinction debt, i.e., time-delayed population extinctions (Tilman et al., 1994; Hanski 2000). Theory predicts that small and isolated populations with dispersal limitations have higher extinction rates than do larger ones because of environmental and demographic stochasticity and genetic deterioration (e.g., Hansson, 2001; Honnay and Jacquemyn, 2006; Hanski, 2007). Fragmentation affects the abiotic and biotic conditions at the edges of forest fragments (Murcia, 1995). The edge effects on the habitat fragments change environmental conditions, such as temperature and wind velocity, depending on the degree on which the surrounding habitat matrix differs from the interior forest habitat (Murcia, 1995). However, no clear pattern has been observed in the effects of the habitat patch size on bryophyte diversity in fragmented landscapes (Berglund and Jonsson, 2003; Pharo et al., 2004; Hylander and Hedderson, 2007; Zartman and Nascimento, 2006; Virtanen and Oksanen, 2007).

Genetic consequences of habitat fragmentation are associated with the reduced population size and increased spatial isolation of populations occupying habitat remnants (Frankham et al., 2003). Reductions in population size create genetic bottlenecks among the remaining individuals, due to small samples of the original gene pool, when the genetic variability of the populations is forced to decrease (Young et al., 1996). Consistent with this, bryophyte populations occupying disturbed habitats have been observed to carry lower levels of genetic diversity compared with populations inhabiting natural-state habitats (Wilson and Provan, 2003; Spagnuolo et al., 2007b). The reduced gene flow among small and isolated populations relates to the functioning of genetic drift and the random fixation of different alleles in different populations, which leads to genetic differentiation among populations (e.g., Slatkin, 1987; Young et al., 1996; Lammi et al., 1999). Yet, even infrequent sexuality combined with dispersal and colonisation could lead to gene flow and prevent the harmful effects of genetic drift (Slatkin, 1987; Young et al., 1996; Bengtsson, 2003). In plants, however, the genetic effects of habitat fragmentation are likely to be complicated due to their sessile nature, longevity, wide variety of reproductive modes and storage capacity (Young et al., 1996).

Species are considered to be sensitive to fragmentation and other external factors depending on their life history traits and the type of the environment a species is adapted to (Kolb and Diekmann, 2004; Verheyen et al., 2004; Honnay and Jacquemyn, 2006; Baldwin and Bradfield, 2007; Fréville et al., 2007). The combination of several traits, such as low reproductive potential, low competitive ability, limited storage capacity, low or restricted dispersal ability, habitat specialisation, high-amplitude of population fluctuations and low population density, are considered to be predictors of species sensitivity to fragmentation (Henle et al., 2004). For forest vascular plants, for instance, habitat fragmentation has been found to have a greater effect on poor colonisers, with dispersal limitations, than on good colonisers (Verheyen et al., 2004), whereas clonality seems to decrease the effects of fragmentation in vascular plants (Kolb and Lindhorst, 2006). In bryophytes, however, the demographic properties that are most sensitive to the effects of habitat fragmentation have not been identified (Pharo and Zartman, 2007).

1.2 Opportunities and constraints of reproductive modes

The reproductive and dispersal potential of a species affect both the population persistence and the colonisation and extinction probability. Sexual reproduction is assumed to be one of the most important characteristics for species survival, because it produces new genotypes capable of dispersal both in space and time in a landscape (Stearns and Hoekstra, 2005; Söderström and During, 2005). A large majority of plants also allocate to asexual reproduction by various means of producing genetically identical offspring, which typically enhance population persistence and short-range dispersal.

A mixed strategy involving both asexual and sexual reproduction is characteristics of numerous bryophytes (Longton and Schuster, 1983). Bryophytes appear to have a high ability to produce potentially independent ramets by lateral growth, vegetative fragments, branching and specialised asexual propagules (During, 1979, 1992; Longton, 1992;

Laaka-Lindberg et al., 2000; Crum, 2001). Clonal reproduction allows populations to persist during unfavourable climatic conditions and in habitats and regions where sexual reproduction cannot occur, for example, in the species' marginal range (During, 1979; Eckert, 2002). Moreover, clonal regeneration also seems to facilitate resistance of water loss and desiccation in bryophytes by reducing the evaporation rate (see, Dilks and Proctor, 1979) by increasing the shoot density within colonies (Bates, 1988; Økland and Økland, 1996; Van der Hoeven and During, 1997; Pedersen et al., 2001).

Many bryophytes do not express sexuality at all or only within a part of their distribution area (Shaw, 2000; Bisang and Hedenäs, 2005). The low frequency of sexual reproduction in bryophytes is linked to unisexuality and environmental constraints (Longton and Schuster, 1983). Consequently, the success of sexual reproduction, which forms a significant part of the life history, encompasses high temporal and spatial variability (Rydgren and Økland, 2002; Rydgren et al., 2006). The presence of water and the close proximity of opposite sexes are essential prerequisites for fertilisation success, because spermatozoids are dispersed to archegonia only over short distances along a water surface (Reynolds, 1980; Longton and Schuster, 1983). Especially among unisexual hepatic species, the lack of sexual reproduction is very common due to spatial separation of sexes and limitations in the production of sex organs (Longton and Schuster, 1983; Laaka-Lindberg et al., 2000; Shaw, 2000; Crum, 2001). Thus, the reliance on asexual reproduction among unisexual bryophyte species is prevalent because many of them tend to be rare world-wide, without the ability to reproduce asexually (Söderström and During, 2005).

1.3 Opportunities and constraints of dispersal

Selection for dispersal ability reflects a balance between the costs and benefits of "staying *versus* dispersing" (Eriksson and Kiviniemi, 1999) and is expected to be linked to adaptation to particular environments (Begon et al., 1996: 527). In clonal plants, dispersal ability and longevity have been found to have a negative correlation (Ehrlen and Groenendael, 1998). Dispersal ability and, consequently, the probability of colonisations are affected by the allocation of resources to reproduction and reproductive organs (e.g., seeds, spores, asexual propagules, shoot fragments) and by their dispersal capacity (Ehrlen and Groenendael, 1998). Crowding, competition among close relatives, resource competition, inbreeding avoidance and the genetic benefits of gene flow are considered to favour dispersal ability further from the source (Dieckmann et al., 1999; Hanski, 2001a). Contrastingly, short-range dispersal is favoured on the basis of local adaptation and the consequent persistence of offspring in an already suitable site with favourable local conditions (Eriksson and Kiviniemi, 1999). In bryophytes, dispersal ability is related to diaspore size. Small spores tend to be more widely dispersed than large ones, but the relative advantage of large diaspores is greater in establishment success (During, 2001).

Besides dispersal in space, dispersal can occur also in time, both by the dormancy of the propagules and vegetative parts of plants and by clonal regeneration, achieving a long life span of the genet (Rees, 1996; During, 2001).

Dispersal and gene flow potential are largely dependent on the prevailing reproductive modes of the organism and the mobility of the gametes and diaspores (Lowe et al., 2004: 106). A slow population turnover rate is considered to decrease the probability of local extinction in forest fragments in the long term compared to short-lived species with a high rate of population turnover (Ovaskainen and Hanski, 2002; Vellend et al., 2006). The short-lived or highly site-specific species with good dispersal ability tend to build metapopulations with a high rate of colonisations and extinctions (Eriksson, 1996). However, the dynamics of epiphytic (and also epixylic) bryophyte species can be characterised as a patch-tracking metapopulation, where local extinctions are caused by patch destruction (see Hanski, 2001b; Snäll et al., 2003, 2005). Contrastingly, long-lived plants with clonal propagation and plants with extensive diaspore banks tend to build up remnant population systems, in which local populations persist over long periods of time through unfavourable conditions (Eriksson, 1996). Plant species with stable dynamic are typically associated with a low reproductive effort, long life span, high competitive capacity, stress tolerance, disturbance resistance, clonality among adults and dormancy (Ehrlen and Groenendael, 1998).

Colonisation rates must be balanced with extinction rates for species survival in a regional scale (Hanski, 1999). In plants, diaspore rain is seldom random, because most diaspores fall within the close vicinity of the parent and their density declines rapidly with distance (e.g., Miles and Longton, 1992; Ouborg et al., 1999; Murrell et al., 2002). Consequently, the colonisation rate and population density decrease with the distance between the focal patch and the source populations (Hanski, 2001a; Snäll et al., 2005). Thus, the connectivity of suitable habitat patches and large patch sizes are likely to increase colonisation rates (e.g., Snäll et al., 2003; Virtanen and Oksanen, 2007). However, dispersal distances, even within a single plant species, can vary considerably in space and time, depending on the prevailing environmental and climatic conditions (Hanski, 1999; Murrell et al., 2002; Tackenberg, 2003; Bullock et al., 2003; Ozinga et al., 2004). Moreover, various physical mechanisms (e.g., elaters, splash-cups) and the small-size of bryophyte diaspores enhance the dispersal distances of diaspores from the source, together with passive work by a set of dispersal vectors (agents), such as wind, water, birds, mammals and insects (Crum, 2001; see Laaka-Lindberg et al., 2003 for review).

Studies on bryophyte dispersal support the expectation that spores are able to disperse further from the source than larger asexual diaspores (McQueen, 1985; Söderström and Jonsson, 1989; Kimmerer, 1991, 1994; Miles and Longton, 1992; Stoneburner et al., 1992; Kimmerer and Young, 1995; Sundberg, 2005; but see Muñoz et al., 2004). Consistent with this, bryophytes with frequent sexual reproduction are genetically less differentiated and exposed to a greater level of gene flow than are mainly clonal species (Van der Velde et al., 2001; Van der Velde and Biljsma, 2003; Korpelainen et al., 2005; Pohjamo et al., unpublished manuscript).

1.4 Aims of the research

The general objective of this thesis is to increase knowledge of the reproductive and dispersal strategies of the substrate-specific forest bryophytes. I have studied in detail the population biology of two substrate-specific liverwort species, *Anastrophyllum hellerianum* and *Trichocolea tomentella*, inhabiting unstable and stable habitats, respectively, in a forest landscape. A further aim was to develop recommendations for conservation measures for species inhabiting unstable and stable habitats in fragmented forest landscape. The life history traits of bryophytes that are assumed to be associated with unstable and stable habitat types are shown in Box 1.

The research is composed of five articles, which are hereafter referred to by their Roman numerals I-V. In each article, I have addressed the specific objectives listed below by collecting research data and analyzing the results.

In the first substudy (I), I studied the population structure of *A. hellerianum* by examining 1) the proportion of shoot types, the proportion of the dead shoots among shoot types and shoot size classes, 2) the relationships between shoot types and size, 3) the relationships between shoot density and shoot types and shoot size classes, 4) the numbers of gemmae on the shoots, and 5) the relationships between gemmae production and shoot density.

In the second substudy (II), I studied the reproductive modes of *A. hellerianum* by examining 1) the proportions of shoot types within the colonies, 2) the frequency of the colony types based on the occurrence of sex-expressing shoots in the colonies, 3) the differences in the proportions of shoot types between the colony types based on the occurrence of sex-expressing shoots, 4) the expressed male-to-female sex ratios for total, living and dead shoots, and 5) the minimum range of sperm dispersal and the frequency of sporophyte production.

In the third substudy (III), I studied the dispersal potential of sexual and asexual propagules of *A. hellerianum* by examining 1) the rate of propagule production in the source colonies, 2) the distribution of the dispersal distances of the propagule types within a 10 m radius from the source, 3) the fraction of propagules dispersed further than 10 m, and 4) the effects of weather conditions on the rates of propagule production and on the dispersal distances of the propagule types.

In the fourth substudy (IV), I detected in *T. tomentella* 1) the genetic diversity of noncoding chloroplast DNA regions in geographically distinct samples, and 2) the suitability of cpDNA markers for population genetic studies.

In the fifth substudy (V), I studied the population genetics and population structure in *T. tomentella* by examining 1) the amount of genetic diversity based on ISSR (Inter-Simple Sequence Repeat) and RAPD (Random Amplified Polymorphic DNA) fingerprinting markers in different regions and in populations of different sizes, 2) the level of gene flow on different spatial scales, and 3) the sporophyte production, the shoot size

distribution, the proportions of dead shoots, the branch density and shoot density of *T. tomentella* and other bryophyte shoots, and their relationships with each other.

Box 1. General life history traits in bryophytes inhabiting temporary and patchy habitats and stable habitats based on During's classification (1979).

Unstable patchy habitat in time and space (colonists in the sense of During 1979)

- Low life expectancy, moderately short life span (1-5 years)
- High reproductive effort both in sexual and asexual propagule production
- Production of specialised asexual propagules
- Low age of first asexual reproduction
- Asexual reproduction in the early life stages, sexual reproduction later
- Absence or rarity of sexual reproduction
- Predominance of asexual reproduction
- Spores small, less than 20 um, asexual propagules larger
- High reproductive output
- Mortality determined by abiotic and biotic factors
- Avoidance strategy for severe stress: stress tolerant propagules, dormancy of the propagules

Stable habitat which lasts very long (perennial stayers in the sense of During 1979)
- Long life span, perennials
- Low or nearly absent reproductive effort for sexual and asexual, localized in small areas
- Age of first reproduction variable, commonly several years
- No specialised propagules
- Spores small, less than 20 um
- Clonal propagation, branching
- Good competitive ability (large shoot size)
- Mortality caused by stochastic habitat changes
- Tolerance strategy of severe stress: vegetative growth and propagation

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2 Material and methods

2.1 Species studied

Anastrophyllum hellerianum

Anastrophyllum hellerianum (Nees ex Lindenb.) R.M.Schust. (Lophoziaceae) is a minute (< 1 cm), unisexual, leafy hepatic (Fig. 1). Sexual reproduction is occasional, whereas asexual reproduction is very common (Schuster, 1969). *A. hellerianum* produces two kinds of small propagules, sexually formed spores and asexually produced gemmae, both of which are 1-celled and about 10–12 μm in diameter (Fig. 2). The spore capsules of hepatics have a special mechanism for enhancing the dispersal of spores. Spore capsules split into valves and, in capsule dehiscence, the spores are flicked actively into the air by elaters, which undergo hygroscopic movements. Asexual gemmae are produced on the tips of gemmiparous erect asexual shoots with no special mechanism for enhancing gemma dispersal.

A. hellerianum mainly inhabits old-growth boreal forests and spruce swamps with high amounts of coarse woody debris (e.g., Ulvinen et al., 2002). Usually it grows on large decaying logs of intermediate decay stages (Fig. 3; Söderström 1993), but it can also occur on smaller decaying wood substrates (Laaka-Lindberg et al., 2005). In the study area in Southern Finland, spores were formed in 2.5–12 % of the colonies from June to September, whereas gemmae were produced in every colony during the whole growth season (I, II, III; Laaka-Lindberg et al., 2005). The mean spore number per capsule has been estimated to vary from 12 000 to 42 000, and the number of gemmae per shoot from 620 to 1900 (I, II, III; see also Jonsson and Söderström, 1988).

A. hellerianum has a circumboreal and continental distribution, with reported occurrences in the tropics (Schuster, 1966, Gradstein, 1993). It has been classified as a 'near threatened' species (NT) in Europe (ECCB web-page 28.9.2007), and also in Finland and Sweden, mainly as a consequence of habitat destruction (Gärdenfors, 2005; Rassi et al., 2001).
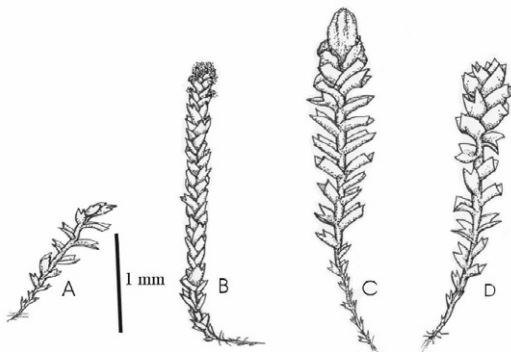

Figure 1. Different shoot types of *Anastrophyllum hellerianum*: A) sterile, B) gemmiparous, C) female sexual and D) male sexual.
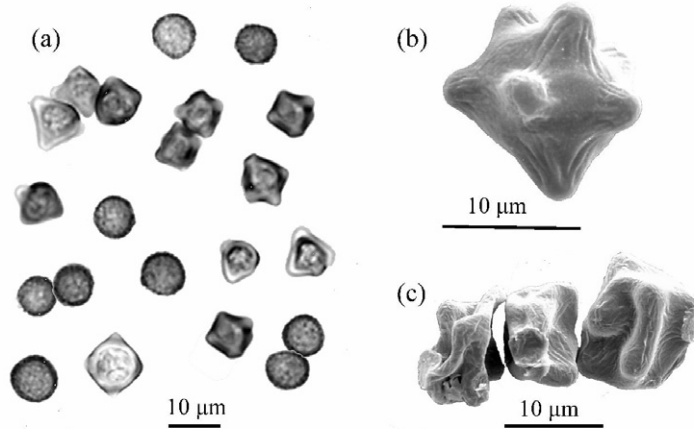
Figure 2. (a) A light microscope picture of the spores (round particles) and gemmae (cubic particles) of *Anastrophyllum hellerianum*. Scanning electronic microscope (SEM) picture of (b) a wet gemmae and (c) dry gemmae.

Figure 3. A decaying log in the Kotinen Nature Reserve area on which *Anastrophyllum hellerianum* forms large patches.

Trichocolea tomentella

Trichocolea tomentella (Ehrh.) Dumort (Trichocoleaceae) is a mesophytic, large, leafy, unisexual hepatic (Fig. 4, 5). Female inflorescences have been widely observed, but male inflorescences very rarely due to their tiny size and short-lived nature (V; Schuster, 1966; Paton, 1999). Sexual reproduction is extremely rare in Europe (Paton, 1999) and rare or occasional in North America (Schuster, 1966). Sporophytes have never been observed in the Baltic countries (Nijole Kalinauskaite, pers. comm.), only once in Scandinavia (in 1887, Hallingbäck 1998), rarely in the UK (Paton, 1999; Mark Hill, pers. comm.), but several times in a population studied in Canada (Fig. 5, Jean Faubert pers. comm). Spores are small (16–20 μm in diameter), and they are released in late autumn (Schuster, 1966; Jean Faubert, pers.comm.). No specialised asexual propagules occur in the species. Regeneration takes place clonally by fragmentation and branching (V).

T. tomentella is widespread in temperate climates in oceanic and suboceanic parts of Europe, North Africa and eastern North America and also in smaller areas in eastern Asia (Schuster, 1966). It is the only representative of the genus *Trichocolea* in Europe (Paton, 1999). It reaches its northern and northeastern distribution range in Finland, where its occurrence is restricted to shaded spring habitats in woodland. In more oceanic regions, the species occurs also in other kinds of moist habitats in pristine and semipristine woodland.

T. tomentella grows on stream banks, seepage areas, decaying logs and the base of ferns in streamside and spring forests, where it patchily forms more or less pure colonies of different sizes (Fig. 6, 7; Table 1 in paper V). Many of the present sites of *T. tomentella* are located in national parks and nature conservation areas. *T. tomentella* is classified as a threatened species in Finland as a consequence of habitat destruction, including spring exploitation (VU, Rassi et al., 2001), and as a rare species in Lithuania (Jukonienė 1996).
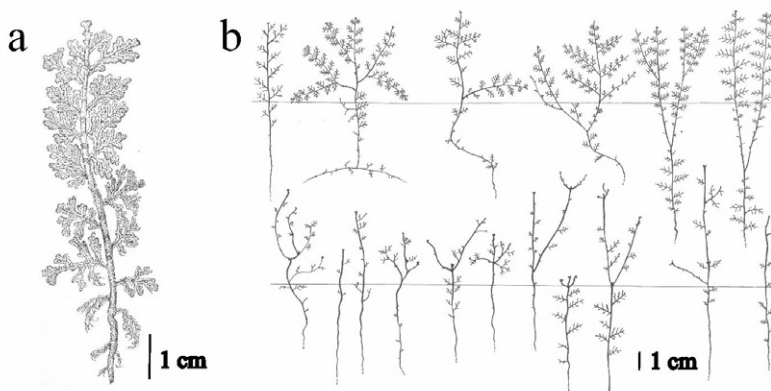

Figure 4. The unisexual *Trichocolea tomentella* and the branch densities of the shoots. Illustration by Nijole Kalinauskaitė.

Figure 5. A sporophyte of *Trichocolea tomentella* in *ex situ* and *in situ* conditions. Sexual reproduction is very rare in the species and was found only in one population in Canada. Photographer Jean Faubert.

Figure 6. A small, light-green patch (about 20 cm^2) of *Trichocolea tomentella* on a streambank in the Red Hill Wood in the United Kingdom.

Figure 7. The streamside habitat of *Trichocolea tomentella* in the Kurtuvėnai
Regional Park in Lithuania. The species forms large patches around stream.

2.2 Study sites and sampling

The study sites were located in the southern boreal vegetation zone in Finland and Canada, and in the temperate deciduous and mixed forest vegetation zones in Lithuania and England (see Ahti et al., 1968).

Study sites and sampling for Anastrophyllum hellerianum studies

In *A. hellerianum*, the field study and sampling were performed in the Lammi commune in Southern Finland in the Kotinen Nature Reserve (I, II, III). This forest area is an old virgin forest with natural canopy layers, a large amount of fallen decaying wood and dead standing trees. The forest area was protected in 1955, the size of the protected area was enlarged in 1987, and it was designated as a nature reserve in 1994. The vegetation of the area is dominated by spruce forest of the bilberry and low herb types (Påhlsson, 1995). The dominant trees are 80-150 years old Norway Spruce (*Picea abies*) mixed with old-growth birch (*Betula spp.*), aspen (*Populus tremula*) and Scots pine (*Pinus sylvestris)* up to 350 years old.

The study area belongs to the Evo recreational area, which is one of the remaining areas in the southern Finland where some old pristine forest patches still exist. Many restoration and conservation actions have been conducted in Evo to maintain biodiversity and achieve an ecological network for different organisms (Anonymous, 2004).

A. hellerianum is relatively common in the Kotinen Nature reserve. It occasionally forms large and uniform colonies on large fallen logs in the middle stages of decay. The material for the population structure studies were collected from 25 colonies of *A. hellerianum* originating from five decaying conifer logs (I, II). For the dispersal study (III), two large, isolated decaying conifer logs with a predominance of both sexual and asexual colonies of *A. hellerianum* were selected as source colonies for the study. The colony sizes of *A. hellerianum* were measured in each study (I, II, III).

Study sites and sampling for Trichocolea tomentella studies

The material for the genetic studies in *T. tomentella* were collected in four different regions from eighteen populations located in Finland, Lithuania, the United Kingdom and Canada, and for the population structure study from eight populations located in Finland and Lithuania (Fig. 8; Table 1 in paper V) (IV, V). Since only large or abundant populations of *T. tomentella* were selected for the population structure study, populations from the United Kingdom and Canada were excluded from the study. The study sites were the surroundings of springs and spring swamp spruce forests in Finland and mixed streamside forests in Lithuania, deciduous woodlands in the United Kingdom, and mixed *Thuja* forests and swamps in Canada (Table 1 in paper V).

For the genetic study, five populations of different sizes were selected within the region based on the information obtained from the databases of the environmental agencies in Finland and UK, and based on the preliminary study by N. Kalinauskaitė in Lithuania in 2001. Additionally, three Canadian populations were included in the genetic study after two years of the initiation of the study in 2003 (see, e.g., Pohjamo, 2003). The populations of *T. tomentella* typically consist of several patches of *T. tomentella*, and in a few cases the seepage areas in the surroundings of the springs were dominated mainly by the species. The materials for the genetic studies were collected from separate patches (distance > 0.5 m) to avoid collecting the same clone multiple times (IV, V). The sampling procedure and number of samples depended on the population characteristics and the number of separate colonies (V). For the population structure study, from five to six samples (patch of 10 cm x 10 cm) per population were collected from separate colonies of *T. tomentella* at regular intervals (V).
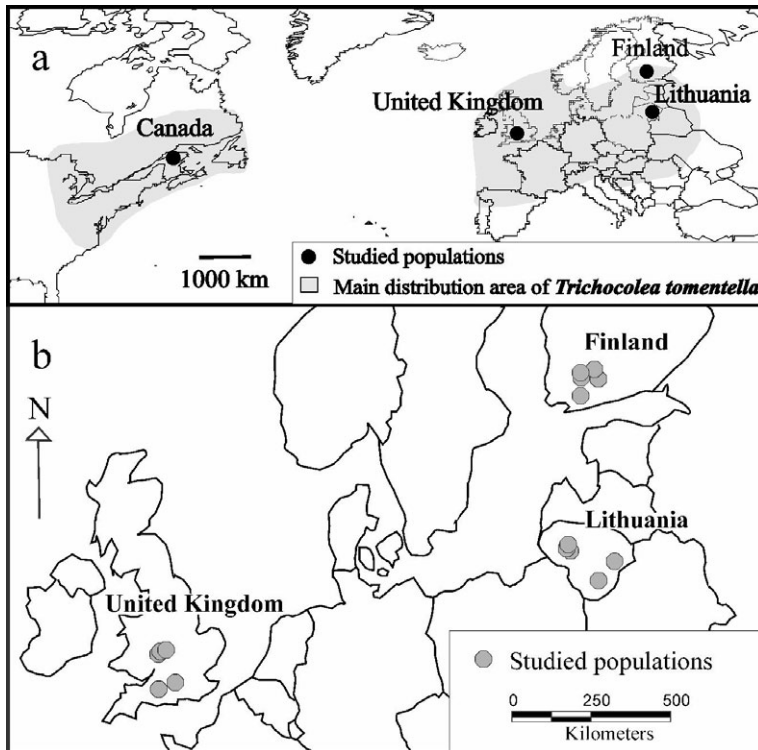

Figure 8. The main distribution area of *T. tomentella* and the study locations in four different regions. The samples for the genetic study were collected from five populations in Finland, Lithuania and the United Kingdom and from three populations in Canada, whereas the samples for the population structure originated from four populations in both Finland and Lithuania.

2.3 Demographic analyses

Shoot size, stage and shoot density

The size and stage traits of the individual shoots of *A. hellerianum* (I, II) and *T. tomentella* (V) were studied to identify the structure of the populations. Around 8000 *A. hellerianum* shoots and around 1400 *T. tomentella* shoots were included in the samples studied.

In *A. hellerianum,* the sporophyte density, the shoot length, the shoot stage classes (sterile, sex-expressing male, sex-expressing female and gemmiparous shoots; see Fig. 1) and entirely non-chlorophyllose dead shoots were recorded.

In *T. tomentella,* the occurrence of sporophytes, shoot length, width of the shoots, numbers of branches per shoot and entirely non-chlorophyllose dead shoots were recorded (V, see Fig. 4, 5). Because the male inflorescences of *T. tomentella* disappear very fast after collecting (Paton, 1999; N. Kalinauskaite, pers. obs.), the gender determination of the shoots was conducted only in a subset of the samples (V).

Shoot densities of the colonies were determined by counting each individual shoot in each sample square of 1 cm^2 in *A. hellerianum* (I, II, III) and in each sample square of 10 cm^2 in *T. tomentella* (V). No other bryophyte shoots were present in the colonies of *A. hellerianum* (I, II), whereas other bryophyte species were present as a minority in most colonies of *T. tomentella.* The other species were identified and their shoot numbers were counted (V).

Propagule counting and cultivation experiments

The estimations of the propagules produced per shoot and per colony give various possibilities to estimate different demographic parameters, such as reproductive effort and dispersal rates. In *A. hellerianum* studies, the average numbers of spores produced per newly matured capsule and gemmae present on individual shoots were counted using a haemacytometer method (I, II, III; method described in Laaka-Lindberg, 1999). Based on these calculations, and other population traits studied, the mean numbers of the propagules per total areas of sexual and asexual colonies were estimated (I, II, III).

The germination ability of bryophyte propagules has been widely tested using culture methods (e.g., Nehira, 1988). The germination rates are easily detectable in liquid media, while the establishment of the propagules is very difficult to observe directly in the field (Duckett et al., 2004). Also in *A. hellerianum*, the germination of the spores and gemmae were tested using liquid culture (II; Nehira, 1988).

Additionally, I conducted some preliminary cultivation tests using solidified media to follow the subsequent development of gemmae in *A. hellerianum*, and the subsequent growth of leaves and shoot fragments of different sizes (0.2 cm-8 cm) in *T. tomentella*

(unpublished data). For the cultivation tests, the shoots were first surface-sterilised for 0.5-1.5 min in 1.0 % sodium hypochlorite containing a drop of detergent, followed by rinsing with sterile distilled water for 3-5 min. The sterilised gemmae and shoot fragments of *T. tomentella* were placed in sterilised petri dishes and glass jars with a solution of agar and half Knop's solution (Nehira, 1988), and incubated at a temperature of about 22° C under diffuse light for six months. The survival of the material was checked weekly.

Statistical testing of demographic traits

The population structures of *A. hellerianum* and *T. tomentella* were analysed by standard parametric or nonparametric statistical methods (see details in papers I, II, III, V). The different statistical tests were performed using SAS, Systat and SPSS software 1) to check the normality of the variables {the Kolmogorov-Smirnov test (V), the Wilk-Shapiro-normality test (I, II)}, 2) to study differences between the traits {ANOVA (I, II), the mixed effect model (II), the Kruskall Wallis-test (V), a binomial test (II), a χ^2 – goodness-of – fit test (II), a pairwise t-test (I, II), the Mann Whitney U-test (V)}, and 3) to study the relationships between different factors {the linear regression (I), the correlation tests (I, V)}.

2.4 Studies on dispersal and gene flow

Minimum sperm dispersal range

The minimum sperm dispersal range of *A. hellerianum* was measured under a dissecting microscope between fertilised and unfertilised females and the closest male plant (II), following the method described in Reynolds (1980).

Direct dispersal experiments (ecological approach)

The dispersal rates of the gemmae and spores of *A. hellerianum* were studied in two field experiments in the forest sites in 2001, and in a controlled experiment without the potential background dispersal on the grassy lawn of the Lammi Biological Station, Helsinki University, in 2002 (III). *A. hellerianum* is an ideal model organism for studying the dispersal rates of gemmae and spores, since both propagule types are of equal size and allow easy identification (Fig. 2).

Propagules were trapped with microscope slides covered with petroleum jelly placed on the ground level around the study plots in eight or six directions at distances up to 10 metres from the source colonies (field experiment design in Figure 2 in paper III). The traps were changed once a day and propagules were scored on a 4 cm^2 area of each slide. The dispersal patterns of the spores and gemmae were analysed using a log-linear model (see Sundberg, 2005), and the cumulative number of propagules that were deposited at

various distances up to 10 m from the source was calculated using the estimated dispersal kernels (III).

During the field experiment, the weather was cloudy and dry during four days and rainy during three days, which allowed us to examine the effects of weather conditions on the dispersal rate of the propagules. The average numbers of gemmae and spores captured daily at each distance were fitted by log-linear regression, in which a variable indicating the type of the day (rainy or dry) was included both in the total amount of propagule deposition and in the strength of distance dependence.

Indirect dispersal studies (genetic approach)

The rarity of sexual reproduction, the absence of specialised propagules and the difficulties of direct dispersal observations of shoot fragments in the field and within a restricted timeframe, led to the use of indirect genetic methods to estimate the dispersal potential of *T. tomentella*.

Three different genetic methods were employed in the genetic study on *T. tomentella*: the sequencing of cpDNA and the ISSR (inter-simple sequence repeat) and RAPD (randomly amplified polymorphic DNA) fingerprinting methods (IV, V). For the genetic analyses, total DNA was extracted from the samples, i.e., the tip of the shoots, using DNeasy Plant Mini Kit (QIAGEN, Inc, Hilden, Germany).

In order to detect chloroplast haplotypes and to test the suitability of cpDNA markers for studies involving an analysis on the possible geographical structuring of cpDNA variation, sequencing of noncoding regions of cpDNA was performed for *T. tomentella* originating from Finland, Lithuania and England (IV). The following regions of cpDNA were sequenced and analysed for possible intraspecific variation: atpB-rbcL intergenic spacer, accD-psaI intergenic spacer, trnT-trnL intergenic spacer, trnL-trnF intergenic spacer, and trnL intron (Table 2 in paper IV). The atpB-rbcL and accD-psaI spacers were examined here for the first time in any hepatic species. Universal primers were tested and found suitable for the amplification of atpB-rbcL intergenic spacer (Chiang et al., 1998) and accD-psaI intergenic spacer (Small et al., 1998). The whole trnT-trnL-trnF area was obtained using one primer pair. The reverse primer used was universal (Taberlet et al., 1991) but a new forward primer was developed based on the complete chloroplast genome available for the hepatic *Marchantia polymorpha* (accession number NC_001319).

As a consequence of the lack of variation within the sequenced cpDNA regions and due to the need to use markers that presumably target highly variable genomic regions, the DNA fingerprinting studies using the ISSR and the RAPD methods were employed to reveal the genetic structure of the populations (see details in paper V). At the initiation of the study in 2001, the RAPD and ISSR methods were commonly used in studies on vascular plant because of their cost effectiveness compared to, e.g., more expensive, laborious and time-consuming microsatellite method. The DNA fingerprinting methods

used have several advantages, such as technical simplicity, no need for prior sequence knowledge, high resolution and cost effectiveness (Weising et al., 2005). Moreover, only small amounts of material are needed for the fingerprinting analysis, thus also facilitating research involving threatened species (Lowe et al., 2004). On the other hand, the dominant nature of the markers and low reproducibility are considered to be drawbacks of the RAPD method (Lowe et al., 2004; Weising et al., 2005). Both ISSR and RAPD primers amplify the regions of the whole genome. The DNA regions amplified by the ISSR markers, however, commonly represent microsatellite hotspots, which are known to be highly polymorphic (Korpelainen et al., 2007; Provan and Wilson, 2007), but randomly generated primers used in the RAPD method represent more conservative segments of the whole genome (Conner and Hartl, 2004). The ISSR markers have not been employed in any bryophyte study published before 2002 (see Hassel and Gunnarsson, 2003).

In total, 230 samples of individual shoots originating from eighteen population in four different regions were analysed in the ISSR-based study, and 324 samples originating from fifteen populations from three regions in Europe were analysed in the RAPD-based study (V). The locations of all sampled shoots within each population were recorded, and the pairwise distances between shoots were determined (V).

In paper V, the standard diversity indices were calculated to reveal the levels of genetic diversity using the ARLEQUIN Software (Excoffier et al. 2005). The relationships between the extent of genetic diversity and population sizes were analysed by the Pearson correlation test. The differences in the level of genetic diversity in populations with different isolation degrees were analysed by the Kruskall-Wallis test using the SPSS statistical program.

Only the ISSR-based data was used for the AMOVA, the Mantel test and the PCA analysis, because the RAPD markers did not provide adequate polymorphism and resolution. The estimations of the gene flow among populations within regions and among individuals within populations were analysed by molecular variance (AMOVA) and the Mantel test using the ARLEQUIN Software (V).

A Principal Components Analysis (PCA) was conducted to reveal the genetic similarities of the individuals in two narrow geographical areas using the SYSTAT program (V). The significances of the genetic similarities were tested by the Mann Whitney U-test.

3 Results and discussion

3.1 Demographic traits

Developmental stage, size and mortality of the shoots in A. hellerianum (I, II)

In *A. hellerianum*, the mean proportions of different developmental stages, sterile, gemmiparous, female and male shoots were 59.0 %, 33.7 %, 4.1 % and 3.2 %, respectively (I). Methodological as well as ecological factors affect the observed distribution of the shoot-stage classes. In the study, sampling was conducted once during a growth season, and the fate of the individual shoots were not followed further over their life span (I). The shoots are likely to change their mode from sterile to gametangia-bearing or gemmiparous when reaching the threshold age and size for reproductive maturity.

In *A. hellerianum,* the size distribution was related to the developmental stages of the shoots (Table 1 and Figure 2 in paper I). The initiation of the reproductive phase of the life cycle, whether sexual or asexual, is affected by the shoot size in *A. hellerianum* (I). The sterile shoots were significantly shorter than the other shoot types, giving support to their designation as juveniles. The shoots with antheridia were on average shorter than the shoots with archegonia, and the threshold size of the shoots bearing gametangia was lower in males than in females. Also, in thalloid hepatics, male plants have been observed to be considerably smaller than females (Crum, 2001), but the opposite is true in the leafy hepatic *Lophozia silvicola* (Laaka-Lindberg, 2001). The production of male sex organs apparently requires fewer resources than does the production of female sex organs (see Laaka-Lindberg, 2001).

Early sexual maturity has generally been considered advantageous, especially in unpredictable habitats (Silvertown and Lovett Doust, 1993). It increases the chance to reproduce and decreases the generation time, thus facilitating the simultaneous existence of many generations in the region (Stearns and Hoekstra, 2005). Some evidence of the required threshold size at first reproduction can be extracted from the proportions among the shoot-size classes for the gemmiparous and sexual shoots in *A. hellerianum* (I). Gemmae are produced occasionally on shoots shorter than 1 mm, indicating a low energetic cost of asexual reproduction, and possibly early maturity (I). The lower age of the first reproduction among asexually reproducing individuals, compared to sex-expressing individuals, promotes persistence and growth in local populations, as detected in the moss *Tetraphis pellucida* (Kimmerer, 1991). By contrast, recruitment of genetically variable offspring resulting in sexual reproduction at a later stage may enhance the long-term survival of the species in a region by increasing the genetic variability of the populations.

In bryophytes, the formation of gametangia has been shown to be affected by several abiotic and biotic factors (e.g., Chopra and Rahbar, 1982; Kimmerer, 1991; Sagmo-Solli et al., 2000). In *A. hellerianum*, sex-expressing shoots, either females or males or both, were present in most of the colonies occupying favourable substrate, which indicates that

other than microclimatic and substrate-related factors induce or inhibit the development of gametangia (II). Female-biased sex expression has been widely detected among unisexual hepatics and mosses at different geographical scales (Bisang and Hedenäs, 2005). Also in *A. hellerianum*, the expressed sex ratio was skewed toward the female sex (1:1.3) in spite of a higher mortality rate among females than males (II). Similarly, a female-biased sex ratio has been observed in a moss population despite the high cost of sporophyte production (Rydgren and Økland, 2002), but also contrastingly as a consequence of lower cost of reproduction in females (Stark et al., 2000).

The proportion of the dead shoots was 4.6 % in *A. hellerianum* (I). The proportions of dead shoots differed between the developmental stages (Table 2 in paper I). The fraction of dead shoots was extremely low among gemmiparous shoots in *A. hellerianum*, (0.02 %), and no trade-off was detected between the gemma production and shoot survival (II). By contrast, sexual reproduction in *A. hellerianum* seems to involve a trade-off between the formation of gametangia and sporophytes and the future survival of the sex-expressing shoots. The fraction of dead shoots was much higher among the sex-expressing shoots than among other shoots (II). Overall, the results suggest that a lower investment is required for asexual reproduction than for sexual reproduction (II).

Female shoot mortality was much higher than that found in males, which suggests a higher cost of reproduction in females (II). The effects of sporophyte production in bryophytes have been observed to include also a reduced size of vegetative offshoots, reduced branching (Rydgren and Økland, 2003) and reduced production of new reproductive organs (Bisang and Ehrlen, 2002). On the other hand, the mortality of the shoots increased with increasing shoot size among all developmental stages indicating also the occurrence of senescence-related mortality in *A. hellerianum* (I).

Developmental stage, shoot size and mortality of the shoots in *T. tomentella* (V)

In *T. tomentella*, female shoots occurred in 83 % of the populations, but male shoots were detected only in one Lithuanian population, where no females occurred (Table I in paper V). It might be possible that males were overlooked during the study and, consequently, the frequencies of the populations with male-expressing shoots are underestimated. However, I was able to check in detail the occurrence of male gametangia using samples (N=110) collected from the populations located in the United Kingdom for the study V. The majority of the shoots of those samples (62.7 %) did not express sex, 27.3 % were identified as females and no males were found (the results are published in Bisang and Hedenäs, 2005). Nevertheless, the absence of sporophytes in the majority of the populations (17/18) may confirm failures in the production of male gametangia in the populations of *T. tomentella*. Moreover, the sex-expression in *T. tomentella* seems to be restricted in undisturbed habitats, and only sterile shoots occurred in most disturbed habitats.

The length of the shoots varied widely among colonies in *T. tomentella* (Figure 4a in paper V). The average lengths of the shoots and the green parts of the shoots were

significantly greater in Finland than in Lithuania (Table 5 in paper V). Significant differences in shoot length were detected also among Finnish populations, but not among Lithuanian populations.

The proportion of the dead shoots was 0.3 % in the studied well-established colonies of *T. tomentella* (V). This may indicate the long life span of the clones and low turnover rate of the colonies, especially under favourable microclimatic conditions, as in the populations studied here. However, mortality can vary between years depending on the prevailing environmental conditions. Especially summer droughts are assumed to increase the mortality rates and cause population fluctuations in forest species (Archaux and Wolters, 2006). *T. tomentella* also tolerates only to a limited degree litter and detritus accumulation (Paton, 1999).

Effects of shoot density in A. hellerianum and in T. tomentella (I, V)

Shoot density varied widely among the logs and colonies in *A. hellerianum* (I, II). Similarly, the shoot densities of *T. tomentella* and the other bryophyte species varied considerably among the populations (Table 4 in paper V). Differences in the shoot densities among sites can be explained by microenvironmental variation between sites (e.g., moisture, humidity and the activity of the micro-organisms), differences in the history of disturbances in the colonies (Pedersen et al., 2001) and differences within colony dynamics (e.g., Kimmerer, 1991; Økland and Økland, 1996).

In bryophytes, positive associations between shoot growth and shoot density are assumed to involve improved microclimatic and moisture conditions for growth within colonies (Proctor, 1982; Bates, 1988; Van der Hoeven and During, 1997; Bergamini et al., 2001), while negative associations involve competition for space, light and resources (Rydin, 1997; Pedersen et al., 2001).

In *A. hellerianum*, the shoot length declined with increasing shoot density (Figures 3 and 4 in paper I). At high densities, shoots were smaller, presumably due to the lower capacity of shoots to grow as compared to sparse colonies. In some studies on bryophytes, a negative density-dependence has been reported between shoot size and density (Clymo, 1970; Collins, 1976). The shoot density had no relationship to the proportions of sterile, gemmiparous or sex-expressing shoots in the colonies of *A. hellerianum*, possibly indicating the effect of another type of regulation than density on the reproduction (I). By contrast, in the moss *Tetraphis pellucida*, Kimmerer (1991) observed a significant relationship between shoot density and reproductive mode. Asexual reproduction predominated at low densities and the production of sexual shoots and sporophytes increased with shoot density. Also, a density threshold would be expected to be involved in a successful fertilisation (Kimmerer, 1991). In *A. hellerianum*, the shoot densities were highly variable, but no differences were detected in density between colonies with and without sporophytes (II).

In *T. tomentella,* shoot density had no relationship to shoot length, but the width of the shoots decreased with increasing density (V). In a forest floor species, *Hylocomium splendens*, Økland and Økland (1996) detected that the density of the bryophyte layer was positively related to shoot size, but branching was reduced at high densities. The shoot density of *T. tomentella* was not correlated with branch densities, presumably due to the benefits of improved moisture conditions and competition capacity (V). Effective ramet production is likely to inhibit the successful colonisation of other bryophyte species within colonies, because the increased shoot density of *T. tomentella* seems to decrease the recruitment of other bryophyte shoots in the colonies (Figure 6 in paper V).

Sexual and asexual reproduction and reproductive output in A. hellerianum and T. tomentella (I, II, III, V)

The relative importance of asexual and sexual reproduction depends on the probability of a successful recruitment of the propagules and detached shoot fragments. Only one shoot is generally produced by a single protonema, originating from a spore or gemma in hepatics (Crum, 2001). This verifies that a large number of propagules and/or detached shoot fragments are needed for successful establishment, especially in patchily distributed suitable substrate.

In *A. hellerianum,* asexual reproduction occurred in every colony type, i.e., nonsexual, unisexual, bisexual without sporophytes and bisexual with sporophytes (Table 1 in paper II), confirming the predicted prevalence of asexual reproduction. By contrast, sexual reproduction was observed only in 12 % of the colonies studied (II). Sexual reproduction was promoted by several colony conditions: medium shoot density, a high proportion of sex-expressing shoots, an even sex ratio and very short distances between individuals representing opposite sexes (II). Moreover, rainy weather conditions were found to increase the probability of the fertilisation success (III). Sexual reproduction in bryophytes also requires specific substrate quality (Laaka-Lindberg, 2000; Laaka-Lindberg et al., 2005), colony structure (I, II; Bowker et al., 2000; Rydgren et al., 2006) and climatic conditions (III; Laaka-Lindberg, 2005).

In bryophytes, the asexual propagules are generally produced in lower numbers per shoot than are spores (During, 1979). The same observation was confirmed in *A. hellerianum*. The average estimate of spores produced per individual shoot (42 000) exceeded that found for gemmae (620-1900) (II, III). Nevertheless, the reproductive output by both spores and gemmae in the colonies is considerable in *A. hellerianum* (I, II, III).

The asexual propagules of bryophytes are often larger than spores and are assumed to facilitate faster germination (e.g., Crum 2001). However, in *A. hellerianum,* gemmae are small and of the same size as spores. Based on the cultivation experiment, gemmae germinated faster than spores indicating their potential for more effective colonisation in *A. hellerianum* (II). Therefore, other factors than propagule size, such as the time of sampling, may account for the differences in germination (Laaka-Lindberg 1999).

In *T. tomentella,* sexual reproduction is extremely rare, presumably due to the spatial separation of sexes and failure in the formation of male gametangia (V). Consequently, the species relies mainly on population persistence by vegetative regeneration by branching and detached vegetative shoot fragments (V). The proportions of the shoots carrying 1-2, 3-4 and 5-9 branches per shoot were 36.6 %, 7.3 % and 1.2 %, respectively. The relatively high production of branches on the shoots indicates effective ramet production resulting in high shoot densities within the colonies and local crowding. The production of individual daughter ramets is a consequence of the continuous decay process of the shoot bases, which in turn separates the branches/ramets from their parents. Effective ramet production has several advantages, e.g., increases in genet size, in the capture of space and resources and in resource storage. Moreover, the cultivation test confirmed that detached ramets and shoot fragments taller than 1.5 cm, can easily continue the growth (unpublished data, M. Pohjamo) indicating that detached vegetative fragments can contribute towards local population growth (V; see Økland, 1995).

3.2 Dispersal and gene flow

Dispersal and gene flow can be studied by direct and indirect methods (Conner and Hartl, 2004). Direct empirical dispersal studies involve observations of diaspore dispersal obtained by, e.g., trapping experiments (e.g., Sundberg, 2005), mark-recapture methods and studying dispersal vectors (e.g., Kimmerer and Young, 1995). The advantage of the direct dispersal method is its reliability, because the actual dispersal is observed. However, the direct methods have some drawbacks (Conner and Hartl, 2004), such as 1) the studies typically encompass a short timescale, whereas dispersal can be episodic and occur only rarely, 2) long-distance dispersal is difficult to study, 3) it might be difficult to distinguish the dispersal agents of a studied species from the agents of other species, and 4) the studies are time-consuming.

By contrast, indirect dispersal methods include *post hoc* methods applying knowledge to investigate gene flow (Korpelainen et al., 2005 for review for bryophytes), spatial genetic structure (Snäll et al., 2004), the spatial distribution of the species with differing reproductive modes (Kimmerer, 1994, Hedenås et al., 2003, Snäll et al., 2003, Laaka-Lindberg et al., 2006), and the colonisation of new uncolonised substrates or areas (Miller and McDaniel, 2004).

In this research, the direct method was used to estimate the dispersal distances by trapping asexual and sexual propagules at various distances from the source in *A. hellerianum* (III), and the indirect methods was used by employing three different genetic methods for revealing the genetic structure of the populations in *T. tomentella* (IV, V).

Studies on the dispersal ranges of bryophyte propagules by direct means, i.e., trapping the propagules at different distances, are usually conducted using a fine-scale system, up to 2.5 to 15 meters from the source colonies, and are mainly concentrated on sexual propagules (e.g., Söderström and Jonsson, 1989; Kimmerer, 1991; Sundberg, 2005 and references therein). The literature survey indicated that only few studies have compared

the dispersal potential of sexual and asexual propagules in a bryophyte species (III; Kimmerer and Young, 1995), and no other study than III, as far as I know, directly compares the dispersal potential of the different propagule types of the same size within a species.

Within colony propagule deposition in A. hellerianum (III)

In *A. hellerianum*, the rate of deposited gemmae was considerably larger than the rate of deposited spores within the source colonies, although only a very small proportion of the standing gemma population (< 1 %) were deposited, whereas a fair proportion of released spores landed within the source colonies (3.6-10.5 %) (Table 3 in paper III). The spore deposition is in accordance with a previous study on six *Sphagnum* species, in which 5.2– 16 % of the spores were deposited within the source colonies (Sundberg, 2005). Such a very localised propagule deposition is expected to facilitate the persistence of the local patch, as, e.g., the decomposition process of the wood creates disturbances that need to be balanced by continuous recolonisation. In addition, the benefits of short-range spore dispersal include the local recruitment of new genotypes as a result of recombination, although its significance may be reduced by matings with close relatives (Mishler, 1988).

Rainy conditions were discovered to considerably increase the release rate of gemmae and their deposition within the source colonies (Table 4 in paper III). Wet conditions are likely to provide a favourable environment for propagule germination and establishment. Because gemmae germinated faster than spores in culture (II) greater establishment efficiency is expected among asexual propagules on the logs (see also Kimmerer 1991). Furthermore, asexual gemmae are genetically identical to their parents and siblings and, thus, presumably well adapted to the local conditions. Because gemma production can occur under suboptimal conditions and in a wider area than the occasional spore production (I, II; Laaka-Lindberg et al., 2005), asexual reproduction is likely to be the most important reproductive mode within the patches in *A. hellerianum*.

Dispersal pattern of propagules in A. hellerianum (III)

The deposition of the *A. hellerianum* spores lacked strong distance-dependence within the first 10 m in the forest sites and in open habitat in Lammi (Figure 3 in paper III). The stronger distance-dependence detected by Sundberg (2005) in *Sphagnum* spores may be a consequence of larger spore sizes and varying spore release mechanisms, and differences in the structure of the landscape and variation in climatic conditions (see also Tackenberg et al., 2003; Ozinga et al., 2004). More than half of the spores of *A. hellerianum* were estimated to disperse further than 10 metres, and some 90 % further than 2 metres from the source. The results are in accordance with previous bryophyte studies on spore dispersal, which have shown that, although the deposition density is highest very close to the source, a considerable proportion of spores disperse further than a few meters (e.g. Söderström and Jonsson, 1989; Miles and Longton, 1992; Sundberg, 2005).

The distance-dependence for the gemmae in *A. hellerianum* was weakest within the forest sites and strongest in the open habitat in Lammi (Figure 3 in paper III). The relatively strong distance dependence in gemma dispersal observed in the open habitat is probably related to the lack of a special release mechanism, where wind is likely to be the most important dispersal vector. However, long-distance dispersal by air currents is potentially enhanced by the very minute size of the gemmae and the location of the gemmae on the tips of the erected-gemmiparous shoots (I).

In both forest sites, the dispersal patterns varied between spores and gemmae (Figure 3 in paper III). Gemmae appeared not to be significantly affected by distances of up to 10 metres from the source (III). However, spore dispersal rates were more affected by the distance from the source. The differences in dispersal patterns between spores and gemmae in the forest sites can be a consequence of spatial and time constraints of sexual reproduction compared with the wider distribution achieved through asexual reproduction. Moreover, gemma release can be extended practically continuously, although its intensity appears to vary depending on prevailing climatic conditions. Rainy conditions facilitate the release of huge numbers of gemmae dispersed at least up to 10 metres, whereas considerably less gemmae seem to be released during the dry season (Figure 4 in paper III). By contrast, a fine-scale study on the dispersal ranges of the spores and gemmae (diameter 40 µm, Smith, 1996) of the epixylic moss *Tetraphis pellucida* suggested that the gemmae disperse only very short distances (Kimmerer, 1991). The difference in the dispersal patterns of gemmae between *A. hellerianum* and *T. pellucida* is likely to be related to the large difference in propagule size.

A previous spatial distribution study in three epixylic hepatics (Laaka-Lindberg et al., 2006), including *A. hellerianum*, conducted in the same forest area as studies I, II and III, support the efficient colonisation ability of gemmae at the forest-stand scale. In the studied area, asexual reproduction predominated in the colonies of *A. hellerianum* and sexual reproduction occurred only in 2.7 % of the colonies. Based on the study by Laaka-Lindberg et al. (2006), the colonies of *A. hellerianum* were closer than expected at distances up to 16 metres and randomly distributed between 16 metres and 18 metres. Over greater distances, the infrequent spore production combined with effective asexual reproduction and clonal growth (I, II, III) produced an over-dispersed pattern. The findings confirmed that the colonisations of *A. hellerianum* are aggregated relatively close to the parental colonies, similar to the pattern of propagule rains (III), but the effective colonisations on all potential, available patches can be expected up to, at least, 26 metres away.

Genetic diversity in T. tomentella (IV, V)

No intraspecific variation was detected when sequencing five noncoding cpDNA regions for a subset of geographically distant samples of *T. tomentella* originating from different geographical regions, although noncoding DNA generally evolves relatively rapidly when compared with coding DNA (IV). Consequently, the studied cpDNA regions are not suitable for population genetic studies in Trichocolea, at least within Europe (IV).

Instead, they may be utilised in investigations on inter-specific or intra-generic levels, as tentatively indicated by the sequence identity obtained for the trnL intron area for the species pair *T. tomentella* and *T. mollissima* (accession number AF071847), equalling 95 %.

The results obtained by DNA fingerprinting methods, which target more variable parts of the whole genome, revealed that the predominantly asexually reproducing populations combined with the restricted gene flow of *T. tomentella* are polymorphic and contain a fair amount of genetic variation (V). This is consistent with several studies conducted using different population genetic methods in many bryophyte populations where sexual reproduction is absent or rare (Shaw, 2000; Van der Velde et al., 2001a,b; Cronberg, 2002; Spagnuolo et al., 2007a; but see Gunnarsson et al., 2005).

The ISSR markers detected more genetic diversity within populations (H_S 0.110-0.283) than did the RAPD markers (H_S 0.039-0.172). Similar results have also been observed in other studies (e.g., Esselman et al 1999). In *T. tomentella*, genetic variability may have arisen by somatic mutations from a limited number of founders in conditions in which the gene flow among populations have been highly restricted. Mutations are likely to explain the high degree of ISSR-based variability, because the DNA regions amplified by the ISSR markers commonly represent highly polymorphic microsatellite hotspots (Korpelainen et al., 2007; Provan and Wilson, 2007). The lower degree of genetic variability detected by RAPD markers is likely a consequence of these markers targeting more conservative segments of the genome (see Conner and Hartl, 2004).

In *T. tomentella*, the naturally small and isolated populations seem to maintain about similar levels of both ISSR- and RAPD-based genetic diversity as compared to the larger populations. Comparably, Gunnarsson et al. (2005) did not detect a relationship between population size and the amount of the ISSR-based genetic variation in the mainly asexually reproducing peat moss *Sphagnum angermanicum*.

In *T. tomentella,* the amount of genetic diversity was higher among the Canadian populations when compared to the European populations. This is consistent with the results of Thingsgaard (2001), who studied the genetic diversity of the rarely sexually reproducing peat moss, *Sphagnum affine,* in Europe and North America. The differences in geographical features between continents and regions affecting colonisation history may explain the differing levels of genetic diversity (Hewitt, 2000). In *T. tomentella*, as expected, the lowest level of genetic diversity was detected among the Finnish populations occurring at the northern margin of the species' distribution. This is probably a consequence of the lower number of founders colonising the northern populations as compared to more southern populations (see Cronberg, 2000; Thingsgaard, 2001).

Gene flow in Trichocolea tomentella (V)

The estimates of genetic differentiation among populations (F_{ST}) reflect the amount of gene flow between populations (Whitlock and McCauley, 1999). Great differentiation

observed among the populations in *T. tomentella* presumably indicates restricted historic gene flow.

The northern populations in Finland were the most differentiated ones, as a consequence of a possible loss of sexuality in the marginal populations and the founder effects (see Eckert, 2002). By contrast, genetic differentiation was expected to be lowest among the Canadian populations because of the occurrence of sexual reproduction in the region (J. Faubert, pers. comm.). However, the great level of differentiation observed there suggests dispersal limitations of spores, although several thousands of small, air-borne spores are typically produced by spore capsules in bryophytes (Crum, 2001). In addition, recruitment from spores is likely a rare event, especially in habitat-limited species (see Sundberg and Rydin, 2002), like *T. tomentella*.

The nature of differentiation and gene flow is assumed to be influenced by the distance between populations, as detected in many studies conducted on bryophytes (Korpelainen et al., 2005 for review). Within a relatively small spatial area, increased levels of gene flow and less differentiation are expected (Conner and Hartl, 2004). However, within a small spatial area of 4 km in Lithuania, individuals originating from three (sub)populations were genetically assigned to their own populations, indicating limited dispersal and only short-range recruitment. This is in accordance with a study by Spagnuolo et al. (2007a), who found that local populations form genetically clear clusters in the Mediterranean seashore populations of the asexually reproducing moss, *Pleurochaete squarrosa*.

By contrast, within a distance of 2 km in the UK, the individuals originating from three (sub)populations were genetically more similar. However, surprisingly low levels of genetic similarities among individuals were observed between the two closest populations (distance 1 km) located along the same tributary stream, indicating dispersal limitations even on such a small scale. By contrast, higher levels of genetic similarities were detected between the two distant populations not located along the same tributary (see Figure 3 in paper V), which can be explained by the founders of the populations having a similar gene pool and by gene flow mediating the dispersal of spores or vegetative fragments.

On a population scale, random colonisation rather than isolation by distance was detected among the individuals of *T. tomentella*. Random colonisation is likely a consequence of the function of dispersal vectors, such as water, mammals or birds, distributing detached shoot fragments and ramets to various distances from the source (Laaka-Lindberg et al., 2003). The successful recruitment of the detached vegetative fragments is likely related to fine-grain disturbances, such as water level fluctuations of streams, trampling or falling twigs and trees, that create gaps for colonisation (see Muotka and Virtanen, 1995; Cronberg, 2002). The high survival rate of ramets and shoot fragments larger than 1.5 cm in *T. tomentella* detected in the cultivation experiment, conducted for vegetative parts of differing sizes (M. Pohjamo, unpublished data), may confirm that detached ramets and large-enough shoot fragments may be easily recruited in favourable microhabitats.

4 Conclusions and implications for conservation

The relative importance of life history traits and population size for species survival are central questions in conservation biology (e.g., Hanski 1999; Frankham et al. 2003). Quantitative data on reproductive and dispersal traits are essential for understanding the importance of the different parts of the life cycle in bryophyte species, especially since the reliance on different reproductive strategies within species may differ between populations and regions (e.g., Longton and Schuster, 1983; Cronberg, 2002). Detailed studies on the life history traits in relation to genetic structure of the species facilitate the design of appropriate conservation and restoration initiatives for the species (e.g., Söderström et al., 1992; Hallingbäck and Hodgetts, 2000; Laaka-Lindberg and Pohjamo, 2001). Based on my findings, the reproductive and dispersal strategies of two substrate specialist species studied in this thesis vary depending on the adaptation to the prevailing habitat. The results may allow generalisations for conservation strategies in declining and threatened bryophytes sharing similar life history traits, although several other factors than habitat specialisation can affect the population dynamics of species.

4.1 Species adapted to unstable habitats

The life history traits and population dynamics of the minute epixylic species, *Anastrophyllum hellerianum,* mainly inhabiting large decaying logs, is consistent with the evolutionary strategy characteristic of species following metapopulation dynamics (see Söderström and Herben, 1997; Hanski, 2001a, b; Snäll et al., 2003, 2005). The high reproductive and dispersal potential of *A. hellerianum* can counteract local extinctions of populations, in spite of high seasonal amplitude of population fluctuations during dry summers. The combination of occasional spore production and practically continuous, massive gemma production facilitates dispersal both on a local scale and over long distances, and it compensates for the great propagule losses that take place preceding successful establishment at suitable sites. However, the long-distance dispersal and establishment probability of spores may be restricted because of environmental and biological limitations linked to the low success of sexual reproduction. In addition, a relationship between the long-term benefits (e.g., long-distance dispersal enabling gene flow) and costs (high mortality of female shoots) of sexual reproduction in *A. hellerianum* was detected. By contrast, the low mortality of gemmiparous shoots suggests the low cost of asexual reproduction. Because gemma production can occur in less favourable conditions than spore production, asexual reproduction dominates over sexual reproduction in the local dynamics of *A. hellerianum*. Colonisations of gemmae seem to be frequent at ranges of up to 30 meters from the source (Laaka-Lindberg et al., 2006).

The substratum limitation is likely to be the main constraint for the long-term survival of *A. hellerianum* populations, primarily in intensively managed forests. Population viability (including occurrence of sexual reproduction) is largely dependent on a dense network of large decaying logs with middle decay stages (Söderström, 1988a, b; Laaka-Lindberg et al., 2005, 2006). However, the local colonies of *A. hellerianum* seem to thrive by asexual reproduction on different types of woody substratum (Laaka-Lindberg et al 2005). Yet, in

the longer term, the substratum limitation is ultimately likely to restrict the population size and cause local extinctions. These extinctions are most likely to occur in small-sized remnant populations of the woodland key habitats, due to extinction debt (Hanski, 2000; Jonsson et al., 2005; Pykälä, 2006). Moreover, edge effect is likely to accelerate the suppression of population sizes in small habitat patches. The edge effect has been estimated to restrict the distribution of epixylic hepatic species up to 50 meters within the edge of forest patches (Moen and Jonsson, 2003; but see Fenton and Frego 2005).

Contrastingly, larger forest fragments with more natural disturbance dynamics, to which the species is adapted, are pivotal to species survival (see, Hanski, 2000; Horne et al., 2006). Effective restoration can be undertaken to improve the conditions of woodland key habitats and the surrounding matrix for epixylic hepatic species by maintaining continuity in the form of large decaying logs and other woody debris close to the remaining populations. Retaining decaying log buffer zones and increasing habitat connectivity should prove to be a simple and effective conservation strategy (Edman et al., 2004; Jonsson et al., 2005). The restoration actions in the surrounding matrix of viable core populations of *A. hellerianum* are likely to be the most cost-effective, because (re-)colonisations of new suitable habitats are likely to increase the population viability by increasing population size (Jonsson et al., 2005). That, in turn, decreases the harmful effects of environmental stochasticity.

4.2 Species adapted to stable habitats

The life history traits of *T. tomentella* follow the traits typical for species inhabiting stable habitats with a low turnover rate of remnant populations (During, 1979; Muotka and Virtanen, 1995; Eriksson, 1996). *T. tomentella* mainly invests in population persistence by effective clonal growth via the formation of independent ramets and in competitive ability by the production of large shoots with a low mortality (see During, 1979), and considerably less in sexual reproduction, gametangia formation and dispersal potential. A high storage capacity, relatively high competitive ability and low-amplitude of population fluctuations without extrinsic effects seem to counteract the local extinctions of *T. tomentella* populations, which is likely to have a slow response to the habitat fragmentation (see e.g., Ovaskainen and Hanski, 2002; Henle et al., 2004).

The presence of relatively high levels of genetic variation in the populations of *T. tomentella*, regardless of the population size, highlights the value of even small populations as important sources of genetic diversity for the species. Thus, the small-sized populations inhabiting stable habitats should not be neglected when establishing conservation strategies for the species and when considering the habitat protection of small spring sites. The genotypes seem to be accumulated over a prolonged period, due to occasional recruitment and clonal propagation in the populations of *T. tomentella*, indicating their origin and adaptation to the local environmental conditions.

The immediate surroundings of springs and streams, the habitats of *T. tomentella*, are woodland key habitats and are included in the habitats types of the Finnish Forest Act.

Thus, conservation management should be carried out "in a manner that preserves the special features of the habitats" (Junninen and Kouki, 2006). However, the protection of the "immediate surroundings" of springs and streams, as stated in the Forest Act, is obviously not sufficient for maintaining the microclimate and moisture conditions of the whole habitat including seepage areas for drought-sensitive species. Because *T. tomentella* seems to persist in undisturbed habitats surprisingly well for long periods of time, maintaining the quality of humid microhabitats favourable for *T. tomentella* by establishing, e.g., buffer strips, are effective tactical measures to minimise local extinctions (see, Hylander et al., 2002; Hylander, 2005; Dynesius and Hylander, 2007).

At the landscape level, the limited dispersal potential of *T. tomentella* is likely to frustrate successful (re-)colonisation in the potential habitats of a recovering forest landscape within the network of woodland key habitats. However, the connectivity of streamside forests in a natural state may increase the level of dispersal of shoot fragments along the water (see Dynesius and Hylander, 2007), as detected in the large populations of *T. tomentella*. Because the suitable habitats for *T. tomentella* are sparsely, but often patchily, occupied, random short-range recruitment seems to be efficient within populations (see Table 1 in paper V). Thus, the restoration actions of spring and streamside habitats close to the populations of *T. tomentella* may contribute to population expansion, which increases the evolutionary potential of the species against environmental and genetic stochasticity. In addition, artificial transplantations of diaspore fragments into suitable microhabitats can be an effective conservation tool for enhancing the long term survival of a species in the region (see Gunnarsson and Söderström, 2007; Mälson and Rydin, 2007), especially in dispersal and habitat limited species, like *T. tomentella*.

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