Population Structure of *Thamnolia subuliformis* and *Dicranum elongatum* in northeastern coastal regions of Wapusk National Park, Manitoba.

By

David Massid Cassie

A thesis submitted to the faculty of Graduate Studies in partial fulfillment of the requirements for the degree of Masters of Science

Department of Botany

University of Manitoba

Winnipeg, Manitoba

October, 2006

Acknowledgements:

I would like to thank my advisor, Michele Piercey-Normore for her endless guidance and suggestions. Her commitment has created an unbelievable educational experience for me. John Markham, Sylvie Renault and Rene Belland also provided very useful feedback. Thanks also to Parks Canada for providing helicopter access to Wapusk National Park and to Manitoba Conservation and NSERC for funding. Without the following list of people, this project would not have been possible: Rhonda Kotelko (for extracting 6 samples), Narinder Kalkat, Jason Robertson (for encouragement on PCR), Mason Kulbaba (for loading 3 DNA samples on Aug.16/05), Matthew Doering, Kyle Fontaine, Sara Beiggi, and all other botany graduate students.

Abstract

Low Arctic populations of *Thamnolia subuliformis* and *Dicranum elongatum* were studied from Wapusk National Park, Manitoba. *Thamnolia subuliformis*, a sterile lichen, is adapted to beach ridge crests where fragmented thallus branches are dispersed by wind over the beach ridge. The bryophyte, *D.elongatum*, grows in moist acidic fen habitats between the beach ridges. Hummocks result from the growth of this moss in low lying areas, where freeze thaw cycles push the stems upward. Since both species grow on exposed beach ridges, they would be among the most susceptible species to the effects of global warming. Therefore, the general goal of this study was to provide an understanding of the species communities and population dynamics of two common species (*Thamnolia subuliformis* and *Dicranum elongatum*) in northeastern regions of Wapusk National Park. Species compositions, habitat parameters and population structure of the moss and the lichen fungus were examined in 141 plots. The presence or absence of an intron at position 1199 within the small subunit (SSU) of the ribosomal DNA (rDNA) in the lichen fungus shows variation across populations. Also in the fungus, the presence or absence of a different putative group-I intron at the end of the SSU rDNA as a result of RFLP of the ITS rDNA, shows variation across populations. Interspersed simple sequence repeat (ISSR) microsatellite markers were screened in fungal tissue manually separated from the algal partner. In the fungus, seven markers have been isolated which show a high degree of polymorphism amongst the samples. Six markers show significant PhiPT values in an AMOVA analysis, suggesting population subdivision. For *D.elongatum*, eight microsatellites have revealed polymorphic banding patterns, all of which have significant PhiPT values in the AMOVA analysis. The

absence of sporophytes, suggesting an absence of or a low level of sexual reproduction, in *Dicranum elongatum* was expected. This was anticipated to be reflected by increased population subdivision with the markers, which consequently appears to be the case. It was expected that *Thamnolia subuliformis* would show little polymorphism because of its asexual reproductive strategy. The high level of polymorphism in *T.subuliformis* detected on the beach ridges was not predicted. This study will serve as baseline data on the genetic population structure and species composition on the beach ridges in northeastern Wapusk National Park and for future studies on the effects of global warming. Also, defining habitat parameters that influence the distribution of species will be beneficial in future studies aimed at describing distributional shifts of species.

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1. Introduction

1.1 *Population Structure*

Populations are dynamic. They are continually changing in both the geographic distributions and their genetic composition. The geographic distributions and genetic compositions of populations are influenced by factors such as reproductive behaviour and migration, making the study of population structure complicated (Gillespie, 1998). The frequency and duration of sexual reproduction coupled with the mixing of alleles (recombination) and dispersal (migration) may generate complex patterns. Assessment of the distribution of alleles in a population will contribute to an understanding of the dynamics occurring within and among populations. Population dynamics can be inferred from a study of the population genetics of a species and interactions with other species in the biological system.

Population structure is defined as the distribution of individuals or separate genetic units. The inferred gene flow between populations is employed to examine dispersal and migration among populations (During, 1979). Weinberg, was a human geneticist and is credited with generating the most important theory (Hardy-Weinberg) of the population genetics discipline (Gillespie, 1998). The Hardy-Weinberg Theory aims at estimating the allele frequencies of homo- and heterozygotes in a randomly breeding population. When populations have deviated from Hardy-Weinberg proportions, one of the assumptions of equilibrium has been violated. These deviations can be a result of

inbreeding, selection, migration (dispersal), genetic drift and evolution. Inbreeding, in violation of random mating, leads to a situation where the alleles in the population remain the same (eg. homozygotes that inbreed will generate only homozygous offspring). Under theoretical Hardy-Weinberg conditions, selection has no influence and allele frequencies can be predicted. However, natural selection can select against deleterious alleles, and thus violate the assumption of no selective pressure. If any deviations from Hardy-Weinberg proportions occur, then populations can be referred to as structured. Consequently, many interpretations of population structure are based on assumptions of equilibrium and models of gene flow (Barker, 2002).

Reproductive Behaviour and Migration (Dispersal)

Population structure is an important concept with regards to genetic variability (Barker, 2002). If a population is reproducing primarily asexually, the gene pool may be severely reduced unless propagules arrive from geographically distant populations. In this case, colonies may arise that contain additional haplotypes. If a population is reproducing sexually, there is a mix of genes and the gene pool may not be as homogenous as in a population of asexually reproducing individuals. The absence of sexual reproduction may be evident by a lack of sexual structures. The absence of sexual structures may be detected by the absence of their products (*ie*. sporophytes in mosses, and apothecia in lichens).

Long distance dispersal and establishment of spores in bryophytes has rarely been observed under natural conditions (Longton, 1994; Miles and Longton, 1987). This does not rule out the possibility that it does occur. Long-distance dispersal of sexual spores by

wind is more plausible than that of asexual propagules because of their smaller size (Longton, 1976), yet studies that examine dispersal distances of spores and vegetative propagules are rare (Longton, 1976; Van Zanten 1976, 1978, 1984). There are several problems associated with long-distance dispersal; 1) a spore must land in a suitable habitat for germination and growth, 2) dessication during dispersal may reduce viability, 3) UV light damage during dispersal if in the jet stream, and 4) the number of viable spores released may be less than the total number of spores released. A series of studies by Van Zanten (1976; 1978; and 1984) simulating trans-oceanic dispersal (subjecting spores to high UV levels and dessication), proposed that long-distance dispersal of spores is possible in species with a trans-oceanic distribution.

Monoicous or diecious reproductive states influence the ability of a species to sexually reproduce and consequently the patterns of variation. Diecious species have male and female sexual structures on spatially separated individuals. In contrast, monoeicous species have both male and female sexual structures on the same individual. Plants that are diecious are faced with the problem of the proximity of the male or female plant to one another. Monoicous species have higher probabilities of fertilization and hence, spore development. Consequently, monoicous species also have an increased chance of self-fertilization, which would maintain a low level of genetic variability as it was in the parent generation. A small gene pool limits the adaptability of a species to environmental changes and the forces of natural selection (Barker, 2002). For example, having only one allele of each gene, as in the haploid stage of bryophytes, directly exposes the alleles to selective pressures (Longton, 1976). If the allele selected against is a deleterious recessive, this would reduce the genetic variation in the population. This

reduction would inhibit the adaptabililty of a species to environmental changes (Gillespie, 1998).

Wapusk National Park

Adaptation to environmental changes should be detectable in harsh environmental conditions with a number of variable niches. Northeastern Wapusk National Park experiences subarctic conditions and may be a suitable place to observe variation due to adaptation.

Wapusk National Park (Figure 1.1) is Canada's seventh largest, and is the largest National Park in Manitoba. Compared with Manitoba's other National Park, Riding Mountain, Wapusk covers 3.5 times the geographic area, with an area of 11,475 km^2. Wapusk National Park is situated 80 kilometers southeast of Churchill, Manitoba between latitude 57ºN to 59ºN and longitude 92º30´W to 94ºW. The park is adjacent to the provincially managed Cape Churchill and Cape Tatnam Wildlife Management Areas (www.pc.gc.ca).

The growing season in Churchill ranges from 100 to 143 days, the mean annual temperature is -7.3°C and annually ranges from 12°C in July to -28°C in January (Dredge and Nixon, 1992). As a result of the short growing season and harsh climate, plant growth of some species is severely reduced.

The park is on a low-lying, poorly drained plain, declining 1.5 meters/km towards Hudson Bay (Dredge, 1992). This is a very young glaciated landscape, in geological terms, continuing to experience isostatic rebound at a rate of up to one meter/century. As a result of this rebound, beach ridges are a major feature and break the uniformity of the

landscape (www.pc.gc.ca). Beach ridges formed during the regression of the postglacial Tyrell Sea and the emergence of the land. The second most recent beach ridge, aside from the current coast, is 1.5 km inland and was formed about 1,300 years ago (Dredge, 1992). The age of beach ridges increases from the coast to inland areas because the elevation above sea level increases. The beach ridges near the coast of Hudson Bay are dissimilar to those occurring further inland, with regards to species compositions. For a detailed account of species compositions on inland beach ridges see Ford *et al.* (2002) and Punter *et al*. (2005).

Open water is prominent in Wapusk National Park, covering half of the land surface as lakes, bogs, fens, streams and rivers. The area is underlain by continuous permafrost, a layer of permanently frozen subsoil, consisting mostly of frozen gravel and finer material. Permafrost is about 80 meters thick at Churchill (Dredge, 1992). The frozen soil inhibits drainage, so water saturates the upper surface as bogs, fens and ponds. There are no deep root systems in the vegetation of the arctic tundra because they can not penetrate the permanently frozen soil. However, there is a variety of shallow root plants, including lichens and bryophytes with no root systems, that are able to tolerate the climate (Brook 2001; Ford *et al.* 2002, Punter *et al*.2005).

Figure 1.1: Wapusk National Park, Manitoba, Canada

Source: www.pc.gc.ca

Objectives

The intent of this study is to acquire baseline data on environmental parameters (pH, aspect, soil type and microtopography) and on the distribution and population structure of *Thamnolia subuliformis* (Ehrh.) W. Culb.*,* and *Dicranum elongatum* Schleicher *ex* Schwagrichen along microtopographical gradients in northeastern coastal regions of Wapusk National Park, Manitoba. These species were chosen because of their abundance in the park. In addition, the population structure of *Dicranum elongatum* and the fungal biont of *Thamnolia subuliformis* will be determined using molecular markers. The mode of reproduction and dispersal for both species will be postulated. Since both species were common on the beach ridges, it is hypothesized that there will be no population subdivision of *Dicranum elongatum* and the fungal biont of *Thamnolia subuliformis*. The information gathered in this baseline study will be used for conservation applications in the park and as a reference for determining human impacts on the heavily used areas for tourism. The generated information will also be used for future studies aimed at understanding, more clearly, the effects of global warming. These exposed habitats in subarctic regions are thought to be the first to show adverse signs to the effects of global warming (Hansen & Lebedoff, 1987). In addition, this study will provide a better understanding of the population genetics of *Dicranum elongatum* and the sterile lichen *Thamnolia subuliformis*.

The general goal of this study was to provide an understanding of the communities and population dynamics of two common species (*Thamnolia subuliformis* and *Dicranum elongatum*) in northeastern regions of Wapusk National Park. The specific objectives, which will be addressed in separate chapters, were:

1) to determine the species compositions of lichens, bryophytes and vascular plants on beach ridges and fens in northeastern coastal regions of Wapusk National Park, Manitoba.

2) to determine the population structure of *Thamnolia subuliformis* using fungal specific SSU primers and interspersed simple sequence repeat (ISSR) microsatellites.

3) to determine the population structure of *Dicranum elongatum* using interspersed simple sequence repeat (ISSR) microsatellites.

2. Literature Review

2.1 *Classification and Reproductive Biology*

Bryophytes are small non-vascular, leafy plants and are the second largest group in the plant kingdom next to the angiosperms or flowering plants (Longton, 1985). They have a worldwide distribution, are diverse, and include liverworts, hornworts and mosses. There are three major orders of mosses. Firstly, there are the granite or lantern mosses (*Andraeaopsida*) with approximately 100 species (Schofield, 1985). They form dark coloured patches on rock or disturbed substrates in mountainous or arctic environments. The second group are the peat mosses (*Sphagnopsida*) with approximately 350 species (Schofield, 1985). They are found mostly in bogs or mires. The third and largest group are the true mosses (*Bryopsida*) with approximately 14,000 species (Schofield, 1985). Families within the *Bryopsida* are differentiated based on morphology, peristomes and cell types. The two main growth forms are acrocarpous and pleurocarpous. The former consists of vertical or apically growing shoots, whereas the latter consists of horizontally or laterally growing shoots. In acrocarpous species the sporophyte arises at the apex of the gametophyte, whereas in pleurocarpous species the sporophyte arises laterally from the stem of the gametophyte.

The lichen symbiosis consists of the mycobiont, or fungal partner, and one or more photobionts, a green alga or cyanobacterial partner. The mycobiont provides protection and increased spatial distribution for the photobiont. In return the mycobiont receives carbohydrates, polyols in the case of green algae and glucose in the case of cyanolichens (Smith and Douglas, 1987). In the case of cyanolichens, the mycobiont receives nitrogen (N) fixed by cyanobacteria. Carbon dioxide (CO_2) produced from

fungal respiration can be used for algal photosynthesis and conversely the production of oxygen (O_2) by algal photosynthesis can be used by the mycobiont for respiration.

The photobionts of the lichen symbiosis are either cyanobacteria in the Monera Kingdom or green algae in the Protista Kingdom (Van Den Hoek *et al*. 1995). The division Cyanophyta contains about 150 genera and 2000 species (Fott, 1971). Cyaobacteria, being prokaryotes, are lacking a nucleus, mitochondria, golgi apparatus, endoplasmic reticulum and vacuoles, but do contain single thylakoids with chlorophyll a, free in the cytoplasm (Campbell and Reece, 2005), thereby permitting photosynthetic activity. Cyanobacteria are found in marine, freshwater and terrestrial habitats on damp soil (Van Den Hoek *et al.* 1995). Green algae, being eukaryotes, contain all cell organelles and have thylakoids in chloroplasts (Capmbell and Reece, 2005). The Phylum Chlorophyta contains around 500 genera and approximately 8000 species found in freshwater, marine and terrestrial habitats (Van Den Hoek *et al*. 1995).

Lichens are classified as fungi and estimates of species numbers range from 13,500 (Hawksworth and Hill, 1984) to 17,000 (Hale, 1974). The majority of lichens are ascomycetes and to a lesser extent basidiomycetes. The latter group constitutes only five known families (Zavada *et al*. 2004). Ascomycetes are fungi that produce eight haploid spores inside sac-like cells called asci. Basidiomycetes are fungi that produce four haploid basidiospores externally on a basidium. Lichenized fungi are mostly ascomycetes that have developed a symbiosis with a green alga or cyanobacterium, or sometimes both.

Ahmadjian (1982) estimates that 50 to 70 % of lichens contain photobionts within the green algae or Chlorophyta; in particular *Trebouxia* and *Trentepohlia.* The

genus *Trebouxia* and *Trentepohlia* are the most common green algal, whereas *Nostoc* is the most common cyanobacteria. *Trebouxia* and *Nostoc* are the most common algal and cyanobacterial partners, respectively, in the lichen symbiosis (Friedl and Budel, 1996).

The main differentiating characteristics of lichens are the growth forms. The main body of the lichen is the thallus which, based on morphology, distinguishes three main groups. These are the crustose, foliose and fruticose growth forms. Typical thalli have an upper or outer cortex, an algal layer, a medulla of fungal mycelium, and occasionally a lower cortex. Crustose lichens are tightly attached to the substrate by the entire lower mycelial layer. No lower cortex is present. They grow on many types of substrates. Foliose lichens are leaf-like, flat and attached usually at more than one point to the substrate. The thallus is usually divided into lobes and an upper and lower cortex is usually present. Fruticose thallus lobes are hair-like, strap-shaped or shrubby and may be flat or cylindrical. The majority have radially symmetrical thalli or appear spherical in cross section.

Poikilohydric Nature of Bryophytes and Lichens

Both bryophytes and lichens are considered poikilohydric. In contrast to homiohydric gymno- and angiosperms, poikilohydric organisms do not have the capacity to maintain internal water levels (Nash, 1996). Lichens and bryophytes rely solely on the surrounding environment for water. This is analogous to cold blooded organisms in the animal kingdom. As animal metabolism slows with lowering environmental temperatures, the water content of lichens and bryophytes changes as moisture levels fluctuate in the surrounding environment. The form of the moisture can be direct

precipitation as rain or snow, or morning dew or fog. Morphological adaptations have developed to optimize water absorbtion from these different forms of water. Since water availability is important for metabolic functions, this poikilohydric nature has an influence on reproduction and life history strategies.

Bryophyte Reproduction

The typical moss life cycle alternates between a haploid (n) gametophyte and a diploid (2n) sporophyte generation. Figure 1.2, illustrates a bryophyte life cycle with numbered references. After spore dispersal (1), the germinating spore (n) develops a branched, filamentous protonema (2). The leafy green gametophyte (n) develops from this first thread and comprises the dominant stage of the life cycle (**3**). All vegetative and reproductive growth arises from the photosynthetic gametophyte. Male and female gametangia, called antheridia and archegonia, respectively, develop (**4,5**) on the stem or in leaf axils depending on the species. Biflagellate spermatizoids (n), developed in the antheridia, are expelled and require water for fertilization of the egg in the archegonium (**5**). One spermatizoid, after swimming down the ventral canal, fertilizes the egg cell in the archegonium (**5**). The zygote (2n) is the first stage of the diploid sporophyte portion of the life cycle (**6**). The zygote divides mitotically to form the sporophyte, which consists of a sporangium, seta and foot (**7**).

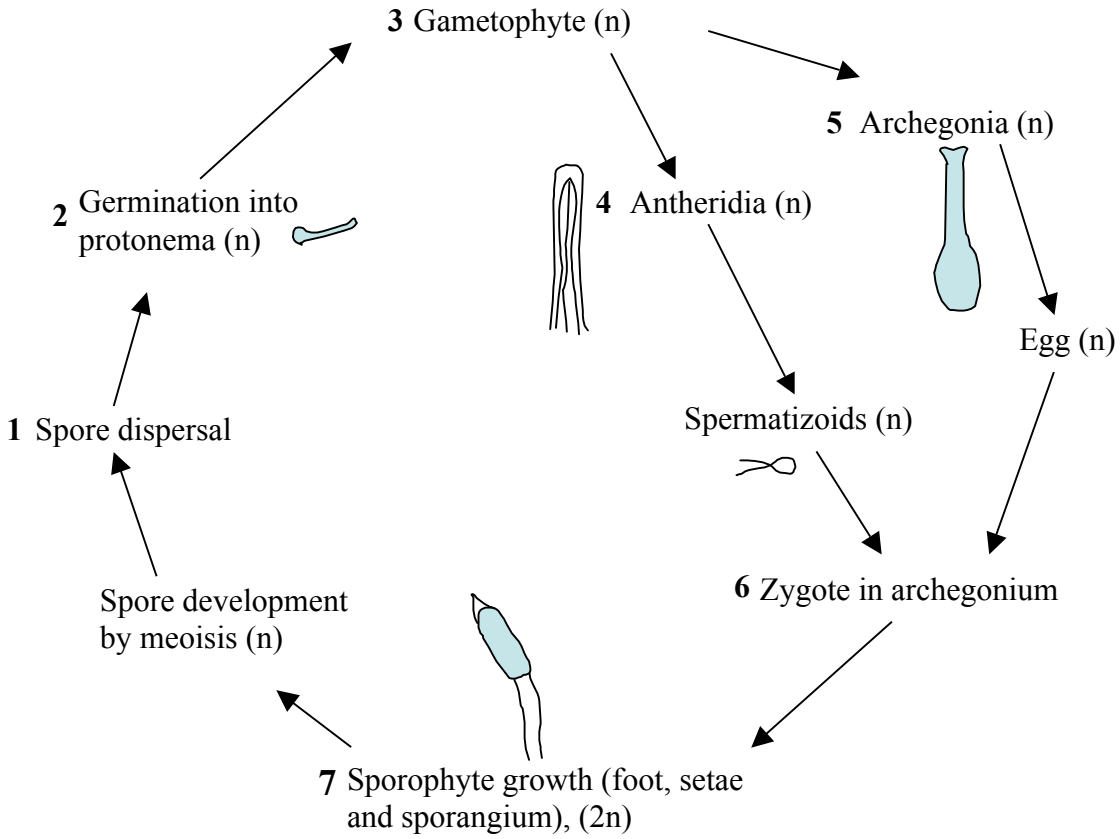


Figure 1.2: Generalized bryophyte life cycle. See text for explanation. Modified from Schofield (1985).

The ventral canal cell in the archegonium forms the haploid (n) calyptra, which covers the capsule during spore development. Meiosis occurs within the capsule, giving rise to haploid (n) spores (**1**). The mechanism by which spores are released from the sporangium is different for the three orders of mosses, and consequently is used in taxonomic identification.

The spore release mechanism in the Sphagnidae is quite unique. As the mature capsule loses water, air pressure increases inside the sporangium. Eventually this pressure will force the operculum off the non-peristomate capsule and the spores will be explosively released. The mechanism in the Andreaeidae is different in that the sporangium itself splits along four furrows and compresses to create bowed openings, thereby allowing spores to be released passively by wind and hygroscopic movement of the capsule. The third family, Bryidae, gowing in moister environments contains a hygroscopic peristome at the opening in the capsule. Under dry conditions, the operculum is shed as a result of water loss by the annulus. The peristome teeth bend outwards and the spores are pushed out of the capsule and are carried by air currents.

Populations can persist as haploid (n) gametophytes as a result of asexual reproduction. This can be accomplished by vegetative diaspores (gemmae) that may arise in structures such as gemmae cups or as clusters on various parts of the gametophyte, such as in leaf axils as in *Pohlia* spp. (Buck and Goffinet, 2000). Gemmae in splash cups require water for dispersal before they fall into a different habitat and germinate into new gametophytes. The continual addition of water to the splash cups via precipitation, suspends the gemmae in solution. When the cup is at volumetric capacity, each additional water droplet displaces the same amount of liquid. This displaced water,

which may or may not contain gemmae, is splashed out of the cup, thereby dispersing the diaspores. Gemmae are haploid (n) like the parent gametophyte and increase biomass without influencing genetic variation.

Growth of new gametophytes from leaves and stems that have dispersed from parent gametophytes has been observed under artificial and natural conditions. This can occur by clonal propagation from leaves that develop secondary protonema (Longton, 1988). Certain taxa have adapted their leave morphology to facilitate dispersal. *Groutiella tomentosa* has 'fragile' leaf apices which frequently break off, *Haplohymenium triste* has leaves that break along cell lines, *Zygodon fragilis* has entire leaves that break off, and *Platygyrium* spp. and *Adelothecium* spp. have leafless stems which grow in leaf axils and are easily shed (Buck and Goffinet, 2000).

Reproduction in the Lichen Association

Reproduction within lichens is quite different from bryophytes, primarily due to the presence of two different organisms. As a result of the controlled parasitism by the fungus in the lichen symbiosis, the algal partner has not been reported to undergo sexual reproduction while in the lichen thallus (Nash, 1996). There are, however vegetative symbiotic propagules produced by each of the bionts, separately and together. Individually, the fungus can produce conidia and the alga can generate new cells by mitosis. One form of vegetative propagation where the bionts are found together is through fragmentation of the thallus. This occurs when a piece of the thallus breaks off. The fragment contains algal and fungal bionts that are already compatible and together in the fragment. Therefore the vegetative propagule is capable of growth into a new lichen.

This is, however, also determined by the microsite in which the fragment falls. The size of the fragment has a direct correlation on the dispersal distance (Walser, 2004). Larger fragments tend to stay close to the parent thallus in the same habitat, whereas smaller fragments are able to be blown more easily by wind, and can disperse further distances to new habitats.

Two vegetative propagules that arise from the thallus surface are isidia and soredia. Both of these are vegetative diaspores that act as separable autonomous subunits of the thallus (Hale, 1983). Isidia are peglike outgrowths of the thallus. These outgrowths contain both medullary and algal tissues surrounded by a cortex. In contrast to isidia, soredia do not contain a cortex. These structures are small (25-100 μm) loose masses of fungal and algal cells that originate in the medulla and algal layer. They differentiate into different packages and are released through pores or cracks in the surface of the thallus. Soredia can be found over the entire thallus or in delimited zones, called soralia (Lawrey, 1984).

Fungal bionts are the dominant partner in the symbiosis and can undergo sexual reproduction. Dominant in this context refers to the fungal tissue forming the major part of the thallus. The algae are found in the medulla beneath the cortex and are intertwined with fungal hyphae. Some fungal hyphae can be differentiated into haustoria, which are parasitic in nature penetrating other cells (Carlile *et al*. 2001). Haustorial fungal hyphae envelop and penetrate the algal or cyanobacterial partner initiating the lichen symbiosis.

Most lichenized fungi belong to the Ascomycetes. An Ascomycete life cycle is illustrated in Figure 1.3**,** with numbered references. Following germination, male and female gametangia (antheridia and archegonia respectively) are produced (**1**). Male

gametes, spermatia, can transfer via wind, water or animal from the antheridium to the trichogyne or ascogonial filament of the ascogonium (**2**). The nucleus of the spermatium, upon landing on the trichogyne migrates into the ascogonium. Nuclear fusion or karyogamy (**3**) eventually takes place in the ascus mother cell of the crozier hook, creating the diploid (2n) zygote. The crozier hook is formed from hyphae that elongate and bend over to form a hook. These hyphae arise as papillae on the inner walls of the ascogonium. The location of sexual reproduction is in a fruiting structure called an ascocarp (**4**), (Lawrey, 1984). The ascospores, produced by meiosis, disperse (**8**), via wind, water or animals and germinate into a new mycelia (**9**). Usually eight ascospores are produced, but the actual number is quite variable.

Meiosis of the diploid (2n) zygote generates 4 haploid (n) nuclei (**6**), which divide mitotically to usually form 8 ascospores (**7**), (Alexopoulos and Mims, 1979; 1996). The spores are dispersed (**8,9**) and the life cycle commences again with new mycelia. It is important to emphasize that this is a generalized Ascomycete life cycle subject to variation, depending on the species in question. This life cycle is assumed to take place in lichenized fungi as well, but has never been completely observed.

Fungi are classified based on their method of spore dispersal. Water droplets that fall on *Nectarial* species (Ascomycetes) and *Fusarium* spp. (Deuteromycetes) cause spores to be released both, as wet spores and as a puff of dry spores. Also, hail hitting the ground creates what is referred to as the 'Tap' effect (Burnett, 2003). When hail comes in contact with the fungus it causes spores to be ejected into the air. These spores must be ejected above the laminar air layer in order for effective dispersal to take place.

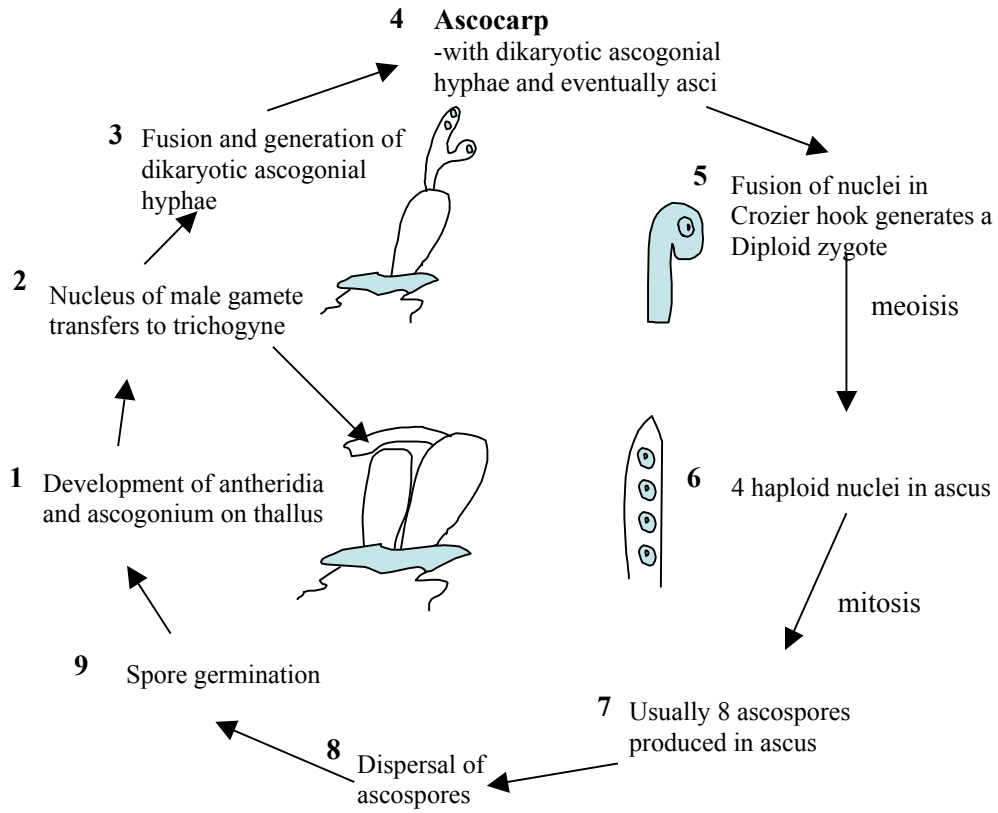


Figure 1.3: Ascomycete life cycle. See text for explanation. Modified from Alexopoulos *et al*. 1996

Basidiomycetes, such as *Agaricus* spp. and *Lycoperdon* spp., release spores using different techniques. *Lycoperdon* spp. or puffballs release spores when water droplets hit the fungus and cause them to be ejected. *Agaricus* spp. in contrast, drop spores out of the bottom gills of the mature fungal cap, to be picked up by air currents flowing underneath. Ascomycetes such as *Sordaria* spp. and *Ascobolus* spp. release spores from the ascus into the air layer above the fruiting body. Air currents catch the spores and carry them to other areas. Other Ascomycetes, such as *Erysiphe* spp. and *Conidiobolus* spp., and Deuteromycetes such as *Cladosporium* spp. and *Peronospora* spp., release spores into mist currents in the laminar boundary layer. The methods of spore dispersal outlined are the main agents by which the Eumycota, which includes Ascomycetes, Basidiomycetes, and Zygomycetes, liberate and disperse reproductive propagules (Burnett, 2003).

The most common photobionts, approximately 50-70% (Ahmadjian, 1982), in lichen symbioses are members of the division Chlorophyta, the green algae. The division contains around 500 genera and approximately 8000 species (Van Den Hoek *et al*. 1995). A generalized life cycle (Figure 1.4) similar to species of *Trebouxia* and *Trentepohlia*, can be derived from that of *Chlorococcum echinozygotum* in the order Chlorococcales, which is found in soil in the Philippines (Van Den Hoek *et al*., 1995).

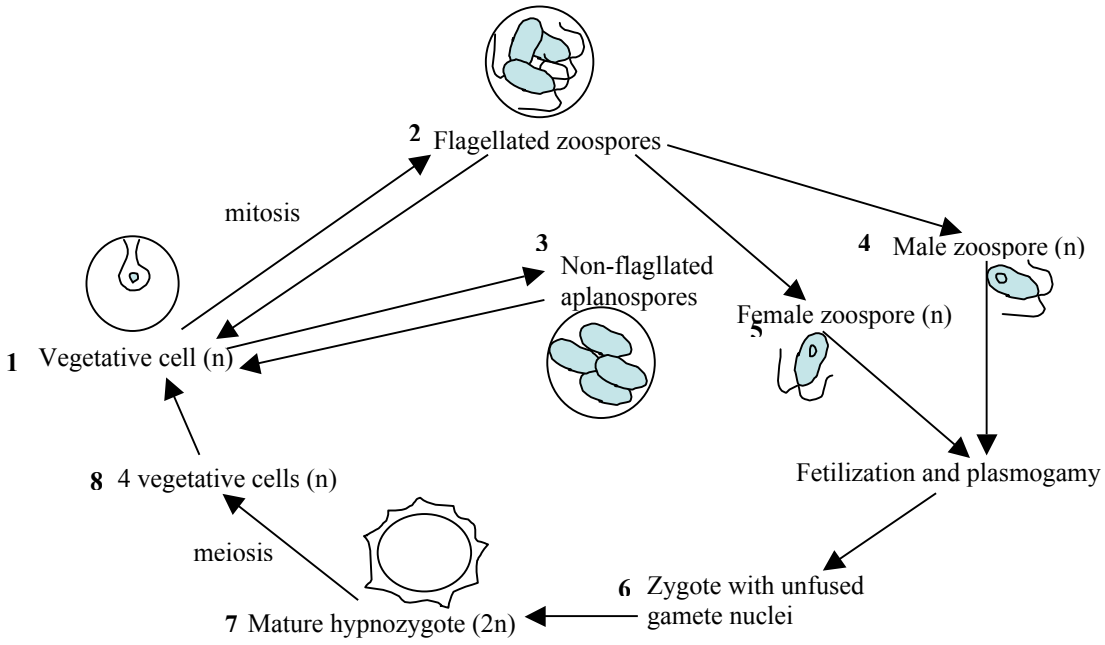


Figure 1.4: Generalized green algae life cycle based on *Chlorococcum echinozygotum*. See text for explanation. Modified from Van Den Hoek *et al*. 1995.

Thamnolia subuliformis-Biology and Growth Patterns

Deuteromycetes are the "imperfect fungi" that have not been observed to reproduce sexually (Alexopoulos *et al*. 1996). Ascomycetes are thought to make up a large portion of Deutermycetes. *Thamnolia,* the white worm lichens, belong to the Deuteromycetes and are known only to be sterile (Culberson, 1963). There have been no reports of sexual reproduction or specialized vegetative propagules within this genus. A discomycete parasite was mistaken for apothecia on *T.vermicularis* by Walker (1970). The primary mode of dispersal and persistence in *Thamnolia* is a result of asexual fragmentation of the branches of the thallus. Lateral branches develop as small bulges on the thallus eventually growing into slender stalks which break loose and become new independent thalli (Kärenfelt and Thell, 1995). The fragile hollow thalli are easily broken by wind and hooves in open tundra regions, where two species of *Thamnolia* are common. Herds of caribou facilitate dispersal by the destructive nature of their hooves. In addition, the absence of high vegetation on the tundra allows winds to reach higher speeds near the ground, which facilitates *Thamnolia* dispersal. Also the frictionless surface of snow between ridges allows fragments to be blown great distances (Thomson, 1972).

Thamnolia vermicularis and *T. subuliformis* are considered 'cladoniiform' lichens (DePriest and Stenroos, 1998) because of shared morphological characters with the genus *Cladonia* Browne. The genus *Thamnolia* has a fruticose growth form. The thallus grows as upright cyclindrical hollow structures called podetia. A central ring called a stereome provides support for the thallus. The stereome usually functions as a mechanism to elevate sexual structures higher than the lichen canopy for spore dispersal from apothecia

at the top of the podetium. In *Thamnolia*, however, which is a sterile lichen, the stereome may function to elevate podetia for easier fragmentation by wind and caribou.

Poelt (1973) placed the genus *Thamnolia* close to the *Cladoniaceae* based on chemical characters. Hawksworth *et al*. (1995) provisionally placed *Thamnolia* in the Lecanorles because it could not be classified conclusively. More recently phylogenetic analyses have placed the genus *Thamnolia* closer to the *Siphulaceae* family (Depriest and Stenroos, 1998), due to the absence of sexual structures and the sequence similarities of the small subunit (SSU). DePriest and Stenroos (1998) placed *Thamnolia* in a separate clade containing the Siphulaceae (*Siphula* and *Thamnolia*), Icmadophilaceae (*Dibaeis*), and Baeomycetaceae (*Baeomyces*). Stenroos *et al*. (2002) further classified *Thamnolia* to the Icmadophilaceae based on the SSU rDNA.

The white worm lichens, *Thamnolia vermicularis* (Sw.) Ach. Ex. Schaerer and *Thamnolia subuliformis* (Ehrh.) W. Culb. are chemotypes (chemical variants with similar morphology), (Brodo *et al*. 2001). *Thamnolia subuliformis* possesses squamatic and baeomycesic acids and fluoresces yellow under long wave UV light. *Thamnolia vermicularis* possesses thamnolic acid and does not fluoresce under long wave UV light. The latter species also discolours pink over time in herbarium specimens. The designation of the two species as chemotypes has received considerable debate (Karenfelt and Thell, 1995). Morphological and chemical data cannot reliably distinguish species. Kärenfelt and Thell (1995) found four distinct morphological types in the tundra regions of the northern arctic coast from Europe to Asia. Differences arose from the thallus either being circular, flattened, slender or highly branched. The authors stated that without genetic analyses, the morphotypes could not be accepted as different species

based on genetics. The species were designated as *Thamnolia vermicularis* and the chemotype *Thamnolia subuliformis,* based on chemistry (Culberson, 1963). In Europe, Minks (1874) treated the two chemotypes as varieties, *T.vermicularis* var. *subuliformis* (Ehrh.) Schaer. and *Thamnolia vermicularis* var. *vermicularis*. The variety concept was also used by Sato (1968a) in South America where he proposed *Thamnolia vermicularis* var. *subsolida* and *Thamnolia vermicularis* var. *solida*.

Thamnolia vermicularis and *T.subuliformis* are erect, fruticose lichens. The podetia generally grow in clumps or can be scattered fragments over the substrate. They are called white worm lichens because the morphology of the podetium resemble that of worms, (Figure 1.5**)**. The podetia are variable, but are mainly hollow and can reach heights of 16 cm and taper drastically towards the apex. Podetia can vary in cross sectional thickness from 1.5-2.0 mm to 10 mm. The photobiont can be composed of cells up to 15 μm in diameter (Kärenfelt and Thell, 1995) and is found in 3 of 4 major clades within the *Trebouxia* genus (Nelsen and Gargas, 2005). This non-specificity of algal partners was also found in members of *Umbilicaria,* in Antarctica, that selected 3 different species of *Trebouxia* (Romeike *et al.* 2002). Ahmadjian (1982) estimates that 50 to 70 % of lichens contain photobionts *Trebouxia* and *Trentepohlia.* The genus *Trebouxia* and *Trentepohlia* are the most common green algal, whereas *Nostoc* is the most common cyanobacterial partner in lichens (Friedl and Budel, 1996).

Figure 1.5: Photo of *Thamnolia vermicularis* showing several fragments and branching patterns. (Photo by D.Cassie).

Dicranum elongatum-Biology and Growth Patterns

The genus *Dicranum* Hedwig. is one of 55 genera in the family Dicranaceae (Vitt, 1984), and is comprised of approximately 26 species in Canada (Ireland *et al*. 1987). They are primarily yellowish green to dark green and can appear shiny or dull in loose to dense tufts. The genus name comes from the Greek word 'dicranon', meaning pitchfork (Ireland, 2002). This name is in reference to the 16 split peristome teeth on the capsule. These mosses are also referred to as the 'broom-mosses' because of the wind blown appearance of the leaves of the gametophyte.

Dicranum elongatum exhibits the typical moss life cycle (Figure 1.2). It is dioicous, meaning that the male and female gametes are developed on separate plants. Male plants are an equivalent size to that of the female plants. This is in contrast to most species of *Dicranum*, where the male plants have been reduced to tiny buds on the leaves or stems of female plants (Longton, 1988). Perichaetial leaves, or those surrounding the archegonia, are abruptly short. Following fertilization in fall and sporophyte development throughout the winter, the seta grows apically to 1.5-2 cm in length terminating in a capsule (1.2-1.8 cm in length), which matures the following summer. The sporophyte arises apically from the gametophyte of *D. elongatum*. The spores are fairly large, 17-22 μm, which is thought to reduce the distance they can disperse. Larger heavier spores may not get carried the same distance that smaller lighter spores get carried by the wind. It is also not known whether spores successfully establish after long distance dispersal (Richardson, 1981). Van Zanten (1976; 1978; 1984) found that moss spores subjected to high UV and dessication are still viable in species that are distributed

inter-continentally. Those that occurred locally could not tolerate the harsh simulated conditions.

Vegetative reproduction in *Dicranum elongatum* includes stem fragments and detached young leaves originating near the stem apices. Over 80 percent of such leaves gave rise to secondary protonemal filaments in culture (Longton and Greene, 1979).

Dicranum elongatum grows in compact tufts, and is a glossy yellowish green to light green, (Figure 1.6**)**. The stems can range between 2-10 cm tall and are covered with matted rhizoids that intertwine with rhizoids of adjacent stems forming a tuft of stems. Leaves are erect and spreading approximately 0.3-0.5 mm in length. Leaves have a strong single costa that when viewed in cross section, has stereid cells (small thick walled cells) above and below a central group of large thin walled cells (Bellolio-Trucco and Ireland, 1990). *Dicranum elongatum* can be readily confused with *Dicranum groenlandicum*. They are both arctic-alpine species with similar distributions. The differentiating character is *D.elongatum* has pitted cells only to about the midpoint of the leaves, whereas *D. groenlandicum* has pitted cels to well beyond the midpoint (Ireland, 2002).

Dicranum elongatum is common in arctic or alpine tundra regions on rock and soil in bogs or fens from 30 m above sea level (asl) to 3700 m asl. It is found in the northern hemisphere in all tundra-containing provinces and territories in Canada, in many northern US states and overseas in Europe and Asia (Ireland, 2002).

In tundra regions, *D. elongatum* develops into hummocks created from the successive annual growth of the moss, coupled with the freeze thaw cycle. The continual freezing and thawing of the substrate creates heaving conditions which generates

microtopographic structures referred to as hummocks. *Dicranum elongatum* is an acrocarpous moss with only the uppermost layer of the moss actively growing (Ireland, 2002). Brown metabolically inactive clusters of stems form the lower layers of hummocks, while green photosynthetic stems and leaves form only the upper outer layer.

Figure 1.6: Photo of *Dicranum elongatum* from Wapusk National Park showing gametophytes and sporophytes.

These hummocks create an array of microhabitats that provide substrate for considerable diversity among bryophytes, lichens and vascular plants. In addition, the presence of such a considerable volume of mosses, including *D.elongatum,* adds an important aspect to the typical wet tundra landscape, referred to as the fen. Fens are low lying peatland where plants are in contact with mineral rich groundwater as a result of snowmelt (Dredge, 1992). This wet environment is very suited for a bryophyte existence. Mosses add to the fen habitat by retaining water and decreasing the pH, which generates suitable habitat for other bryophytes.

2.2: Ecological Physiology

Ecological physiology is the physiological response induced in organisms by environmental features, such as (1) temperature, (2) moisture, (3) nutrient status and (4) light. The responses depend on factors such as species physiological adaptations, habitat and seasonal variations. Seasonal variations include temperature and photoperiod. In the northern hemisphere there are low temperatures associated with increasing photoperiod in the spring. In the summer, however, the temperature and photoperiod are increased, whereas in fall and winter, the temperature drops significantly and the photoperiod becomes reduced. Moisture and nutrient status relate to each other. In spring periods, moisture is abundant and nutrients are moderate. As summer approaches, water becomes more limiting, yet nutrients become more available due to decomposition (Grossnickle, 2000).

Temperature Stress

Dicranum elongatum is a shade intolerant species, usually growing on rocks or soil in bog or fen areas of the tundra. These microsites are subject to intense radiation. Oechel (1976) studied arctic mosses and the seasonal patterns of radiation and responses induced in various species by the temperature fluctuations. The optimal temperature for photosynthesis varied throughout the growing season. In early July the optima was 13 ºC, in late July it was 21ºC and near the end of August it had dropped to 14ºC. The net CO_2 exchange was also determined to be 0.1 mg/hr in early July, 0.6 mg/hr in late July and 0.5 mg/hr in late August. In spring, lower temperatures and low radiation generate lower rates of photosynthesis. In summer, temperature and photoperiod increase thereby permitting higher daily rates of photosynthesis. Seasonal changes in light and temperature determine photoperiod and consequently photosynthetic activity.

During winter and after long periods of dessication there is a strong decrease in the rates of photosynthesis. The cushion form of *Dicranum elongatum* provides improved moisture retention during periods of dessication. Oechel (1976) also found that as atmospheric temperature and light increased from winter to summer, the optimal temperature for photosynthesis at light saturation and field capacity (maximal water content) increased. Since day length is longer and the sun is positioned closer to the zenith in summer months, the temperature is higher. At full light saturation and optimal leaf water content, photosynthesis will proceed at higher temperatures in the summer.

Low temperature tolerance of bryophytes has been studied by Vesanto (1988) and Proctor (1982). Vesanto (1988) measured the recovery of net photosynthesis after a cold treatment of -10°C for two hours. When the cold treatment was performed during the

growing season, the youngest segments of the shoots did not survive. However, when the cold treatment was performed in autumn and spring, the young shoots were resistant to these freezing temperatures. Proctor (1982) reinforced this finding and showed that bryophytes are able to maintain photosynthesis at very low temperature; but photosynthetic rate is not as high as during more favourable temperatures. This suggests that seasonal aclimation exist for *Dicranum elongatum* to temperature extremes at different times of the season. These physiological responses are not solely a result of temperature, but also of thallus moisture levels.

Temperature in microhabitats in tundra regions is influenced by light intensity, climate and atmospheric moisture conditions (relative humidity). The adaptation of lichens to high temperature has been extensively studied by Lange (1953). Tundra lichens are continually exposed to direct light as a result of the absence of high vegetative cover in tundra to shade out UV radiation. As a result, the light thallus color of some lichens such as *Thamnolia* may reflect excess UV radiation for protection against overheating (Kershaw, 1971a) and UV damage.

Tundra soils are incident to considerable radiation. As a result of long periods of daylight during summer, heat penetrates further down into the soil. As ambient air temperature decreases as a result of decreasing light during the fall, a temperature inversion is created. The inversion is when the ambient air, which is warmer during the day becomes cooler at night. This becomes cumulative by continual heating and cooling as the growing season progresses. The ambient air temperature can be drastically different than the temperature at the surface of the lichen canopy (Kershaw and Field, 1975). This is a result of the high thermal insulating properties of soil. The same authors

also noted that as a result of temperature variations, moisture levels may also vary because of the influence of temperature on relative humidity. These two factors affect photosynthesis and respiration. The influence of one environmental parameter on another must be recognized when attempting to diagnose physiological adaptations.

Seasonal variation in optimal temperatures for photosynthesis is present in some lichens. The optimal photosynthesis temperature for *Cladonia rangiferina* is 10°C higher in summer compared to winter (Tegler and Kershaw, 1980). This seasonal trend is present in most vegetation including *Thamnolia,* but with different optimal temperatures for different species.

As podetia of fruticose lichens continue to grow vertically, the lower portions become older. The increased density of newly formed podetial branches shade lower branches and light availability changes. Lechowicz (1983) found that photosynthesis declines with age. This decline may be attributable to thallus age or a decrease in optimal environment for the alga. For example, 1 year old branch whorls of *Cladonia stellaris* had a photosynthetic rate of 0.76 mg carbon dioxide/g/hr, whereas 15 year old whorls had a rate of 0.02 mg carbon dioxide/g/hr. With less light penetrating the base of the lichen canopy, photobiont size and number would be reduced and consequently less photosynthesis would take place. Nash *et al.* (1980) confirmed this finding with research on the podetia of *C.stellaris* and *C.rangiferina*. They found the upper 2-2.5 centimeters of the fruticose canopies of both species to have the greatest photosynthate production. The photosynthesis rates were markedly different between the two species. In members of the *Thamnolia* genus the podetia grow in dense clusters but do not grow in a vertical mat as do the reindeer lichens. *Thamnolia* tends to spread horizontally and laterally,

rather than have a vertical fruticose thallus as in Cladonia species. However, in dense clusters, the upper podetia should exhibit this trait of higher photosynthetic rates than the shaded lower podetia.

The influence of light on photosynthesis also has a significant effect on the production of secondary compounds. *Cladonia stellaris* and *C.rangiferina* kept at low illumination in a growth chamber for a year yielded a 50 percent loss in perlatolic and fumarprotocetraric acid respectively (Fahselt, 1981). It has not been assessed whether thamnolic acid in *Thamnolia vermicularis* or squamatic and baeomycesic acid in *Thamnolia subuliformis* are affected. Secondary compounds may have detrimental properties to vascular plant growth (Lawrey, 1984), and in their decline or absence, lichens are subjected to increased competition. Some compounds increase hydrophobicity in the medulla and other light absorbing compounds are found in the upper cortical layer and screen the amount of light reaching the algal layer (Elix, 1996) Some of these compounds may in fact protect the thallus from UV radiation (Rundel, 1978; Hawksworth and Hill 1984).

Tolerance of lichens to temperature changes are strongly correlated with thallus hydration. Kappen and Lange (1970) found that most fruticose lichens were able to withstand cooling to -196ºC in the dry state and to -75ºC in the wet state. This is a function of the destructive effects of freezing as water crystals enlarge and destroy biological tissues. Therefore, the temperature at which the thallus is dehydrated will define the thermal stress limits which the species must survive. Conversely, the temperature at which maximal thallus hydration occurs will define the required optimum for net photosynthesis (Kershaw, 1985).

Moisture Stress

Absorption of water takes place over the entire surface of the moss. Approximately 80-90 percent of the water retained by saturated mosses is held externally in leaf axils and in capillary films on leaves and stems (Busby and Whitfield, 1978). Although the majority of bryophytes are relatively tolerant of dessication, growth is usually confined to seasons which maintain the tissues in a fully hydrated state for significant periods of time. Differences in the relation of water content to water potential can be attributed to differences in cell wall structure or in cell matric or osmotic potential (Busby and Whitfield, 1978). This can be attributed to the presence or absence of leptoids and hydroids in different species (Ireland, 2002). Due to their poikilohydric nature, isolated shoots dry out unless in contact with an external moisture supply (Richardson ,1981). Recovery may take place over a period of hours or days, and it may not be entirely complete (Busby and Whitfield, 1978). After drought conditions, cells of *Pleurozium schreberi* recovered their usual structure after 1 to 4 hours (Noailles, 1978). The order of successive cell recovery was that vacuoles were first to recover and then cytoplasm, mitochondria, golgi bodies, endoplasmic reticulum, chloroplasts, and nucleus. The rapid return to the hydrated structure indicated the protection of organelle membranes during drought and rehydration. Most mosses are poikilohydric and will exhibit this trait at some level. Specific studies of *Dicranum elongatum* recovery have not been made, but assumptions can be made based on other studies.

It is apparent that mosses are adapted to dessication events and it is these adaptations that produce a wide range of conditions in which mosses are photosynthetically active. The function of the turf carpet in mosses may be to maintain

high internal levels of humidity (Carleton and Dunham 2003) by capillary wicking. This humidity can supply much of the needed moisture during dry down periods to carry out photosynthesis. The unsophisticated transport systems and water relations of bryophytes have limited their capacity to monopolize resource capture and dominate vegetation in productive, undisturbed habitats (Proctor, 1982). Habitats, such as the tundra fen are subject to extreme cold and water stress; conditions most vascular plants are unable to endure.

The metabolism of poikilohydric lichens is dependent on the thallus being moist, emphasizing the importance of relative humidity of the habitat (Ahti, 1966). Percent thallus saturation or dehydration is a determinant of photosynthetic activity. Optimal photosynthesis was reported when *C.rangiferina* was at 55 percent saturation, *Cladonia uncialis* at 75-85 percent saturation and *C.mitis* at 55-75 percent saturation (Lechowicz *et al*, 1974). In addition, photosynthesis was inhibited above 90 percent thallus saturation for all three species, and reduced to <10 percent for thallus saturation below 20 percent. The overall trend is that thallus saturation levels and photosynthesis are dependent on each other.

Life History Strategies

Life history strategies are adaptations by species to colonize, reproduce and persist in a specific habitat (During, 1979). They include descriptions of (1) reproductive effort, (2) fecundity, (3) reproductive life span, (4) age at first reproduction, and (5) juvenile and adult mortality schedules (Wilbur *et al*. 1974). *Dicranum* is considered to be a stress-tolerator and a ruderal, whereas *Thamnolia* is considered to be a stress-tolerator

in the Competitor-Ruderal-Stress Tolerator (CRS) model (Grime et al. 1990). In this model there are three categorical values, competitors (C), which are continuously abundant, but are subject to a reduction in distribution if resources are exploited; ruderals (R), which are only temporarily abundant; and stress-tolerators (S), which are continuously scarce. Some characteristics of Stress tolerators that Grime et al. (1990) describes are existing for long periods as an established phase, spore production is intermittent over a long life history, there is a small proportion of annual production devoted to spores, growth rate is slow, photosynthesis and uptake of minerals is opportunistic, and they are adapted to seasonal changes in regard to photosynthesis and mineral uptake.

Reproductive effort is a measure of the allocation of resources to reproduction. Production of sexual reproductive structures usually results in a cost to vegetative growth. In harsh climatic conditions, such as the tundra, mosses and lichens reproduce frequently by vegetative means to conserve resources (Piercey-Normore, 2005). Fecundity refers to the frequency and number of offspring produced in a given period. For mosses and lichens, sexual reproduction usually generates hundreds of thousands of spores (Longton, 1976). The age an organism lives to may directly relate to how many times reproduction occurs (Wilbur *et al*. 1974). The age at which sexual reproduction first occurs, depends partly on the time lapse between dispersal and germination of the spore. It also depends on the duration of the dispersal period and the occurrence of a dormancy period. In addition, the developing spore from a moss or lichen, must be in a suitable habitat for gametophyte or thallus growth, respectively, to persist.

The production of bryophyte spores and specialized asexual propagules is highly restricted in severe environments and regeneration is thus mostly dependent on gametophore branching and fragmentation (Longton, 1988). Acrocarpous mosses, like *D.elongatum*, produce vegetative diaspores, such as gemmae, more often than do pleurocarpous mosses (Schofield, 1981). However, *D. elongatum* does not produce gemmae. Acrocarpy may be an adaptation to water stress because the tight cushions allow for better uptake and retention of water. Moss cushions retain water during dessication periods (Schofield, 1981). This may make the water accessible for uptake by capillary movement up stems.

The trade-off between production of vegetative propagules or sexual propagules can be examined with regards to habitat. Allocation of resources to vegetative growth or sporophyte production, in the case of *D.elongatum*, varies depending on age and habitat. The frequency of spore production is a function of growth, age and biomass and is intermittent over a long life history; also, the proportion of annual production devoted to spores is small (Grime et al., 1990). Asexual fragmentation in *Thamnolia* may be an adaptation to habitat. The amount of energy that would be required for sexual reproduction may be limited in harsh habitat conditions. Crustose lichens produced the greatest number of sexual structures (~95%), followed by foliose (~35 %) and fruticose (4%) growth forms in Wapusk National Park (Piercey-Normore, 2005). This additional energy in the foliose and fruticose forms can then be allocated to thallus growth. According to Kappen *et al*. (1988), a stress-tolerant life strategy is advantageous in environments with limited soil water availability and extreme temperatures, which is characteristic of tundra environments. *Thamnolia*, occurring in arctic conditions, may

have lost the abililty to produce sexual structures throughout evolutionary history (Karenfelt and Thell, 1995).

2.3: Population Genetics in Bryophytes and Lichens

Population genetics is the study of the adaptability of a given species to change, based on the current gene pool of alleles and the pressures of natural selection (During, 1979). Population genetic structure and diversity studies have primarily focused on vascular plants (Houle and Delwaide 1991, Menken et al. 1995) and pteridophytes (Soltis and Soltis 1987, Soltis and Soltis 1988, Rumsey et al. 1999, Suter et al. 2000). The impetus has been placed on these types of plants simply due to their size and dominance in numerous habitats (During, 1990). Bryophytes, lichenized and non-lichenized fungi, and algae have received less attention. Bryophytes are the second largest group of land plants next to the flowering plants (During and Van Torren, 1987). Population genetic research that has been carried out has focused on *Sphagnum* (Daniels, 1993), *Sphagnum troendelagicum* (Stenoien and Flatberg, 2000), *Sphagnum angustifolium, Sphagnum fallax, Sphagnum lindbergii and Sphagnum isoviitae* (Stenoien and Sastad, 1999), *Sphagnum angermanicum* (Gunnarrson *et al*. 2005), (Sastad *et al*. 2000, Cronberg 1988, *Sphagnum pulchrum, Sphagnum recurvum* var. *mucronatum and Sphagnum compactum* (Daniels, 1982;1985a;1985b), *Sphagnum capillifolium* (Natcheva and Cronberg, 2003), *Polytrichum juniperinum* (Innes, 1990), *Polytrichum commune* (Derda and Wyatt, 1990), *Polytrichum formosum* (Van Der Velde *et al*. 2000 and 2001, Van Der Velde and Bijlsma 2000 and 2001), *Hylocomium splendens* (Cronberg 2002, Cronberg *et al*. 1997), Liverworts (Szweykowski *et al* 1981a, 1981b, Daniels 1982, 1985a, 1985b) and other

bryophytes *Plagiomnium ciliare* (Wyatt *et al.* 1989), *Plagiomnium medium* (Wyatt *et al*., 1992), *Plagiothecium* (Hofman *et al*. 1992), *Climacium americanum* (Meagher and Shaw, 1990), *Mielichhoferia elongata* (Shaw, 1991), *Pellia borealis* (Zielinski, 1984, 1986).

Dicranum elongatum reproduces sexually and asexually and may persist in colonies as a result of fragmentation or meiotic spores. Being dioicous, *D. elongatum* is faced with the problem of the spermatizoids from the antheridia of one gametophyte being transferred successfully to the archegonia on another gametophyte. If the male and female plants are too far apart, the spermatizoids cannot successfully transfer because they require a waer film for the transfer to occur. If either antheridia or archegonia are produced in small numbers, successful sperm transfer will also be inadequate (Longton, 1976). Longton (1994) postulated that one reason for the low level of sporophyte production is a result of the absence or rarity of antheridia. In antheridial absence, archegonia cannot be fertilized because sperm are not present. Being dioicous this creates a scenario where fertilization is rare because of the proximity and rarity of male gametophytes.

Dicranum elongatum is a haploid organism, meaning that it primarily persists in the gametophyte phase of the life cycle and contains half of the genetic material of a diploid organism. As a result of fragmentation or clonal propagation, one would expect the genetic variablility to be very low and the ability to adapt to changing conditions to be limited. Anderson (1963) attributed this low genetic variability to the haploid state of the dominant gametophyte generation. There would be a strong selection against any allele that might be deleterious in a given environment. This is in contrast to diploid

organisms, where the presence of two alleles may hide deleterious recessive alleles in heterozygotes (Ennos, 1990).

A series of studies by Zielinski and Wachowiak-Zielinski (1994, 1995) have found that another bryophyte, *Pleurozium schreberi,* which reproduces almost exclusively by fragmentation and diaspores in Europe, maintains a level of genetic variation typical of sexually reproducing species. This genetic variation has been discovered in other haploid bryophytes such as *Hylocomium splendens* (Cronberg *et al*., 1997) and *Plagiomnium ciliare* (Wyatt *et al*., 1989). These results do not support the previous consensus that haploid species have a decreased level of genetic variability (Longton, 1994). In the three studies on *P. schreberi*, the percentage of polymorphic loci was 45.8%, 86% and 71 % respectively. These studies showing high levels of variation took place in the boreal forest of Poland. In boreal forests mosses form a nearly continuous understory. In mature stands the closed canopy creates cool temperatures and shades the soil favoring the growth of pleurocarpous mosses such as *Hylocomium splendens, Ptilium crista-castrensis* and *Pleurozium schreberi* (O'Neill, 2000).

Genetic diversity and population structure of lichenized fungi has also received little attention. Past research has focused on genetic variation within and among populations of *Lobaria pulmonaria* using simple sequence repeats (SSR), (Zoller *et al*., 1999) and the recombination and clonal propagation of *Lobaria pulmonaria* (Walser *et al*., 2004) due to its threatened status in Europe. Further studies on *Usnea filipendula* populations in western Germany using RAPDs (Hiebal *et al*., 1999) have yielded results supporting reinvasion by the species. Genetic techniques such as restriction fragment length polymorphisms (RFLP's) have brought to light the genetic variation within local

populations of *Candida albicans* (Xu *et al*. 1999). Piercey-Normore (2006) found multiple algal haplotypes in 45 % of the 290 lichen thalli using RFLP's. Information generated from genetic studies of lichenized fungi provides information on where and how variation exists within and among populations.

Thamnolia vermicularis and *T. subuliformis* are sterile, reproducing by fragmentation and usually persist in colonies or dense clusters. Apothecia or fungal sexual structures have never been encountered under natural or artificial conditions. Lichen associations contain genetic material from both the haploid fungal and the haploid algal partners.

Genetic variation in the genus *Thamnolia* has not been examined. *Thamnolia* is considered a 'cladoniiform' lichen (DePriest and Stenroos, 1998), because of characters such as a hollow podetium and the presence of a stereome. The genus *Cladonia* is extremely diverse containing over 500 different species (Ahti, 1966). Early population genetic work has been done on the *Cladonia chlorophaea* complex by DePriest (1992, 1993a, 1993b, 1994) finding genetic variation in the small sub-unit (SSU) of ribosomal deoxyribonucleic acid (rDNA) expressed by the presence or absence of introns. Other reindeer lichens such as, *Cladonia arbuscula* and *Cladonia subtenuis* have been found to contain genetic variation in the presence/absence of introns (Robertson and Piercey-Normore, in press) and in the intron sequence (Printzen and Ekman, 2003).

Few studies have examined genetic variation in algal bionts. Kroken and Taylor (2001) studied two species of *Letharia,* a fruticose lichen in western North America, and found genetic variation of the algal bionts. There were six to seven phylogenetic species of *Trebouxia jamesii* associating with five of the six phylogenetic species of *Letharia*.

There were also three phylogenetic species of *Trebouxia jamesii* found only in two species of *Letharia* in California (Kroken and Taylor, 2001). Romeike *et al*. (2002) investigated four species of *Umbilicaria*, a foliose lichen, from the Antarctic Penninsula. There were five distinct haplotypes of *Trebouxia jamesii* found, based on the ITS region between 480 and 660 base pairs (bp), associating with 4 species of *Umbilicaria*, (Romeike *et al*., 2002).

Global Warming

Studies in genetic population structure of species provide information for management considerations and toward an understanding of the effects of global warming. Baseline data on microsite preference, population structure and genotypic diversity of bryophytes and lichens will allow for future comparisons. Inhabiting tundra regions, which are believed to be the first to show signs of global climate change (Hansen and Lebedoff, 1987), bryophytes and lichens are the most prominent organisms and distributional changes may be a result of increasing global temperatures.

The implications of increased temperature, characteristic of global warming, have been demonstrated in oceanic habitats. A reduction in the density of symbiotic algae in coral reefs has been directly correlated to low and high levels of ultraviolet (UV) light (Banaszak and Trench, 1995; Lesser and Schick, 1989). Increased oceanic temperature, as a result of global temperature increases, causes coral to become bleached because the algae cannot survive the higher temperatures. Studies by Douglas (2003) and Kinzie *et al*. (2001) have directly correlated increasing temperature with the decline in *Symbiodinium* algae, the symbionts in corals.

Global warming is expected to have an influence on areas of high latitudes, such as open tundra (Hansen and Lebedoff, 1987). The climate in the tundra severely limits all plant growth to within 30 cm of the ground (Scott, 1995). Ultimately, the plants are exposed to higher temperatures as a result of high levels of UV radiation, and should exhibit the greatest impact. Knowledge of the present distribution and genetic variation of tundra species will aid in future studies where their distributions may be shifted north due to increasing temperatures.

Chapter 2

General Materials and Methods

Study Site and Sampling

Field work was carried out in Wapusk National Park for a period of six days in June, 2005. Helicopter access to permanent Parks Canada camp Nestor I was provided by Parks Canada. The study area was beach ridges and fens within walking distance of Nestor I. The sites to be sampled were all located on the tundra in the northeastern region of Wapusk National Park near Nestor I (Table 2.1). They were located approximately 7 km or greater from the western coast of Hudson Bay. Sites were located randomly as to ensure no bias in the sampling regime and the sites were approximately 1-2 km apart. Fourteen, 75 m transects were surveyed perpendicular to the length of beach ridges. This layout ensured that the transition from fens to ridges were sampled. 0.5 m x 0.5 m plots were surveyed every 7.5 m along each of the fourteen transects, generating 140 plots. Site 1 consisted of transects 1, 2, and 3; Site 2 consisted of transects 4, 5, and 6; Site 3 consisted of transects 7, 8, 9, 10 and 11; and Site 4 consisted of transects 12, 13, and 14, (Figure 2.1).

Soil type and pH were determined for each plot that occurred in a fen or on a ridge top for each transect. The Manitoba Forest Ecosystems Classification (FEC) guide (www.mb.gov.ca) was utilized for identification of soil type. The pH was determined by mixing approximately 10 grams of soil with distilled water. After a homogenous mixture was generated from mixing, the pH was measured with pH strips accurate to within one tenth of a point between pH 6 and 7 (Fischer Scientific). The percent cover of bryophytes, lichenized fungi, vascular plants, grasses, bare soil, rock, wood and detritus were determined. The percent cover was assessed by the following classes; 1-0 to 20%,

Figure 2.1: Transect locations in Wapusk National Park, Manitoba. Transects are represented by 'T' from 1 to 14. Light coloured areas are beach ridges, dark areas are fen areas and light areas are inland lakes and bogs. Nestor I was located between transects 1 and 14 on the beach ridge.

Table 2.1: Latitude and longtitude locations of the 14 sampled transects in northeastern coastal regions of Wapusk National Park, Manitoba. The letters **B** and **E** denote the beginning and end of transects, respectively.

Site	Transect	Latitude	Longtitude
1	1 B	N 58 40' 2.1"	W 93 11' 15.1"
	1 E	N 58 40' 3.79"	W 93 11' 18.06"
	2 B	N 58 40' 11.8"	W 93 11' 21"
	2 E	N 58 40' 11.8"	W 93 11' 21.4"
	3 B	N 58 40' 11.8"	W 93 11' 21.4"
	3 E	N 58 40' 11.8"	W 93 11' 24.4"
2	4 B	N 58 41' 11.22"	W 93 11' 16.73"
	4 E	N 58 41' 13.52"	W 93 11' 18.49"
	5 B	N 58 41' 12.7"	W 93 11' 21.7"
	5 E	N 58 41' 10.14"	W 93 11' 20.58"
	6 B	N 58 40' 57.7"	W 93 11' 29"
	6 E	N 58 40' 57.61"	W 93 11' 19.97"
3	7 B	N 58 40' 43.6"	W 93 11' 30.7"
	7 E	N 58 40' 43.4"	W 93 11' 26.1"
	8 B	N 58 40' 37.9"	W 93 11' 38.8"
	8 E	N 58 40' 37.7"	W 93 11' 43.5"
	9 B	N 58 40' 42.3"	W 93 11' 56.6"
	9 E	N 58 40' 41.8"	W 93 11' 52.2"
	10 B	N 58 40' 53.1"	W 93 12' 10.5"
	10 E	N 58 40' 53.4"	W 93 12' 6.2"
	11 B	N 58 40' 55.6"	W 93 12' 15.1"
	11 E	N 58 40' 58"	W 93 12' 15.5"
4	12 B	N 58 38' 49"	W 93 11' 30.8"
	12 E	N 58 38' 49.7"	W 93 11' 35.2"
	13 B	N 58 38' 40.3"	W 93 11' 46.7"
	13 E	N 58 38' 40.3"	W 93 11' 51.4"
	14 B	N 58 38' 55"	W 93 11' 51.3"
	14 E	N 58 38' 54.6"	W 93 11' 46.8"

2-20 to 40 %, 3-40 to 60 %, 4-60 to 80% and 5-80 to 100%. If any section of a plant was within the boundary of the plot, it was sampled as if were entirely in the plot.

All bryophytes, lichens, and vascular plants were initially identified to species using field guides and a hand lens. Those species that could not be readily identified in the field were collected to be determined later. Vouchers of all lichens were collected and brought back to the lab for analysis utilizing a dissecting microscope and appropriate chemical tests. *Thamnolia subuliformis, Thamnolia vermicularis* and *Dicranum elongatum* specimens were collected in every plot encountered. All specimens collected were placed in paper bags and labelled with the site, transect and plot number. The samples were stored at room temperature and air dried at Nestor I throughout the sampling period. Upon returning to Churchill, the samples were placed in a drier for 12 hours at 80ºC at the Parks Canada compound.

Lab Methods

DNA Isolation

Individual lichen podetia or moss gametophytes identified as either *Thamnolia subuliformis* or *Dicranum elongatum* by morphological characteristics and in the case of the lichen, UV tests, were used for DNA extraction. The CTAB-DNA extraction protocol was modified from Grube *et al*. (1995). Lichen tissue samples were ground to a homogenous solution in N-Tris (hydroxymethyl) methyl-2-aminoethanesulfonicd acid (TES) buffer [250 µL 1M Tris-HCl (pH 7.5), 100 µL 0.5M ethylenediaminetetraacetic acid (EDTA), 250 µL 20% sodium dodecyl sulfate (SDS), 400 µL sterile distilled H_20]. Bryophyte tissues were ground to a powder in liquid nitrogen prior to the addition of the

TES buffer. The separation of DNA from cells was accomplished by warming the solution, with 70µL 10% CTAB (cetyltrimethyl ammonium bromide) and 140 µL 0.5M NaCl, to 65°C for a period of 1 hour. The solution was extracted two times with chloroform:isoamyl alcohol (24:1). The DNA was precipitated in 0.2 vol. of 0.5M NaCl and 2.5 vol. of 100% Ethanol (EtOH) and resuspended in 50 µL of sdH_2O. DNA was examined and quantified on a 1% agarose gel stained with ethidium bromide (EtBr). All samples were extracted yielding a sample size of n=95 for *Thamnolia subuliformis* and n=37 for *Dicranum elongatum*.

PCR Amplification

Amplifications of DNA fragments from the fungus and bryophyte were performed in 20 µL reaction solutions for scoring and restriction endonuclease digestions, and in 300 µL volumes for DNA sequencing. Amplification reaction mix consisted of amplification buffer [200 µM Trizma base (pH 8.4) and 500 µM potassium chloride], 200 µM of each deoxyribonucleotide triphosphate (dNTP), 2.0 µM $MgCl_2$, 0.5 µM of primer, 2.5 units of Taq DNA polymerase (Invitrogen, Burlington, ON, Canada) and 10-50 ng of DNA. In this study interspersed simple sequence repeats (ISSR) (UBC primer set #9, Nucleic Acid Protien Service Unit, University of British Columbia) were utilized to screen for molecular variation in *Thamnolia subuliformis* (see Chapter 4) and *Dicranum elongatum* (see Chapter 3). Sequencing protocols can also be found in Chapter 4.

Electrophoresis and Quantitation

DNA extractions, fungal PCR product prior to digestion, and purified PCR product for sequencing were quantified using agarose gel electrophoresis. Gels contained 1 to 1.5 % agarose (Fisher Scientific, Fair Lawn. NJ) in 1X TBE (0.089M Tris, 0.089M boric acid, 2 mM EDTA) buffer and were stained with 25 mg/ mL ethidium bromide (EtBr). DNA extracts were mixed with 6X bromophenol blue (BPB) loading dye and run at between 120 and 140 volts. Images of the gels were taken using the AlphalImager™ 2200 UV transilluminator (Fisher Scientific, Nepean, ON, Canada).

The 1650 base pair (bp) band contains 8% of the mass (ng) on a gel when using 1 Kb Plus DNA Ladder (Invitrogen, 9885 Town Centre Dr., San Diego, California). Comparing the intensity of bands within sample lanes with this reference allowed for the quantity of DNA to be estimated.

Haplotype Scoring and Genetic Analysis

Amplified fungal and bryophyte microsatellite bands were scored as binary and haplotype (the genetic makeup of a single chromosome) data and analyzed using analysis of molecular variance (AMOVA) in GenAlEx 6.0 (Peakall and Smouse, 2005) with a genetic distance resemblance metric. Variation was analyzed among transects (ϕ_{PT}), which is analogous to F_{ST} (Weir and Cockerham, 1984) when studying diploid organisms. Cluster analysis of the binary microsatellite datasets were each analyzed individually by the use of HEIRCLUS in Syntax 5.0 (Podani, 1994). Syntax was also employed to generate minimum spanning trees for haplotype data of each microsatellite primer, in both the fungal partner of *Thamnolia subuliformis* and the bryophyte

Dicranum elongatum. Haplotypes were mapped onto geographic maps of the northeast corner of Wapusk National Park. UPGMA dendrograms, principal coordinates analyses and non-netric multidimensional scaling were done using ORDIN in Syntax.

Chapter 3

Population structure of *Dicranum elongatum* in northeastern coastal regions of Wapusk National Park, Manitoba

The traditional view of bryophyte populations is that they have a reduced level of genetic variation compared to vascular plants (Derda and Wyatt, 1999), due to the dominant haploid phase of the life cycle (Crum, 1972). This reduction in variation is a result of the range of haplotypes being reduced by the presence of only one allele of each gene (Longton, 1976; Ennos, 1990). As a result of reduced genetic variability and similar morphological characteristics of extinct and extant taxa (Mishler, 1988; Wyatt *et al*. 1989), bryophytes have been referred to as 'evolutionary failures' (Crum, 1972) and show a low evolutionary rate (Ennos, 1990). According to Longton (1985) organisms that have been in existence since the Devonian and are the second largest group of land plants are "some failures!".

A low level of genetic variation in bryophytes is anticipated to be a reflection of their mode of reproduction (Van Der Velde *et al*. 2001). Dioicous species rarely produce sporophytes as a result of the low probability of male and female gametophytes to be found growing together (Longton, 1976; Mischler, 1988). As a result, asexual reproduction is believed to occur frequently in bryophytes, leading to low genetic variability (Mishler, 1988). Monoicous species, in contrast, are assumed to have low levels of genetic variation due to the high frequency of self-fertilization (Longton, 1976; During, 1990). As a result of the assumed low genetic variation in bryophytes, they have received little consideration in population genetic studies (Ennos, 1990).

In contrast to the traditional view of bryophytes, considerable variation has been encountered using isozymes (Daniels, 1985; Wyatt et al. 1989; Cronberg et al. 1997; Derda and Wyatt, 1999; Van Der Velde and Bijlsma, 2000; Cronberg, 2002) RAPDs

(Selkirk et al. 1997; Stenoien and Sastad, 1999; Skotnicki et al. 2000; Stenoien and Flatberg, 2000) and ISSR microsatellites (Van Der Velde et al. 2001a; Van Der Velde et al. 2001b; Hassal and Gunnarsson, 2003; Gunnarsson et al. 2005) suggesting they are just as genetically diverse as the angiosperms (Stoneburner et al. 1991; Wyatt and Derda, 1997). To date, few studies of genetic population structure in arctic and subarctic-alpine plants and bryophytes have been carried out (Molau, 1993).

Dicranum elongatum, being found in the Low Arctic tundra regions of northeastern coastal Wapusk National Park, and being sensitive to environmental changes makes it a prime candidate for study. In open tundra and fen areas, *D.elongatum* forms hummocks, which create microhabitats for many other vascular plants, bryophytes and lichens (Ford *et al*. 2002). *Dicranum elongatum* is found throughout the northern hemisphere, where male and female gametangia are found on separate gametophytes. Because *D.elongatum* is dieocious, the transfer of sperm to archegonia is difficult (Longton, 1985) and consequently sporophytes are rarely produced (Ireland, 2002). Acording the Ireland (2002), no specialized vegetative propagules are produced aside from gametophyte fragments. The present study, therefore, aims at describing the population structure of *D.elongatum* in this region of Wapusk National Park by the use of ISSR microsatellite primers.

Materials and Methods

One sample of *Dicranum elongatum* was collected from each of 37 plots, comprising 11 transects, in northeastern coastal regions of Wapusk National Park. In this study, transects were treated as individual populations, and will be referred to as such.

An alternative analysis was performed by grouping 3 transects together as a population. Total genomic DNA was extracted using a protocol modified from Grube *et al*. (1995) and outlined in Chapter 2. Prior to extraction, gametophytic stems were washed with sterile distilled H_20 to remove any parastic fungi or algae, to prevent contamination by foreign DNA (Hassel and Gunnarson, 2003). The amplification of inter SSR sequences of *D. elongatum* was with ISSR microsatellite primers (UBC). For each microsatellite primer utilized, annealing temperature, $MgCl_2$ concentration gradients, as well as dilution series of template DNA were carried out to determine the optimal reaction conditions, (Table 3.1). Amplification conditions on the Techne Genius thermal cycler (Fisher Scientific, Nepean, ON, Canada) and Whatman-Biometra® T-Gradient thermal cycler (Montreal Biotech Inc., Kirkland, PQ, Canada) were as follows: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 60 seconds, annealing at 4 different temperatures for 90 seconds depending on the melting temperature of the primer (see Table 3.1), and extension at 72°C for 120 seconds.

Table 3.1: Primers used in ISSR-PCR of *Dicranum elongatum* DNA, showing primer sequences, annealing temperatures (T_m) and optimal mass (ng) of DNA used in the final reaction (diluted with sdH_2O), and optimal $MgCl_2$ concentration used in the final reaction.

Primer	Sequence	T_m (°C)	DNA Mass (ng)	$MgCl_2$ Concentration
ISSR 808	AGA GAG AGA GAG AGA GC	49	10-50	1.5 mM
ISSR 811	GAG AGA GAG AGA GAG AC	51	10-50	3.5 mM
ISSR 826	ACA CAC ACA CAC ACA CC	51	10-50	1.5 mM
ISSR 834	AGA GAG AGA GAG AGA GYT	49	10-50	1.5 mM
ISSR 836	AGA GAG AGA GAG AGA GYA	49	10-50	1.5 mM
ISSR 840	GAG AGA GAG AGA GAG AYT	49	10-50	3.5 mM
ISSR 842	GAG AGA GAG AGA GAG AYC	55	10-50	3.5 mM

Total genomic DNA and PCR products were visualized by agarose gel electrophoresis (outlined in Chapter 2). Agarose gel electrophoresis of amplified PCR products for each of the seven microsatellite primers were scored to produce a binary data set and a haploid data set. The binary data set was scored by the presence or absence of bands at each length on the gel. The haplotype dataset was scored by comparing all bands between samples, to designate different haplotypes. This binary dataset and the corresponding haplotype dataset were analyzed using analysis of molecular variance (AMOVA) in GenAlEx 6.0 for Excel. The genetic distance between all samples created a resemblance matrix which was then subjected to a within and between analysis of designated populations. All other multivariate analyses, including the UPGMA cluster analysis, were produced using Syntax 5.0 (Podani, 1994).

Results

Dicranum elongatum was found in 37 plots in 11 out of 14 transects. Transects 2, 6 and 12 contained no species of *Dicranum*. Sites were distinguished as being those transects that were spatially clustered together (Figure 3.4). Plots where *D.elongatum* was sampled were occasionaly on the sides of beach ridges, but were more common on plateaus near inland lakes and fens. Within sampled plots, *D.elongatum* was only encountered growing as hummocks, usually supporting an array of other vegetation including: vascular plants- *Andromeda polifolia, Arctostaphylos rubra, Ledum decumbens, Pinguicula vulgaris, Rhododendron lapponicum*; lichens-*Cladonia amaurocraea, C. arbuscula, C. pocillum;* lichens on vascular plants-*Lecanora circumborealis, Micarea mileana, Ochrolechia upsaliensis, Peltigera rufescens,*

Pertusaria dactylina, Rinodina turfacea; lichen fragments (lichens not attached to any substrate)- *Alectoria ochroleuca, Bryocaulon divergens, Cetraria islandica, F.cucullata, Flavocetraria nivalis, Sphaerophorus fragilis, Stereocaulon rivulorum, Thamnolia subuliformis;* and bryophytes- *Aulocomium acuminatum, A. palustre, A. turgidum, Drepanocladus revolvens, Encalypta rhaptocarpa, Hylocomium splendens, Pleurozium schreberi, Pohlia nutans,* and *Tomenthypnum nitens.* For a more detailed account of the flora in northeastern coastal regions of Wapusk National Park, see Chapter 5.

Microsatellite Polymorphism

The seven ISSR primers revealed a total of 46 scorable bands of different lengths (Table 3.2) amongst the 37 samples, from 11 populations. Bands were considered polymorphic if they were present or absent between different samples. Of the 46 bands, 35 (79.54%) bands were polymorphic (variable) and 11 (21.46%) were monomorphic. Five of the primers (808, 826, 834, 836, 840) encompassing 30 bands, showed polymorphic levels greater than or equal to 75 %. Three primers (808, 826 and 836) exhibited all scored bands to be polymorphic. Primers 811 and 842 had lower levels of polymorphism (42.86 % and 55.55 %, respectively) due to the presence of 4 monomorphic bands.

Based on the individual primer haplotypes (Table 3.3), the greatest number of haplotypes (7) were encountered with primer 826 and population 11. Populations 10 and 11 showed the greatest number of haplotypes for all primers and population 10 showed the highest number of unique haplotypes. Three of the seven banding patterns can be seen in Figure 3.1.

Table 3.2: ISSR primers used in this study of *Dicranum elongatum*, with number of bands scored, number of poly- and monomorphic bands, base pair (bp) range and percent polymorphism. Percent polymorphism was calculated by dividing the number of polymorphic bands by the total number of bands.

ISSR Primer	Number of scored bands	Number of polymorphic bands	Number of monomorphic bands	Bp range	Percent polymorphism
808	6	6	-	300-750	100
811	7	3	4	300-700	42.86
826	5	5	-	200-700	100
834	6	5	1	200-650	83.33
836	5	5	-	250-700	100
840	8	6	2	200-1000	75
842	9	5	4	200-700	55.55
Total	46	35	11	---	79.53

Table 3.3: Haplotype data for *Dicranum elongatum* for the seven ISSR primers.

(Pop.#=population number or transect, n=sample size)

		Number of Haplotypes							Number of Unique Haplotypes*						
Pop.#	*n*	808	811	826	834	836	840	842	808	811	826	834	836	840	842
1	5	5	3	3	2	3	3	4	2	--	1	---	1	1	1
3	2	1	1	1	1	1	1	1	1	--	---	1	1	---	---
4	2	1	1	1	1	1	1	1	---	--	1	---	---	---	1
5	3	3	2	1	3	2	3	2	2	--	---	---	---	1	1
7	2	1	1	1	1	1	1	1	---	--	---	1	---	1	---
8	2	1	1	1	1	1	1	1	1	--	---	---	---	1	---
9	2	2	1	2	2	2	2	1	---	--	---	---	1	---	---
10	7	5	4	4	4	3	6	5	4	--	1	1	1	3	2
11	8	5	3	5	7	3	5	5	3	--	1	1	1	---	1
13	2	2	2	2	2	1	2	2	1	1	1	---	---	2	2
14	2	2	1	2	1	2	1	2	---	---	2	---	1	---	---

(* = haplotypes were considered unique only if they were encountered once in the entire study within that population)

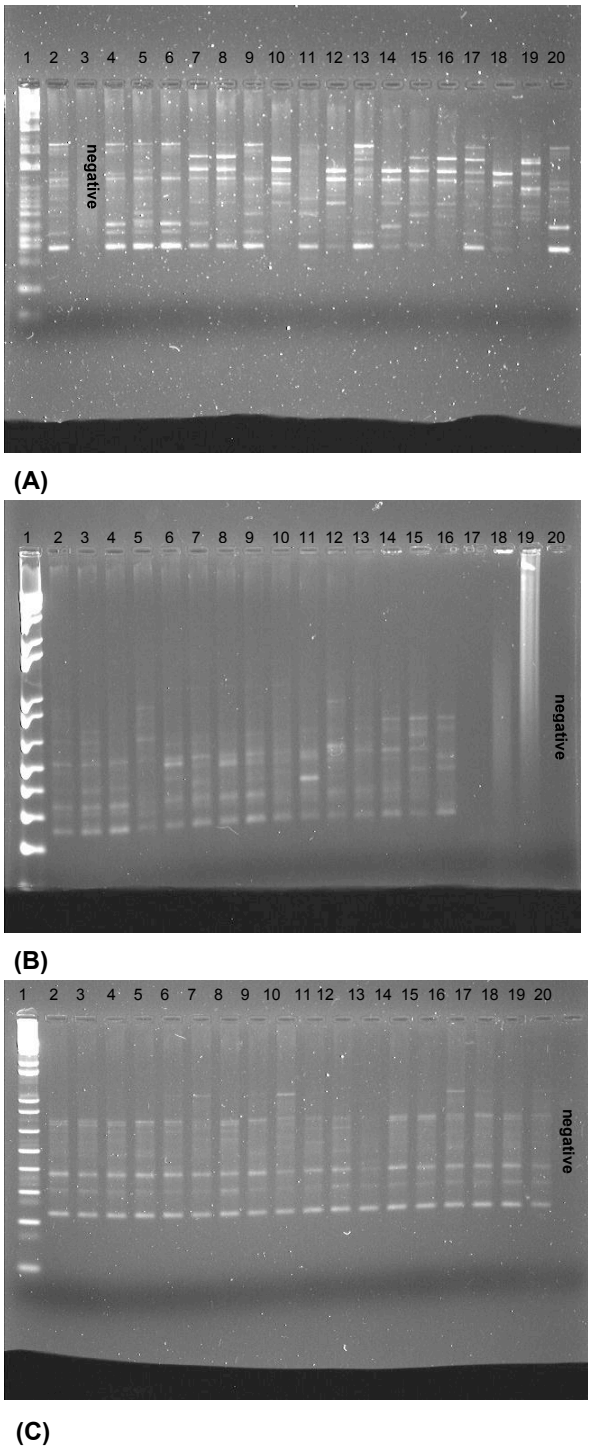


Figure 3.1: Banding patterns of primers 836, 834 and 840 for *Dicranum elongatum* in northeastern coastal regions of Wapusk National Park, Manitoba. PCR products were run on 1.5% agarose gels stained with EtBr at 120 volts for 90minutes. (A) 836, (B) 834 and (C) 840. Lane 1 contains 1 Kb DNA ladder and lanes 2-20 contain samples.

It appears that as the number of samples/population increases, the number of haplotypes increases accordingly. Populations 3, 4, 7, 8, 9, 13 and 14 all have a sample size of n=2 and the number of haplotypes is ≤ 2. In all the sampled populations, the number of haplotypes appears to be 1 or 2 less than the sample size. The number of haplotypes encountered across all populations in primers 808, 826, 834 and 836 were 19 (n=37), 15 (n=37), 12 (n=37), and 11 (n=37), respectively (Figure 3.2).

Accordingly, these four primers had the highest polymorphic levels based on the binary data. There is a direct relationship ($r = 0.85$, $p=0.047$) between the level of polymorphism and the number of haplotypes encountered. The UPGMA dendrograms (Figure 3.2) show that primer 808 with 19 haplotypes has only 1 which occurs close to 20 % ; 826 with 15 haplotypes has 1 occurring 29.73%; 834 with 12 haplotypes has all less than 20% and 836 with 11 has 1 occurring 24.32% and 2 others at 18.91 % and 16.21 %. In essence, no haplotype is encountered more than 30% in any of the primer analyses. The most common haplotypes found with each primer (808, 826, 834 and 836) are found consistently in populations 7, 10 and 11 followed by populations 8 and 9 with a slightly lower frequency.

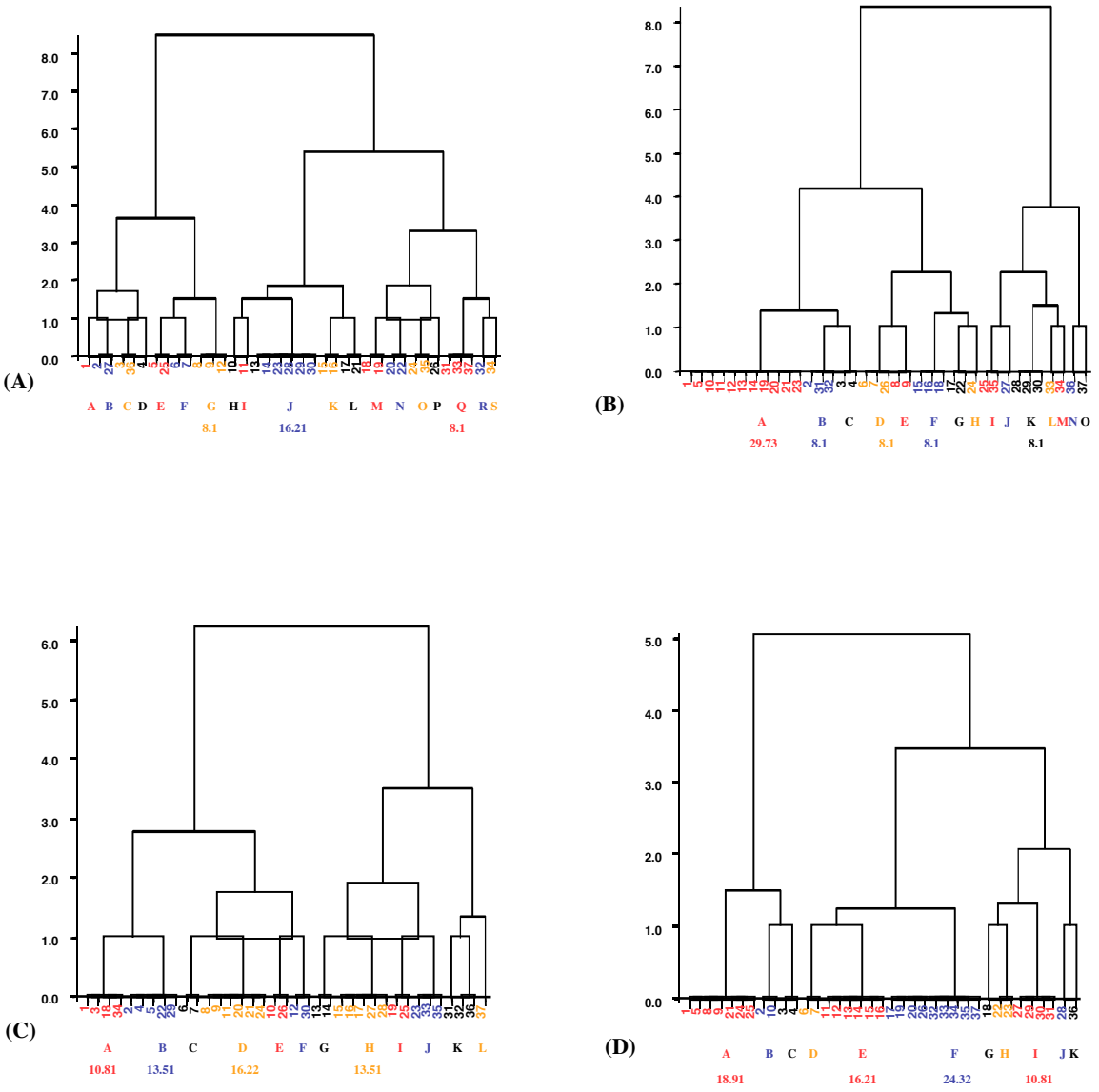


Figure 3.2: UPGMA dendrograms of n=37 samples of *Dicranum elongatum* for primers (A) 808, (B) 826, (C) 834 and (D) 836 based on haplotype data. Letters indicate different haplotypes, numbers are haplotype % (only >8% shown). Colors are for ease of cluster identification. Clustered numbers are samples and correspond to populations (Pop.1=1-5; Pop.3=6,7; Pop.4=8,9; Pop.5=10-12; Pop.7=13,14; Pop.8=15,16; Pop.9=17,18; Pop.10=19-25; Pop.11=26-33; Pop.13=34,35 and Pop.14=36,37).

Population Subdivision

All microsatellite primers had significant ϕ_{PT} values ($p < 0.05$) in the AMOVA analysis, based on both binary and haplotype data of 11 populations, Table 3.4. Significant ϕ_{PT} values suggest that populations are subdivided. Primer 836 showed the highest degree of subdivision or among population variation, for binary data (ϕ_{PT}=0.503) as well as haplotype data (ϕ_{PT}=0.328).

Primers 840 and 826 exhibited subdivision levels (ϕ_{PT} =0.460 and 0.414, respectively) lower than primer 836 (ϕ_{PT} =0.503). The within population variation for primer 808 haplotype data was the highest at 82 %, which may reflect the larger number of haplotypes encountered with primer 808 than other primers. The low degree of among population variation in primer 808 (ϕ_{PT}=0.181) suggests that these 19 haplotypes are unique to specific populations (Figure 3.2). The within population variation based on haplotype data, for all individual primers, ranged from 67 to 82 %, and the among population variation ranged from 18 to 33 %.

In the combined analysis of all primer haplotype datasets, the minimum spanning tree (Figure 3.3) illustrates the clustering of samples with identical haplotypes. The most common haplotype H (29.73%) is found in populations 7, 10, 11 and 13. The AMOVA analysis of all primers combined revealed levels of population subdivision (ϕ_{PT}=0.249, p=0.01) (Table 3.4) similar to those for the individual primers. The among and within population variation for all combined primers were 25% and 75%, respectively. A subsequent AMOVA analysis, Table 3.5, yielded similar results but was based on 4 populations.

Table 3.4: AMOVA analysis of *Dicranum elongatum* ISSR primers. Analysis was on n=37 samples (comprising 11 populations) with 9999 permutations on both binary and haplotype datasets. (* indicates significant p-values at the 95 % level, Among=among populations, Within=within populations, df=degrees of freedom, SS=total sum of squares, MS=mean sum of squares).

						Binary Data	Haplotype Data
ISSR Primer	**Df**	**SS**	**MS**	**Estimated variance**	**Percent Variance**	ϕ_{PT}	ϕ_{PT}
836 Among	10	18.7	1.87	0.443	50	**0.503***	----
836 Within	36	11.4	0.44	0.437	50	**----**	----
836 Among	10	7.86	0.79	0.149	33	**----**	**0.328***
836 Within	36	7.93	0.31	0.305	67	**----**	**----**
808 Among	10	22.5	2.25	0.368	26	**0.258***	**----**
808 Within	36	27.5	1.06	1.059	74	**----**	----
808 Among	10	6.83	0.68	0.088	18	**----**	**0.181***
808 Within	36	10.4	0.4	0.398	82	**----**	**----**
826 Among	10	21	2.1	0.453	41	**0.414***	**----**
826 Within	36	16.7	0.64	0.64	59	**----**	----
826 Among	10	7.84	0.78	0.145	31	**----**	**0.313***
826 Within	36	8.24	0.32	0.317	69	**----**	**----**
834 Among	10	18.7	1.87	0.347	32	**0.315***	**----**
834 Within	36	19.6	0.75	0.752	68	**----**	----
834 Among	10	7.15	0.72	0.108	23	**----**	**0.228***
834 Within	36	9.5	0.37	0.366	77	**----**	**----**
811 Among	10	7.62	0.76	0.118	24	**0.237***	**----**
811 Within	36	9.9	0.38	0.381	76	**----**	----
811 Among	10	5.61	0.56	0.087	24	**----**	**0.237***
811 Within	36	7.29	0.28	0.28	76	**----**	**----**
840 Among	10	23.7	2.37	0.538	46	**0.460***	**----**
840 Within	36	16.4	0.63	0.632	54	**----**	----
840 Among	10	7.49	0.75	0.119	25	**----**	**0.247***
840 Within	36	9.46	0.36	0.364	75	**----**	**----**
842 Among	10	16.2	1.62	0.312	33	**0.339***	**----**
842 Within	36	15.8	0.61	0.609	66	**----**	----
842 Among	10	6.48	0.65	0.093	21	**----**	**0.211***
842 Within	36	9.04	0.35	0.348	79	----	**----**

Table 3.5: AMOVA analysis of *Dicranum elongatum* ISSR primers. Analysis was on n=37 samples (comprising 4 populations) with 9999 permutations on both binary and haplotype datasets. (* indicates significant p-values at the 95 % level, Among=among populations, Within=within populations, df=degrees of freedom, SS=total sum of squares, MS=mean sum of squares).

						Binary Data	Haplotype Data
ISSR Primer	**df**	**SS**	**MS**	**Estimated variance**	**Percent Variance**	**PT**	**PT**
836 Among	3	5.71	1.903	0.145	16	**0.164***	
836 Within	33	24.344	0.738	0.738	84		
836 Among	3	2.831	0.944	0.068	15		**0.148***
836 Within	33	12.953	0.393	0.393	85		
808 Among	3	3.302	1.101	0.000	0	**0.000**	
808 Within	33	37.455	1.135	1.135	100		
808 Among	3	1.982	0.661	0.025	5		**0***
808 Within	33	15.207	0.461	0.461	95		
826 Among	3	6.210	2.070	0.139	13	**0.127***	
826 Within	33	31.466	0.954	0.954	87		
826 Among	3	2.318	0.773	0.044	10		**0.096***
826 Within	33	13.763	0.417	0.417	90		
834 Among	3	8.642	2.881	0.246	22	**0.215***	
834 Within	33	29.628	0.898	0.898	78		
834 Among	3	2.276	0.759	0.040	8		**0.084***
834 Within	33	14.372	0.436	0.436	92		
811 Among	3	1.805	0.602	0.016	3	**0.032**	
811 Within	33	15.709	0.476	0.476	97		
811 Among	3	1.356	0.452	0.013	4		**0.035**
811 Within	33	11.536	0.350	0.350	96		
840 Among	3	7.461	2.487	0.186	16	**0.158***	
840 Within	33	32.647	0.989	0.989	84		
840 Among	3	2.305	0.768	0.040	8		**0.083***
840 Within	33	14.641	0.444	0.444	92		
842 Among	3	5.861	1.954	0.144	15	**0.154***	
842 Within	33	26.139	0.792	0.792	85		
842 Among	3	1.615	0.538	0.015	3		**0.033***
842 Within	33	13.898	0.421	0.421	97		

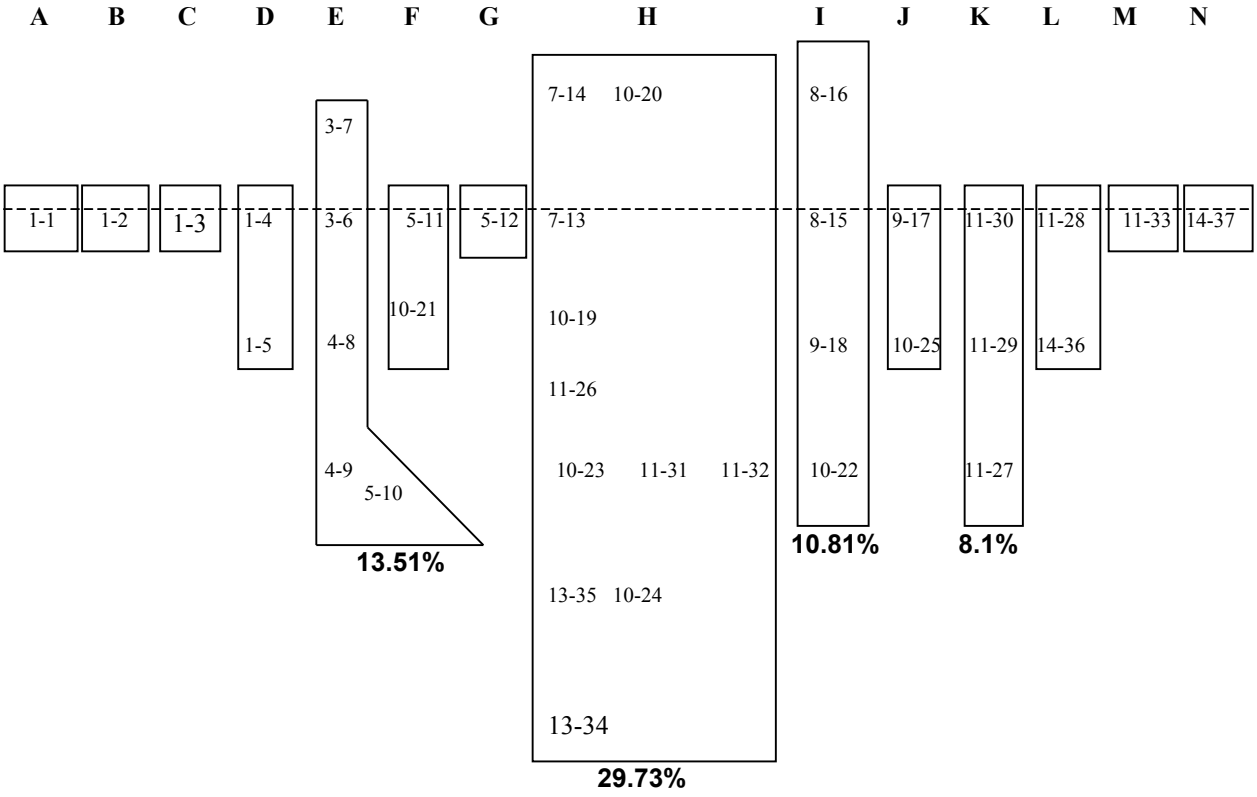


Figure 3.3: Minimum spanning tree of combined haplotypes for *Dicranum elongatum* in northeastern coastal regions of Wapusk National Park. Clustered numbers are populations followed by sample. Letters indicate different haplotypes and numerical values below are the percentage of that particular haplotype (only values >8% are shown).

Pairwise Population Subdivision

Based on the pairwise distance matrix of populations (Table 3.6), populations 7 and 8 show the greatest subdivision (ϕ_{PT}=0.406, p<0.01) and populations 10 and 3 show the second greatest subdivision (ϕ_{PT}=0.350, p<0.01). Populations 10 and 11 show subdivision with all populations except for population 9 and the lowest subdivision (ϕ_{PT}=0.074, p<0.01) with each other, suggesting they are the most similar.

The distribution of haplotypes spatially over the study area (Figure 3.4) revealed interesting results. Seven of fourteen haplotypes are unique (occurring only once) in specific populations (A, B, C, D, G, J, M and N). The other seven haplotypes appear to be rare (occurring in at least two separate populations). Haplotype E seems to be dispersing amongst populations 3, 4 and 5, whereas haplotype F is found in population 5 and 10 only. Haplotype H is the most prominent (29.73%) and is found in populations 7, 10, 11 and 13. Populations 8, 9 and 10 contain haplotype I, and Population 11 contained a unique haplotype K from three different plots. The last haplotype, L, was found in only two populations, 11 and 14.

Table 3.6: Pairwise comparisons of the 11 populations of *Dicranum elongatum* in northeastern coastal regions of Wapusk National Park with 999 permutations. Only significant ϕ_{PT} values are shown. Samples were not found in Pop.2.

	Pop.1	**Pop.3**	**Pop.4**	**Pop.5**	**Pop.7**	**Pop.8**	**Pop.9**	**Pop.10**	**Pop.11**	**Pop.13**	**Pop.14**
Pop.1	0										
Pop.3	---	0									
Pop.4	0.338	0.34	0								
Pop.5	0.118	---	---	0							
Pop.7	---	0.32	0.32	---	0						
Pop.8	---	0.34	0.34	---	0.41	0					
Pop.9	---	---	---	---	---	---	0				
Pop.10	0.058	0.35	0.28	---	0.25	0.31	---	0			
Pop.11	0.094	0.35	0.37	0.17	0.28	0.29	---	0.074	0		
Pop.13	0.148	---	---	---	---	---	---	---	---	0	
Pop.14	---	---	---	---	---	---	---	---	---	---	0

Figure 3.4: Populations (transects) of *Dicranum elongatum* in northeastern coastal regions of Wapusk National Park. Letters indicate the 14 different haplotypes revealed in the combined primer analysis.

Discussion:

Population Subdivision in Dicranum elongatum

The majority of populations of *Dicranum elongatum* are genetically distinct (Table 3.4, 3.5, & 3.6) suggesting that no gene flow is occurring. However, some gene flow was reported (insignificant ϕ_{PT} values in Table 3.4). The low level of gene flow was expected since no sporophytes were observed in this region of Wapusk National Park. The presence of reproduction and sporophytes only in seasons which are favourable may account for the low level of gene flow reported. In addition, populations in these regions of Wapusk National Park may have lost the ability to produce sporophytes when the initial populations dispersed north to these areas.

The results in this study suggest that *Dicranum elongatum* reproduces only rarely by sexual means (by meiotic spores) and more commonly by asexual means (gametophyte fragmentation) in northeastern coastal regions of Wapusk National Park. Populations of *D.elongatum* contained high levels of variation within population (67-82%, 75% in the combined analysis) and genetic variation among population (18-33%, 25% in the combined analysis, Table 3.4). High variation is not typical of the traditional view of low genetic variation in haploid bryophytes (During, 1990). Some species exhibiting a low level of variation are as follows: *Polytrichum formosum* Hedw. in Danish and Dutch populations using SSR's and allozymes (Van der Velde *et al.* 2001; Van der Velde and Bijlsma, 2000), *Hylocomium splendens* (Hedwig) Schimper in Baltic uplift islands using allozymes (Cronberg, 2002), and in Scandinavia using allozymes (Cronberg, 1997). However higher levels of variation similar to those of vascular plants have been reported in other bryophytes such as: *Plagiomnium ciliare* (C. Muell.) Kop.

(Wyatt *et a*l. 1989), *S. capillifolium* (Natcheva and Cronberg, 2003), and *Polytrichum commune* (Derda and Wyatt, 1999) using isozymes, *Sphagnum angeranicum* (Gunnarsson *et al*. 2005) using ISSR, *S. troenelagicum* (Stenoien and Flatberg, 2000), *S.angustifolium* and *S. fallax* (Stenoien and Sastad, 1999), *Sarconeurum glaciale* (C. Muell.) Card. and Bryhn (Selkirk *et al*. 1997), and *Bryum argenteum, B. pseudotriquetrum, Ceratodon purpureus, Hennediella heinnii* and *Saconeurum glaciale* (Skotnicki *et al.* 2000) using RAPDs.

Dispersal in Dicranum elongatum

A few identical haplotypes were distributed among populations in the study area that were geographically distant from one another, suggesting that dispersal was occurring or had historically occurred. The level of genetic homogeneity between these populations is indicative of efficient dispersal and maintenance of allelic variation. Populations 3, 4 and 5; 7, 10, 11 and 13; and 8, 9 and 10 appear to have efficient gene flow between them (Figure 3.4). The method of gene flow cannot be assessed. Vegetative propagules in the form of fragments are common in this species (Ireland, 2002), and may be a mode of dispersal in northeastern regions of Wapusk National Park. Spores from sexual reproduction may be arriving from distant populations out of the study area, but were not encountered locally because sporophytes were not encountered in this study. This may have been a result of the time of year (May) that sampling took place. Examination of other specimens of *Dicranum elongatum* collected in more southerly populations in Wapusk National Park from the University of Manitoba herbarium, it became evident that sporophytes on *Dicranum elongatum* are produced

within the park. Populations around Owl River (57° 50'N, 93 49'W) and Skidmore Lake (58° 47' 31" N, 93 22' 56" W) contained sporophytes around June 25th, 2005. Young sporophytes should have been visible in May if they were developing. Another explanation for the absence of sporophytes may be the populations are too close to the coast and the environment is suboptimal for development.

Populations 7, 10 and 11 (Figure 3.4) are situated in fairly close proximity to one another (1-1.5 kms) when compared to the entire sampling area (9-10 kms). Population 7 is the most easterly on the beach ridge, while 10 and 11 are more westerly in a lower fen area. It seems plausible that spores could be blown between these populations if spores were produced. Population 3 is separated from population 4 and 5 by about 5 kms. These three populations are located on a north-south line parallel with the beach ridge which may facilitate spore dispersal. Populations 8, 9 and 10 are in close proximity to each other so spore dispersal may have a higher probability of occurring. Small spores, if exposed to low temperatures and high ultraviolet radiation, have a reduced viability (as a result of dessication) for germination after long distance dispersal (Van Zanten 1976, 1978 and 1984), yet experimental results support the notion that the majority of spores are deposited only within a couple of meters of the parent sporophyte and fewer are deposited with increasing distance (McQueen 1985, Soderstrom and Jonsson 1989, Kimmerer 1991, Miles and Longton 1992, Stoneburner et al. 1992, Wyatt 1977, and Miles and Longton 1987). Although dispersal and successful germination of spores has not been documented under natural conditions (Longton 1976), sexual reproduction has been supported by parentage studies (Van der Velde et al. 2001) and may be occurring in this region of Wapusk National Park. In the absence of sporophytes, sexual reproduction

seems to be illogical. Meiotic spores may be arriving via long distance dispersal, accounting for the multilocus similarities between populations 3, 4 and5; 7, 10 and 11; and 8, 9, and 10.

It is thought to be difficult for dioicous species such as *D.elongatum* to produce meiotic spores because of the spatial separation of the male and female gametophytes (Longton, 1976). Male gametes must swim through water to reach the archegonium (Wyatt, 1982) and it is believed that these distances can be no more than a couple of centimetres (Longton, 1976). Since male and female gametophytes grow in close proximity in *D.elongatum* as hummocks, fertilization may be possible. Hummocks may act as sponges, that may create the liquid medium necessary for gamete transfer.

The distribution of unique haplotypes (A, B, C, D, G, J, M and N) (Figure 3.4) suggests that dispersal of asexual fragments may not be common between populations. The distribution of these haplotypes is too far apart to suggest dispersal of gametophyte fragments by wind, which are known to disperse only within a couple meters of the parent gametophyte (Longton 1976). Asexual reproduction may be responsible for population growth and persistence on a local scale or within populations. Three different plots, each separated by 7.5 meters, in population 11 revealed the same haplotype, which was unique to this particular population. This would suggest that spore arrival may have generated the population, but asexual fragmentation has generated new gametophytes on the local scale. This process agrees with Kimmerer (1993) who found that asexual reproduction predominates in colony maintainance, while sexual reproduction is more effective in establishing new colonies. In contrast to this situation, vegetative propagules may be dispersing between populations via rodent and caribou vectors.

Population Growth

Populations 10 and 11 had the greatest number of haplotypes of all populations. One explanation is that these populations of *D.elongatum* are receiving genetic material from numerous other distant sources. Upon spore arrival and germination into a new gametophyte, asexual fragmentation may be involved in population expansion. These new gametophytes may also produce gametangia and interbreed with other gametophytes in the population. This process may generate new patterns of the alleles for the population, which may then expand by means of asexual fragmentation, generating the distribution of haplotypes observed. It is also plausible that closer populations, outside the study populations, are a source of some genetic variation in populations 10 and 11. These closer populations may contain haplotypes different from those reported in this study. Gametophyte fragments carried by animals may be sources of gene flow to these populations. Gametophyte fragments are known to disperse by wind only within meters of the parent gametophyte (Longton 1976). Animal movement, however, may facilitate further dispersal distances of gametophyte fragments. Rodents, such as mice or lemmings, burrowing in hummocks may catch gametophyte fragments in their fur. Their movement over the tundra landscape may deposit these fragments, thereby generating spatially segregated populations with the same haplotype. Beach ridges serve as caribou migration routes. The destructive nature of caribou hooves may fragment mosses in hummocks thereby liberating gametophyte fragments. These fragments may then adhere to fur of the caribou. As migration proceeds, possibly to the lower fen areas of populations 10 and 11, these fragments may fall off and generate new gametophytes (and haplotypes) in these populations.

Significance of Allelic Variation

Thus far, the distribution of haplotypes has been assessed and the modes of dispersal have been postulated. The main question remaining is where does the variation encountered in *D.elongatum* come from? Persisting predominantly as a haploid gametophyte, there is only one allele of each gene present in the plant. This directly exposes recessive deleterious mutations to the forces of natural selection (Wyatt *et al*. 1989, Longton 1976). These recessive alleles cannot be masked as heterozygotes as in the diploid sporophyte (Ennos, 1990). *Dicranum elongatum* is subjected to high direct levels of ultraviolet radiation in low Arctic tundra regions of Wapusk National Park. High light levels may induce a higher level of recombination, yielding levels of variation found in this study. Extreme temperatures, greater than 80°C, are known to cause mutations (Schmaulhausen 1949; Sankaranarayanan 1982; Walker 1984) as well as higher rates of recombination (Parsons, 1988). Mutation rates may be high in *D.elongatum* as a result of intense solar radiation, of which, UV radiation is known to cause mutation (Vrieling *et al*. 1989). With increasing global temperatures, these proposed mutation and recombination rates are expected to increase, thereby generating populations of *D.elongatum* that may be more adaptable to changes in environmental conditions because deleterious mutations will be selected against. Mutations and recombination rates are known to be mechanisms that generate genotypic diversity (Meyers 2005). The level of variation in *D.elongatum* may also be an adaptation to selective pressures, since alleles of the haploid gametophyte are directly exposed to selection. The higher level of genetic variation encountered in this study, may be beneficial in low arctic habitats, such as Wapusk National Park, which are expected to be

the first affected by climate changes such as global warming (Hansen and Lebedoff, 1987).

Chapter 4

Population structure of the fungal biont of *Thamnolia subuliformis* in Wapusk National Park, Manitoba

Introduction

Lichens are symbiotic associations between primarily an ascomycete fungus with either a green alga or a cyanobacterium (Hawksworth and Honegger, 1994). Early genetic work focused on enzyme variation in *Cetraria arenaria* Karenfelt (Fahselt and Hageman, 1983), *Umbilicaria mammulata* (Ach.) Tuck. (Hageman and fahselt, 1984; Hageman and Fahselt 1986a, 1986b), *Cladonia cristatella* Tuck. (Fahselt, 1986), *Peltigera rufescens* (Weis.) Mudd. (Brown et al. 1989) and *Tuckermanopsis americana* (Sprengel) Hale*, Usnea subfloridana* Stirton and *Evernia mesomorpha* Nyl. (Fahselt, 1988). With the development of molecular techniques, characterizing populations of lichens has become more common (Walser *et al*. 2003). Nuclear ribosomal DNA in lichenized fungi has been used to describe the genetic variation in many species. In particular, the presence or absence of introns in the SSU has been utilized in studies on *Letharia vulpina* (L.) Hue (Hogberg *et al.* 2002), *Cladonia subtenuis* (Abbayes) Mattick (Beard and DePriest, 1996), *C. subcervicornis* (Vainio) Kernst. (Printzen and Eckman, 2003), and *Parmelia sulcata* Taylor (Crespo *et al*. 1998). The SSU of lichen-forming ascomycetes has revealed group 1 insertions, which are short sequences (200-500 bp) that often have the ability to catalyze their own excision from preRNAs (Cech, 1988). This region in the SSU is known to contain group 1 introns in green algae, fungi, ciliates, red algae and different amoebas (Friedl *et al*. 2000), as well as positions 1199, 1210, 1389 and 1506 in ascomycete fungi (Gargas *et al*. 1995, Piercey-Normore, 2004).

The ITS region of rDNA has also been used to describe the population structure of lichens. It has been used as a character in phylogenetic studies at the family level: *Parmeliaceae* Ach. (Crespo and Cubero, 1998) and *Umbilicariaceae* Hoffm. (Ivanova *et*

al. 1999), at the genus level: *Xanthoria calicola* and *X.parietina* (L.) Th. Fr. (Franc and Karenfelt, 1998), and *Ramalina americana* Hale (LaGreca, 1999), to determine genetic variation in populations of the threatened lichen *Lobaria pulmonaria* (L.) Hoffm. in Switzerland (Zoller *et al*. 1999), to characterize the genetic diversity of algal and fungal bionts in *Umbilicaria* species in Antarctica (Romeike *et al.* 2002) and to describe the population structure of the mycobiont in the *Peltigerineae* Willd. (Goffinet and Bayer, 1997). Other techniques to determine the population structure of lichens are RAPDs and RFLPs. RAPDs have been utilized to characterize the genetic variation of *Biatora helvola* Korber *ex* Hellbom (Printzen *et al*. 1999) and *Usnea filipendula* Stirton (Heibel *et al*. 1999). DePriest (1993) characterized different haplotypes in the *Cladonia chlorphaea* (Florke ex Sommerf.) Sprengel complex by using restriction fragment length polymorphisms of the rDNA. RFLPs were also used by Piercey-Normore (2004) to distinguish algal haplotypes in *Cladonia gracilis* (L.) Willd., *C. rangiferina* (L.) Nyl. and *C. multiformis* G. Merr and by Piercey-Normore (2006) to distinguish algal haplotypes in *Evernia mesomorpha* Nyl.. With the development of microsatellite markers, characterizing populations has become more affordable and predictable (Walser *et al*. 2004). These markers have been used to determine recombination and clonal propagation levels in populations of *Lobaria pulmonaria* (Walser *et al*. 2004), genetic variation in populations of *L. pulmonaria* in Canada and Switzerland (Walser *et al*. 2003), genetic variation in *L. pulmonaria* as a result of forest disturbance (Werth *et al*. 2006), phylogenetics of the pathogenic fungus *Coccidioides immitis* (Fisher *et al.* 2000), and population structure of the anther smut fungus *Microbotryum violaceum* (Bucheli *et al*. 2001). With the development of these techniques the knowledge of lichen genetics

has significantly increased and according to Printzen (2000) this sort of information is a prerequisite for interpreting the genetic viability of populations of lichen-forming fungi.

It is generally assumed that genetic variation is an important factor in the ability of a species to adapt to varying environmental conditions (Meyers, 2005), and according to Walser *et al*. (2003) little is known about the process that generates and maintains genetic diversity in lichens. High latitudes, such as those in Low-Arctic habitats of Wapusk National Park, are expected to be the first to show signs of global warming (Hansen and Lebedoff, 1987); and since lichens are highly sensitive to environmental changes and are prominent in Wapusk National Park (Punter *et al*. 2005), they are ideal organisms for studying the effects of global warming. An understanding of how *Thamnolia subuliformis* will respond to changing temperatures will depend partly on the genetic variation of the population. According to Dyer and Murtagh (2001) it is important to have knowledge of the extent of genetic variation in populations of high latitude lichens for studying adaptability to changes.

Thamnolia subuliformis is a sterile lichen existing as fragments on the tops and sides of beach ridges in Wapusk National Park. The thallus is chalky white, referred to as white worms, is slender, hollow and tapering toward the apex. It is widespread especially in arctic-alpine regions of the Northern Hemisphere, but also in cold temperate regions in the Southern Hemisphere (Karenfelt and Thell, 1995). Being a sterile lichen, it is anticipated genetic variability will be low and consequently this species may not adapt to changing conditions caused by global warming. The aim of the present study is to describe the population structure of *Thamnolia subuliformis* in northeastern regions of Wapusk National Park, using ISSR microsatellite PCR, RFLP of ITS rDNA and

screening the SSU rDNA for the presence of putative group-I introns. This information will serve as baseline data for future studies to detect changes in the environment caused by global warming or human intervention.

Materials and Methods

In this study three different experiments were carried out. The SSU of rDNA was screened for the presence of putative group-I introns and the region containing this intron was sequenced. Secondly, the ITS region was amplified with two different primer sets and digested using the endonuclease restriction enzyme *HaeIII*. Digested products were purified and sequenced. Lastly, ISSR microsatellites were amplified using fungal extracts. In the first two experiments whole podetia were extracted, whereas with the microsatellites, the fungal biont was manually separated from the algae and extracted.

Introns in SSU rDNA

Amplification of a region of the nuclear small subunit (SSU) of rDNA of the fungal biont of *Thamnolia subuliformis* (n=95) was accomplished using primers 0819-5' (5'-GAATAATAGAATAGGACG-3') and 1750-3' (5'-AAACCTTGTTACGACTTTTA-3'), (Gargas and DePriest 1996). Three smaller segments within this SSU region were also amplified using the following primer sets, (1) 0819-5' (5'-GAATAATAGAATAGGACG-3') to 1203-3' (5'-GAGTTTCCCCGTGTTGAGTC-3'), (2) 1184-5' (5'-GACTCAACACGGGGAAACTC-3') to 1597-3' (5'-GATGACTCGCGCTTACTA-3'), and (3) 1566-5' (5'-CAACGAGGAATTCCTAGT-3') to 1750-3'. For an illustration of the primer positions and directions in the SSU see Figure 4.1. All primers were selected from those

designed by Gargas and DePreist (1996). Reaction conditions in the Techne Genius thermal cycler (Fisher Scientific, Nepean, ON, Canada) were an initial denaturation at 95˚C for five minutes, followed by 38 cycles of: denaturation at 95˚C for 60 seconds, annealing at 56˚C for 60 seconds, and extension at 72˚C for 90 seconds. The amplified region 1184-5' to 1597-3' was purified and sequenced using BigBye Terminators, Version 3.0, on a 377 and 377XL ABI DNA Sequencing Instrument (University Core DNA and Protein Services, University of Calgary, Calgary, AB, Canada).

RFLP of ITS rDNA

Amplification of internal transcribed spacer (ITS) region of rDNA was accomplished using forward primers AL1700F[1] (5'-CCCACCTAGAGGAAGGAG-3', Helms *et al.* 2001) and 1780A (5'-CTGCGGAAGGATCATTGATTC-3', Piercey-Normore and DePriest 2001) each with non-specific reverse primer ITS4-3' (5'-TCCTCCGCTTATTGATATGC-3', White *et al*. 1990). The amplification conditions in the Techne Genius thermal cycler (Fisher Scientific, Nepean, ON, Canada) were an initial denaturation at 95°C for 5 minutes followed by 40 cycles of denaturation at 95°C for 60 seconds, annealing at 54°C for 90 seconds and extension at 72°C for 120 seconds. DNA optimization was accomplished by carrying out a series of amplifications in which $MgCl_2$, annealing temperature and DNA dilution were varied, (Figure 4.2).

[1] Algal specific primer AL1700F was used but amplified the fungal DNA rather than algal DNA-see Results for explanation.

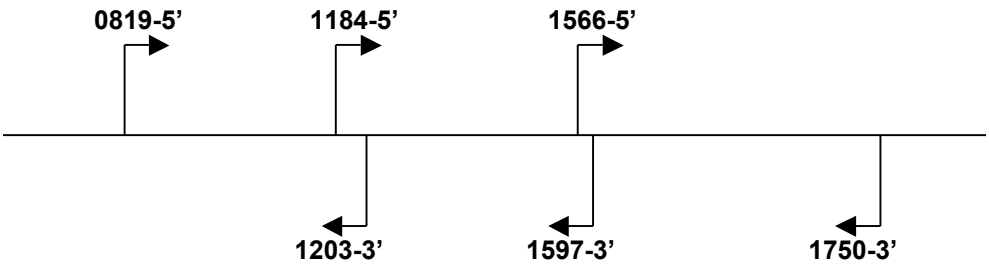


Figure 4.1: Illustration of primer annealing positions and directions in the SSU rDNA of *Thamnolia subuliformis*. Primer name (*ex.* 0819-5') indicates bp number where annealing takes place in SSU, followed by 5' or 3' indicating forward or reverse directions, respectively.

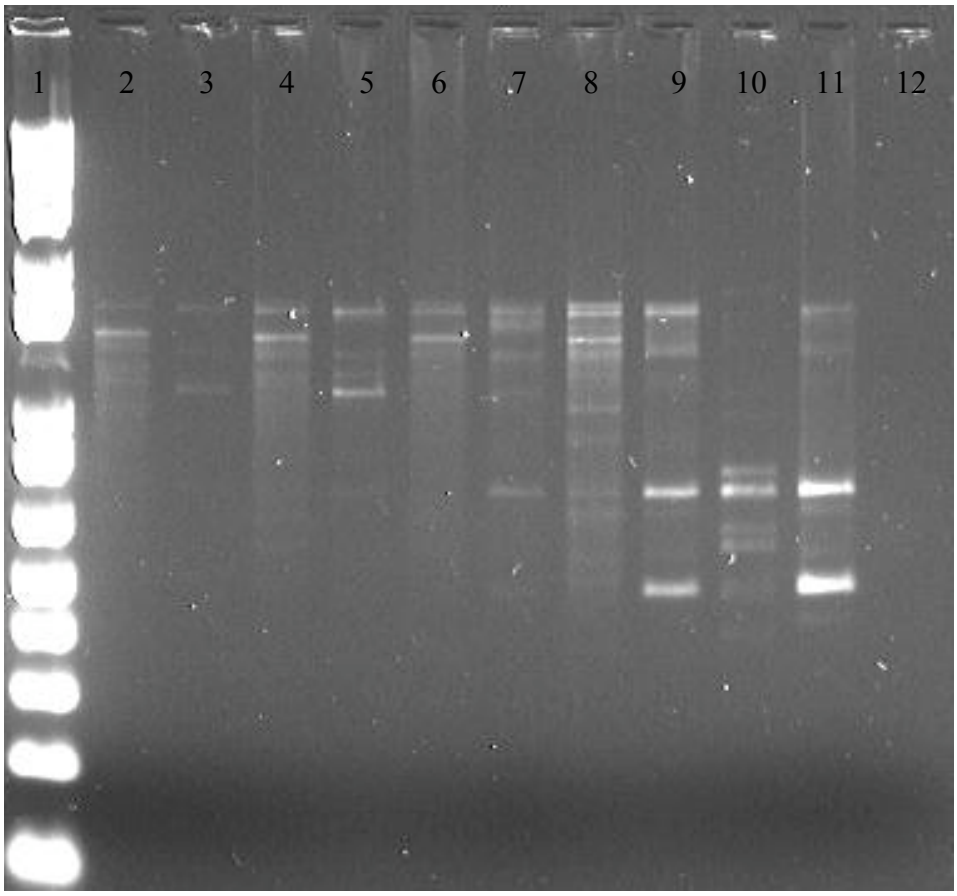


Figure 4.2: Agarose gel showing the $MgCl_2$ and annealing temperature gradient for ISSR primer 827 on 2 different samples of *Thamnolia subuliformis* from northeastern regions of Wapusk National Park. Lane 1=1 Kb DNA ladder, Lanes 2-11=3.5 mM $MgCl_2$ at temperatures of 38°C (Lanes 2-3), 39°C (Lanes 4-5), 40°C (Lanes 6-7), 41°C (Lanes 8-9), and 42°C (Lanes 10-11), Lane 12 is negative.

The amplified products were digested with endonuclease restriction enzyme HaeIII (GG`CC) because of identified cut sites in sequences in Genbank. Approximately 300 ng of DNA was digested in 1x/µL 10X React buffer 2, and 1U/µL restriction enzyme for four hours at 37°C. Enzyme was deactivated by increasing the temperature to 65°C for ten minutes in a water bath.

Fungal hyphae were manually separated from the algae using a dissecting microscope. A subsample of two samples per transect, yielding a total of 24 samples, were separated. The thalli were washed with sterile distilled water to remove any debris or foreign DNA sources prior to the separation. The individual thalli were soaked in 100% ethanol for one to two minutes. This made the thalli more pliable and hydrated. The ethanol softened the fungal tissue allowing for the algae to be scraped away with a razor blade. DNA of the generated fungal samples were extracted utilizing the protocol outlined in Chapter 1, differing only by being resuspended in 25 µL instead of 50 µL. The fungal extracts were amplified using ISSR molecular markers (Table 4.1).

Table 4.1: Primers used in ISSR-PCR of *Thamnolia subuliformis*, showing primer sequences, annealing temperatures ($\mathbf{T_m}$), optimal mass (ng) of DNA used in final reaction (diluted in sdH_2O), and optimal $MgCl_2$ concentrations used in final reaction.

Primer	Sequence	T_m (°C)	DNA Mass (ng)	Final $MgCl_2$ Concentration
ISSR 812	GAG AGA GAG AGA GAG AA	47	10-50	1.5 mM
ISSR 827	ACA CAC ACA CAC ACA CG	42	10-50	3.5 mM
ISSR 855	ACA CAC ACA CAC ACA CYT	54	10-50	2.5 mM
ISSR 864	ATG ATG ATG ATG ATG ATG	39	10-50	3.5 mM
ISSR 868	GAA GAA GAA GAA GAA GAA	45	10-50	2.5 mM
ISSR 869	GTT GTT GTT GTT GTT GTT	39	10-50	2.5 mM
ISSR 878	GGA TGG ATG GAT GGA T	48	10-50	2.5 mM

Results

Introns in SSU rDNA

Thamnolia subuliformis was found in 93 out of 140 plots (66.43%). It was abundant only as thallus fragments on beach ridge tops and sides and was found in all transects, covering the entire study area. Screening of three regions (0819-5'-1203-3', 1184-5'-1597-3', and 1566-5'-1750-3', see Figure 4.1 for illustration) of the fungal SSU rDNA revealed one region (1184-5'-1597-3') with fragment lengths greater than the expected 413 base pairs of the SSU coding region. Initially, these primers generated two different bands and were thought to represent two different fungal haplotypes within the same thallus. The two bands (Figure 4.3) generated from using primers 1184-5' and 1597-3', were sequenced and the longer band was found, as a result of a BLAST search, to be significantly similar to the same region reported from *Thamnolia subuliformis* (e-score=0.0). The shorter band was the SSU coding region of the algal biont, which was 100 % similar to the same region reported in *Trebouxia jamesii* (e-score=1e-107).

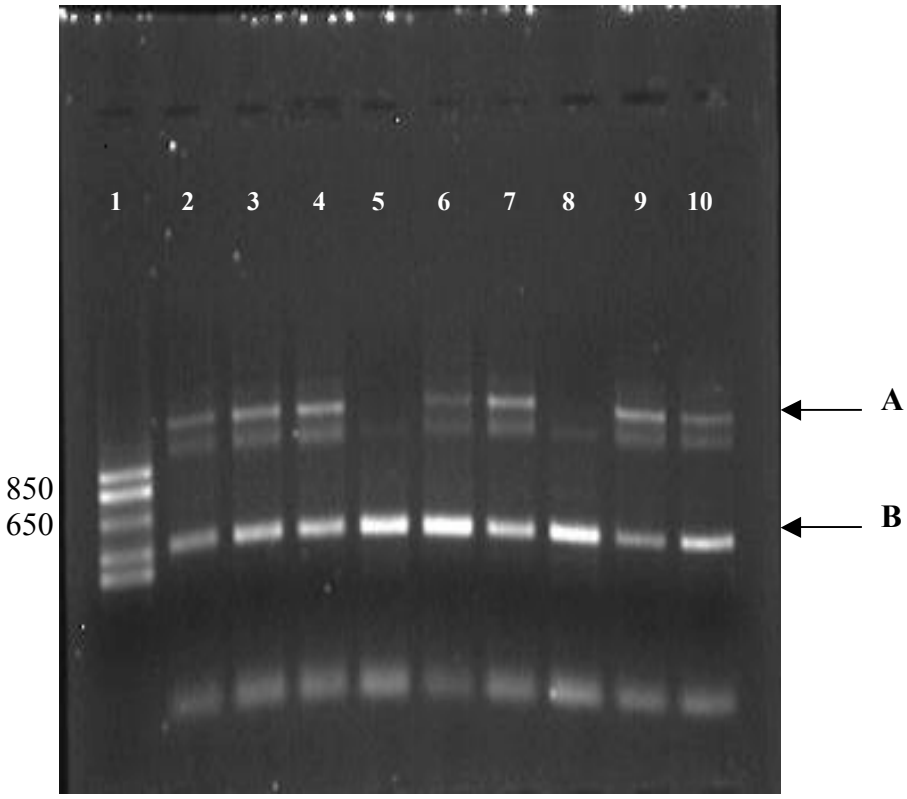


Figure 4.3: Agarose gel showing the two different bands (one double band) amplified using primers 1184-5' and 1597-3'. Band A was sequenced and determined to be most similar to the region reported from *Thamnolia subuliformis*, whereas band B was sequenced and determined to be most similar to the region reported from *Trebouxia jamesii*. Lane 1 contains DNA ladder, lanes 2-10 contain DNA samples.

In the fungus, the intron was present in this region from 18 out of 93 samples (20%) and absent in 75 out of 93 (80%). The intron containing *Thamnolia* samples were distributed in the northern area of the study site, (Figure 4.4**)**. Based on the presence or absence of the intron, the populations were found to be subdivided in an AMOVA analysis (ϕ_{PT}=0.173, p=0.012). Intron containing samples were found specifically in populations four, five, six, seven and eight.

RFLP of ITS rDNA

Digested fragments from the amplified ITS rDNA region using the two different primer sets (1780A-3' to ITS4-5', and AL1700F-3' to ITS4-5') yielded two different banding patterns, (Figure 4.5). The two forward primers (1780A-3' and AL1700F-3') overlapped by approximately 30 bps. When both regions were amplified, only those amplified using AL1700F-3' yielded a long band and two shorter bands. Those samples amplified with 1780A-3' yielded three shorter bands. This difference was interpreted to be the result of a putative group-I intron close to the SSU. The forward algal primers 1780A and AL1700F anneal at the 3' end of the SSU and at position; 1737-1754 in the SSU (Helms *et al*. 2001), respectively. The reverse universal primer (ITS4) is located at position 738-757 at the 5' end of the LSU rDNA. The putative group-I intron must therefore be located between the forward primers AL1700F and the 5' end of the ITS1. The digest of the putative group-I intron containing samples yielded three fragments of lengths approximately 650, 250 and 280 base pairs (bp), whereas the samples without the putative intron yielded fragments of lengths approximately 210, 240 and 280 bp. Putative group-I intron containing samples, or haplotype B from Figure 4.5**,** were

Figure 4.4: Populations of *Thamnolia subuliformis* in northeastern regions of Wapusk National Park, Manitoba. The letter 'T' denotes transects and flags indicate the location. The intron located between 1737-1780 is a putative group-I intron resulting from RFLP of the ITS rDNA. The intron located between 1184-1597 is the result of an amplification of the SSU rDNA. Map is modified from Google Earth.

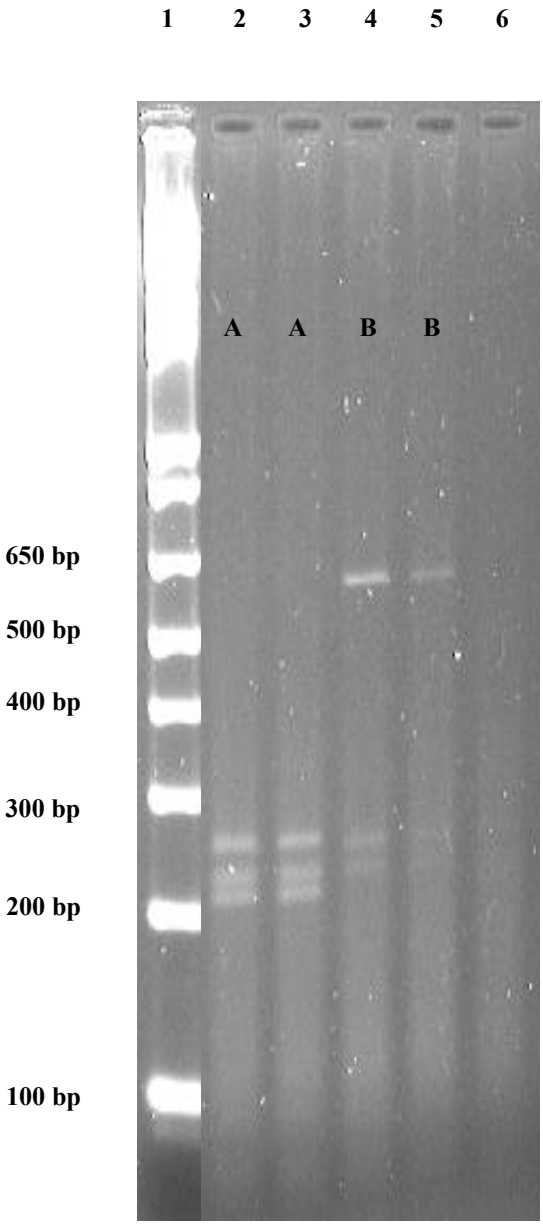


Figure 4.5: Digested fragments of the ITS rDNA region in *Thamnolia subuliformis* (n=95) from northeastern coastal regions of Wapusk National Park using HaeIII. Lane 1- 1 kb DNA ladder, lanes 2 and 3- digest of region 1780A-ITS4, lanes 4 and 5- digest of region AL1700F-ITS4, Lane 6- negative.

distributed only in the northern regions of the study area, (Figure 4.4). Haplotype A was found in all populations sampled.

Microsatellite Polymorphism

The seven ISSR primers revealed a total of 54 scorable bands of different lengths (Table 4.2) amongst the 93 samples, from 14 populations. Two primer banding patterns can be seen in Figure 4.6. Of the 54 bands, 52 (96%) bands were polymorphic and 2 (4%) were monomorphic across populations. Polymorphism was determined by the variablility of a band across all populations. Six of the seven primers, encompassing 52 bands, showed polymorphic levels of 100 percent, and only primer 855 contained monomorphic bands. Fragment sizes were variable, ranging from 200 to 1650 bps.

Table 4.2: ISSR primers used in this study of *Thamnolia subuliformis*, with number of bands scored, number of poly- and monomorphic bands, base pair (bp) range and percent polymorphism.

Primer	# Scored Bands	# Polymorphic Bands	# Monomorphic Bands	bp Range	% Polymorphism
812	12	12	---	300-1650	100
827	4	4	---	400-1500	100
855	6	4	2	200-1000	66
864	7	7	---	650-1650	100
868	10	10	---	400-1650	100
869	10	10	---	200-1000	100
878	5	5	---	400-850	100
Total	54	52	2	200-1650	95.14 (mean)

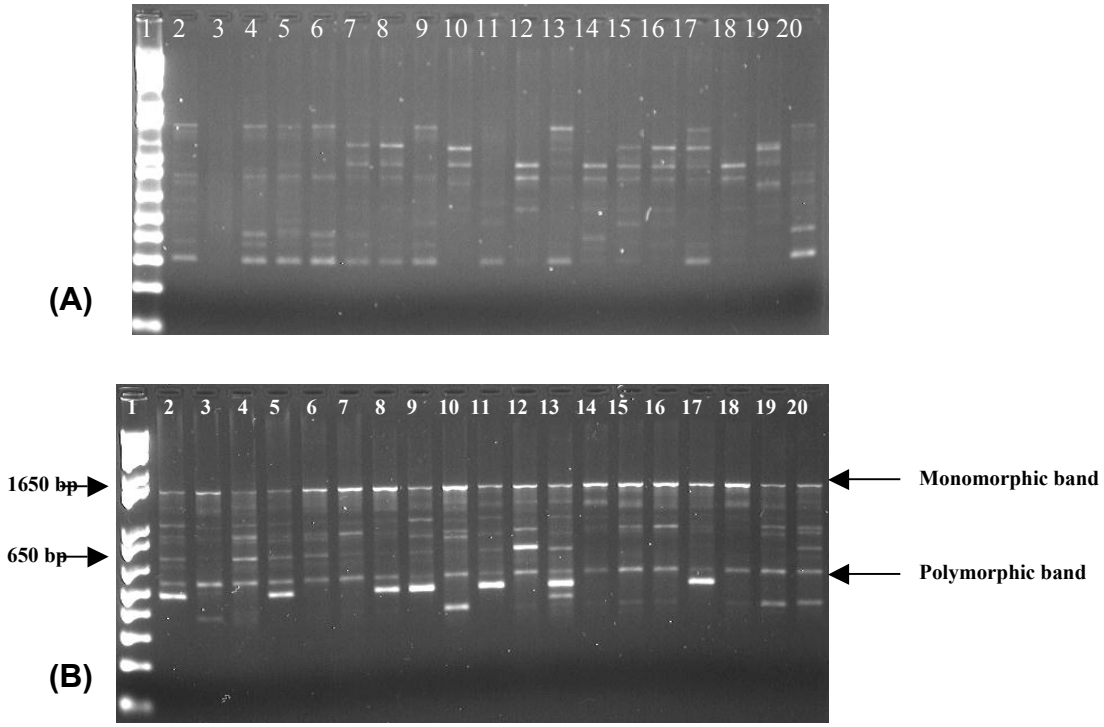


Figure 4.6: Examples of fungal ISSR for (A) ISSR primer 812 and (B) ISSR primer 868 for *Thamnolia subuliformis* in northeastern coastal regions of Wapusk National Park, Manitoba. PCR products were run on 1.5% agarose gels stained with Ethidium bromide and electrophoresed at 120 volts for 90 minutes. Lane 1 contains 1 kb DNA ladder, lanes 2 to 20 contain DNA samples (2 samples /population for populations 1, 2, 3, 4, 5, 6, 7, 8, and 9.

Unique Haplotypes

Based on the primer haplotypes (Table 4.3), no geographic regions of high polymorphism were evident. Primers 812, 868 and 869 yielded unique haplotypes in all populations, whereas primers 827, 855, 878 and 864 generated very few. The sequences for primers 812, 868 and 869 are all G and A rich suggesting that mutations in the genome or single base substitutions are most likely to be replaced by G or A nucleotides. Mutations leading to new haplotypes may be the result of G and A substitutions in the genome. From Table 4.3 it is also apparent that populations 6 and 11 have the greatest number of unique haplotypes, followed by populations 4 and 8 to a lesser extent.

The large clusters of samples from the UPGMA dendrogram of primer 855, Figure 4.7, represent clusters of similar haplotypes. The dendrogram for primer 855 (Figure 4.7B) shows 5 haplotypes: A (25% of the total number of haplotypes), B (29.16%), C (12.5%), D (8.3%) and E (4.2%). Haplotype A is distributed amongst populations 1, 2, 4, 5, 6 and 11, whereas haplotype B is present in populations of closer proximity (6, 7, 9 and 10). Primer 812 supports different unique haplotypes in each population. The A-G rich sequence of primer 812 (as well as 868 and 869) may correspond to variable motifs in the genome, thereby generating the level of variation reported (Table 4.1).

Table 4.3: Haplotypes associated with the ISSR primers used on *Thamnolia subuliformis* in northeastern regions of Wapusk National Park. Pop.#=population number, *n*=sample size, in each cell x(y) denotes number of haplotypes and number of unique haplotypes*, respectively.

Pop.#	*n*	812	827	855	864	868	869	878	Total
1	2	1 (1)	2 (0)	2 (0)	2 (2)	2 (1)	2 (0)	2 (2)	13 (6)
2	2	2 (1)	2 (0)	2 (1)	2 (1)	2 (1)	2 (0)	1 (0)	13 (4)
3	2	2 (1)	2 (0)	1 (0)	2 (0)	2 (2)	2 (1)	2 (1)	13 (5)
4	2	2 (2)	1 (1)	2 (0)	2 (0)	2 (2)	2 (1)	2 (1)	13 (7)
5	2	2 (2)	2 (0)	2 (0)	2 (1)	2 (1)	2 (2)	2 (0)	14 (6)
6	2	2 (2)	2 (1)	2 (0)	2 (1)	2 (2)	2 (2)	2 (1)	14 (9)
7	2	1 (1)	2 (0)	1 (0)	1 (1)	1 (1)	1 (1)	1 (0)	8 (4)
8	2	2 (2)	2 (0)	1 (1)	2 (1)	1 (1)	2 (2)	2 (0)	12 (7)
9	2	2 (2)	1 (0)	1 (0)	2 (1)	2 (2)	2 (1)	1 (0)	11 (6)
10	2	2 (2)	1 (0)	1 (0)	1 (0)	2 (1)	2 (1)	2 (0)	11 (4)
11	2	2 (2)	1 (1)	2 (0)	2 (1)	2 (2)	2 (1)	1 (1)	12 (8)
12	2	1 (1)	1 (0)	1 (1)	1 (0)	1 (1)	1 (0)	1 (1)	7 (4)

*=haplotypes were considered unique if they were only found in a single population.

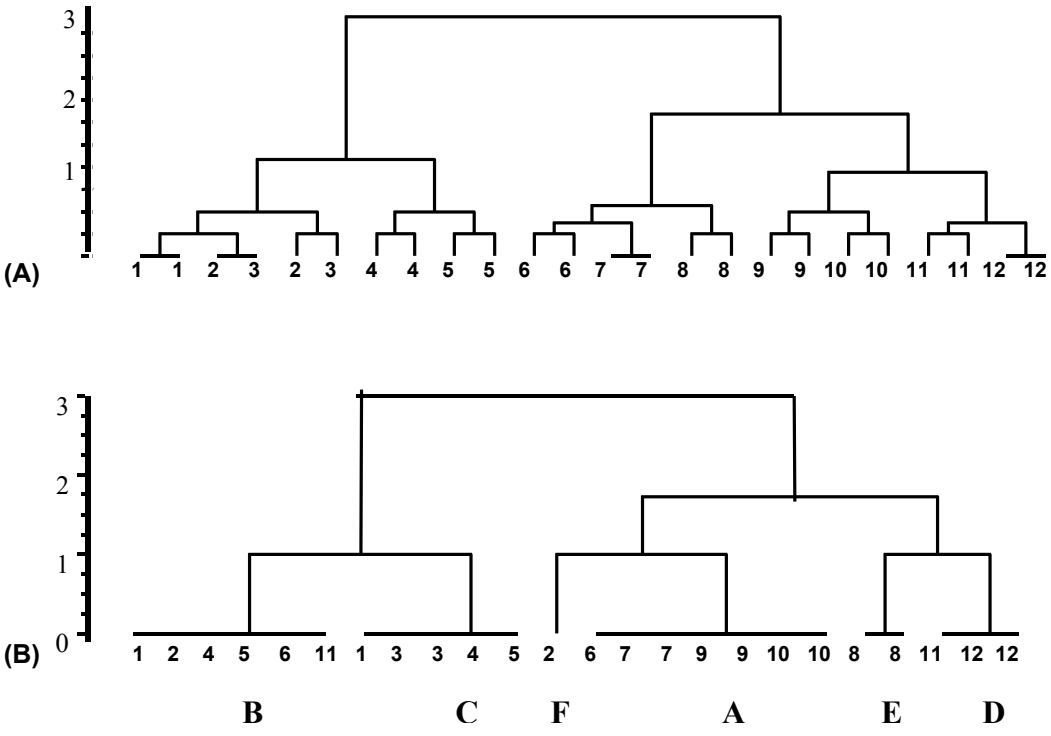


Figure 4.7: UPGMA dendrograms of 24 samples of *Thamnolia subuliformis* for (A) ISSR primer 812 and (B) ISSR primer 855 based on haplotype data from northeastern regions of Wapusk National Park. Numerical values correspond to population numbers, letters indicate haplotypes.

Population Subdivision

Almost all microsatellite primers had significant ϕ_{PT} values ($p < 0.05$) in the AMOVA analysis, (Table 4.4), based on 12 populations, and based on 4 populations (Table 4.5). Some discrepancies arose between the binary and haplotype dataset analyses. The analysis using 12 populations yielded higher subdivision values than those for the analysis with 4. However, both analyses support the same result of population subdivision in a sterile lichen. Primer 827 showed the highest degree of subdivision (ϕ_{PT}=0.584, p=0.002), followed by primer 868 (ϕ_{PT}=0.553, p=0.001). Overall, the range of percent variance among populations was 19-40 % for haplotype data and 23-58 % for binary data. The variance was higher within populations, 60-81 % for haplotype data and 42-77 % for binary data. In essence, the population variance is primarily explained by differences within populations, but variation among populations is also considerable. This level of variation was unexpected in a sterile species such as *Thamnolia subuliformis*.

The individual primer analyses revealed 3 primers (812, 868 and 869) supporting unique haplotypes in each populations, whereas 4 primers (827, 855, 864 and 878) supported similar haplotypes in multiple populations.

Table 4.4: AMOVA analysis of 12 populations (24 samples) of *Thamnolia subuliformis* using variation detected from ISSR primers (Among=among populations, Within=within populations, df=degrees of freedom, SS=sum of squares, MS=mean sum of squares). (* indicates significant p-values at the 95% level)

						Binary Data	Haplotype Data
ISSR Primer	**df**	**SS**	**MS**	**Estimated Variance**	**Percent Variance**	**ϕ_{PT}**	**ϕ_{PT}**
812 Among	11	6.833	0.621	0.123	25%	---	**0.247***
812 Within	12	4.5	0.375	0.375	75%	---	---
812 Among	11	39.833	3.621	0.894	33%	**0.327***	---
812 Within	12	22	1.833	1.833	67%	---	---
827 Among	11	7	0.636	0.172	37%	---	**0.371***
827 Within	12	3.5	0.292	0.292	63%	---	---
827 Among	11	15.708	1.428	0.527	58%	**0.584***	---
827 Within	12	4.5	0.375	0.375	42%	---	---
855 Among	11	6.417	0.583	0.167	40%	---	**0.4***
855 Within	12	3	0.25	0.25	60%	---	---
855 Among	11	11.917	1.083	0.167	18%	**0.182**	---
855 Within	12	9	0.75	0.75	82%	---	---
864 Among	11	6.042	0.549	0.087	19%	---	**0.189***
864 Within	12	4.5	0.375	0.375	81%	---	---
864 Among	11	14.667	1.333	0.25	23%	**0.231***	---
864 Within	12	10	0.833	0.833	77%	---	---
868 Among	11	6.792	0.617	0.121	24%	---	**0.244***
868 Within	12	4.5	0.375	0.375	76%	---	---
868 Among	11	33.458	3.042	1.083	55%	**0.553***	---
868 Within	12	10.5	0.875	0.875	45%	---	---
869 Among	11	5.708	0.519	0.051	11%	---	**0.109**
869 Within	12	5	0.417	0.417	89%	---	---
869 Among	11	26.417	2.402	0.492	26%	**0.258***	---
869 Within	12	17	1.417	1.417	74%	---	---
878 Among	11	7.25	0.659	0.184	39%	---	**0.387***
878 Within	12	3.5	0.292	0.292	61%	---	---
878 Among	11	17.375	1.58	0.436	38%	**0.381***	---
878 Within	12	8.5	0.708	0.708	62%	---	---

Table 4.5: AMOVA analysis of 4 populations (24 samples) of *Thamnolia subuliformis* using variation detected from ISSR primers (Among=among populations, Within=within populations, df=degrees of freedom, SS=sum of squares, MS=mean sum of squares). (* indicates significant p-values at the 95% level)

						Binary Data	Haplotype Data
ISSR Primer	**Df**	**SS**	**MS**	**Estimated Variance**	**Percent Variance**	**PT**	**PT**
812 Among	3	19.500	6.500	0.731	26%	---	**0.256***
812 Within	20	42.300	2.117	2.117	74%	---	---
812 Among	3	2.000	0.667	0.033	7%	**0.067***	---
812 Within	20	9.333	0.467	0.467	93%	---	---
827 Among	3	9.375	3.125	0.431	44%	---	**0.443***
827 Within	20	10.833	0.542	0.542	56%	---	---
827 Among	3	2.833	0.944	0.094	20%	**0.196***	---
827 Within	20	7.667	0.383	0.383	80%	---	---
855 Among	3	6.25	2.083	0.225	23%	---	**0.235***
855 Within	20	14.667	0.733	0.733	77%	---	---
855 Among	3	2.583	0.861	0.087	20%	**0.202***	---
855 Within	20	6.833	0.342	0.342	80%	---	---
864 Among	3	4.667	1.556	0.093	8%	---	**0.085**
864 Within	20	20.000	1.000	1.000	92%	---	---
864 Among	3	2.042	0.681	0.043	9%	**0.091***	---
864 Within	20	8.500	0.425	0.425	91%	---	---
868 Among	3	10.792	3.597	0.323	16%	---	**0.163***
868 Within	20	33.167	1.658	1.658	84%	---	---
868 Among	3	1.958	0.653	0.031	6%	**0.062***	---
868 Within	20	9.333	0.467	0.467	94%	---	---
869 Among	3	11.750	3.917	0.389	20%	---	**0.197***
869 Within	20	31.667	1.583	1.583	80%	---	---
869 Among	3	1.708	0.569	0.020	4%	**0.042**	---
869 Within	20	9.000	0.450	0.450	96%	---	---
878 Among	3	6.708	2.236	0.213	18%	---	**0.182***
878 Within	20	19.167	0.958	0.958	82%	---	---
878 Among	3	2.417	0.806	0.065	13%	**0.135***	---
878 Within	20	8.333	0.417	0.417	87%	---	---

Combined Haplotype Analysis

In the combined analysis of all primer haplotype datasets, the minimum spanning tree and the UPGMA dendrogram revealed similar results, (Figure 4.8). The AMOVA analysis also revealed levels of population subdivision (ϕ_{PT}=0.276, p=0.000) similar to those for the individual primers, (Table 4.4). Among and within population variation for the combined analysis, were 28 % and 72 %, respectively. This is within the range of among and within values for the individual primers. The combined analysis supports a high number of unique haplotypes amongst all the populations.

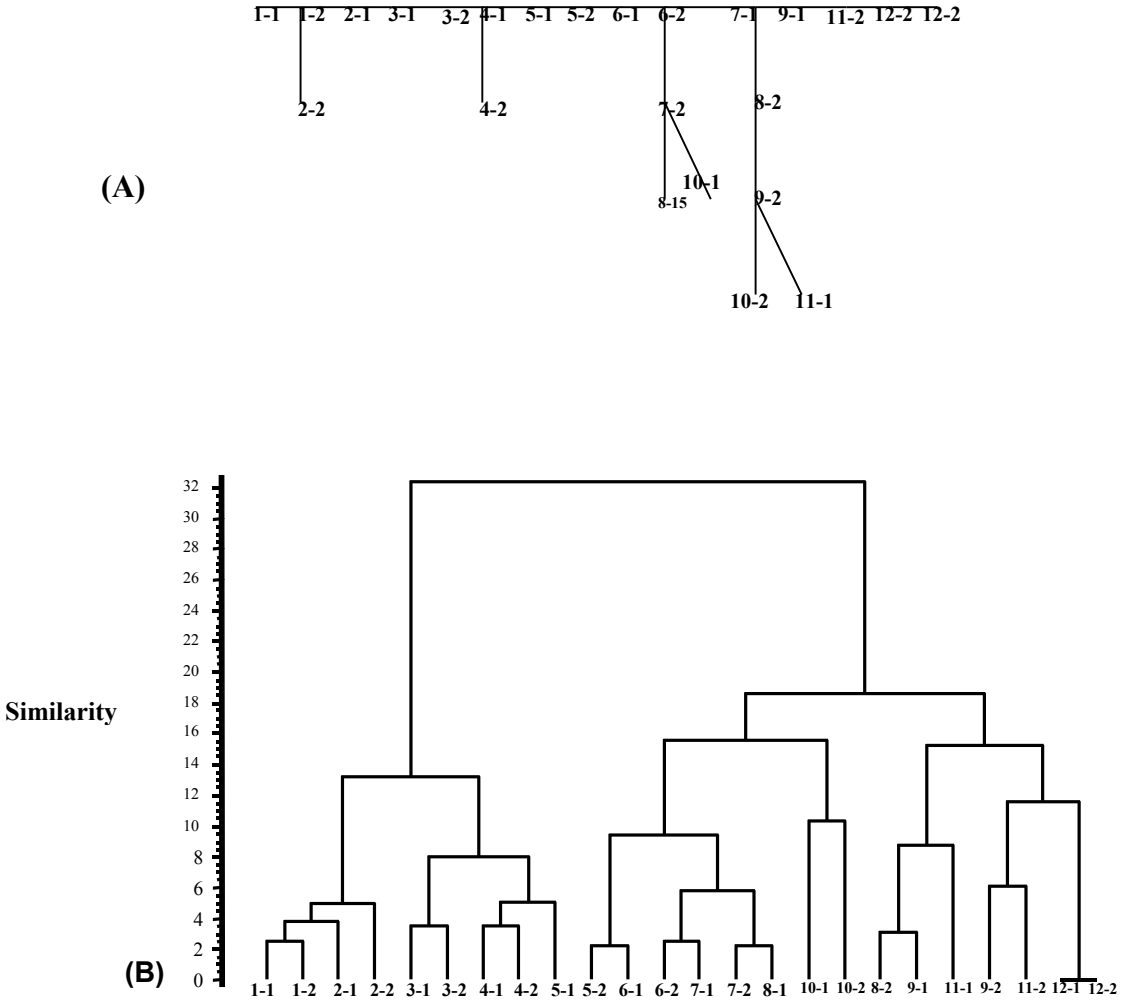


Figure 4.8: (A) Minimum spanning tree and (B) UPGMA dendrogram of combined haplotype datasets for *Thamnolia subuliformis* in northeastern regions of Wapusk National Park, Manitoba. Clustered numbers represent populations, followed by sample number.

Discussion:

Genetic Variation in Thamnolia subuliformis

The results in this study suggest that *Thamnolia subuliformis* in northeastern regions of Wapusk National Park has a level of genetic variation not typical of a sterile lichen. Populations of *T.subuliformis* contained high levels of within population (51-79 % averaged across all individual primers; 72 % in the combined analysis) and among population (21-49 % averaged across all individual primers; 28 % in the combined analysis) genetic variation. Twenty-three out of twenty four samples were genetically distinct. Since sexual structures have not been encountered in this species of lichen-forming ascomycete (Karenfelt and Thell, 1995), it is assumed that no sexual reproduction has occurred. In the study area, *T.subuliformis* was only found as fragments and appeared to be unattached to the substrate. By dispersing as fragments, the lichen reduces the need for the fungus to find a suitable alga for relichenization. Both algal and fungal bionts are within the fragment and are already adapted to these low-arctic habitats of Wapusk National Park. Reduced relichenization events may be a strategy for survival under harsh environmental conditions (Romeike *et al*. 2002).

No Gene Flow in T.subuliformis

Only two primers showed no population subdivision (dispersal), (Table 4.4) suggesting that little dispersal is occurring overall. The lack of gene flow was unexpected since the presence of single branched podetia on the beach ridge appeared as fragments, which suggested dispersal. However, these may not be fragments but rather young lichens that have stunted growth due to harsh conditions.

Low levels of dispersal of *T. subuliformis* may be possible through wind or animal movement. Limited resistance by other vegetation would facilitate wind dispersal by fragments (Karenfelt and Thell, 1995). The vegetation (vascular plants, grasses, lichens and mosses) in this region of the park is very low to the ground (Scott, 1995) and therefore may not inhibit dispersal. This dispersal scenario coupled with the observations of large quantities of thallus fragments throughout the study area, would suggest a situation where there would be no population subdivision (due to efficient dispersal). Another mechanism that may facilitate dispersal of thallus fragments is animal movement. As caribou travel throughout the park on beach ridges their hooves come in to contact with many thallus fragments. Smaller fragments may be broken off, which may then adhere to the fur on the lower legs of the caribou. These fragments may be deposited in vegetation in other areas, blow off during movement or wash off in ponds or lakes, float by wave action to shorelines and get blown to other areas. Larger fragments get hung up on vegetation more easily than smaller ones. Long-distance dispersal may be facilitated by hooves breaking the thallus fragments into smaller pieces. Caribou eat *T.subuliformis* in spring and fall periods (Kärenfelt and Thell, 1995) as a minor part of their diet and would benefit from this lichen being widely distributed. Fragmentation by caribou may benefit the lichen by creating an efficient dispersal system.

With an efficient dispersal system, it is expected that populations of ascomycetes will be genetically identical (Fisher *et al.* 2000), because haplotypes will be evenly distributed. However, in this study, populations of *T.subuliformis* were differentiated based on variable microsatellite markers, SSU insertions in rDNA and ITS RFLPs.

Group-I Introns

The nuclear SSU rDNA of some lichen-forming ascomycetes contains group 1 insertions, which are short sequences (200-500 bp) of a conserved primary and secondary structure at the RNA level that often have the ability to catalyze their own excision from preRNAs (Cech, 1988). They have been reported in the fungal biont of *Lecanora dispersa, Calicium tricolor* and *Porpidia crustulata* (Gargas *et al*. 1995), *Cladonia chlorophaea* complex (DePriest and Been, 1992; DePriest, 1994), *Cladonia cristatella, C.cervicornis* and *C.leporina* (DePriest, 1992), species of the *Umbilicariaceae* (Ivanova *et al*. 1998), group I-like insertions in *Cladonia gracilis* and *Cladonia rangiferina* (Piercey-Normore 2004, Piercey-Normore *et al*. 2004), degenerate group-1 introns in *Arthonia lapidicola* (Grube *et al*. 1996), as well as non-lichen forming *Monilinia fructicola* (Côté *et al*. 2004). The insertion position in the SSU of *T.subuliformis* was between positions 1184 and 1597 bp. Based on work by Gargas *et al*. (1995) there are three possible group 1 intron insertions in lichenized ascomycetes in this region (1199, 1210, and 1389) that may explain the length differences seen in *Thamnolia*. The region of the SSU was sequenced and determined to be significantly similar to *Thamnolia subuliformis* based on a BLAST search in Genbank (e-score=0.0). When compared to the SSU rDNA of *Lecanora dispersa* (Gargas *et al.* 1995), the insertion was determined to be at position 1199. This insertion is most likely a group 1 intron because out of the 17 known insertion positions in SSU rDNA, 15 are classified as group-I introns (Gargas *et al*. 1995). The insertion at the 3' end of the SSU rDNA between positions 1754 and 1780 bp, has not yet been reported to be an intron (Gargas *et al.* 1995; DePriest 1993). The insertion is approximately 648 bp long and is present in 29 out of 95 samples. The length

of the insertion may be an indication of two group-I introns, since these introns are known to be between 200-500 bp each (Cech, 1988). Without further sequencing, folding and ribozyme activity assays, this insertion cannot be confirmed as a group-I intron.

Historical Gene Flow in Thamnolia subuliformis

In *Thamnolia subuliformis* where sexual reproduction is currently absent, variation may be an indication of a past recombining population. Other non-lichenized ascomycetes such as *Alternaria* spp. (Berbee *et al*. 2003), *Coccidioides immitis* (Burt *et al*. 1996), *Candida albicans* (Graeser *et al*. 1996), and *Aspergillus flavus* (Geiser *et al*. 1998) that have not been reported to sexually reproduce, have been suggested to have had a history of recombination because genetic variation is not typical (Berbee *et al*. 2003). *Thamnolia subuliformis* may also have undergone sexual reproduction in the past but has lost the ability to sexually reproduce in this harsh climate of northeastern regions of Wapusk National Park. This can be tested by subjecting this species to a series of temperatures in a laboratory to see if sexual reproduction takes place. This situation seems to be similar in European populations where work by Karenfelt and Thell (1995) has also found no indication of sexual reproduction. Perhaps this species, following the last glaciation, began to follow the retreat of the ice by the dispersal of fragments. In doing so the population may have dispersed too far north where conditions were unfavorable for sexual reproduction, and consequently the lichen lost the ability to produce meiotic spores. Instead, perhaps the lichen had to generate a new way to

recombine populations, thereby creating a level of genetic diversity able to adapt to the pressures of natural selection. This process may be a parasexual one.

Parasexuality occurs when nuclei in a heterokaryon, which is a fungal cell containing two separate nuclei, fuse and undergo mitotic recombination or haploidization when the chromosomes segregate (Pontecorvo 1959). Parasexuality is known to reshuffle genetic material in fungi in the absence of meiosis (Caten, 1981). The same author has demonstrated that under laboratory conditions, basidiomycetes such as *Schizophyllum commune*, *Stereum hirustum*, and *Armillaria gallica*, can undergo somatic fusion, diploidization and haploidization. Parasexuality has not been reported under natural conditions (Anderson and Kohn, 1998), but Zeigler *et al*. (1997) observed comparable behaviour as that obtained in laboratory conditions for *Magnaporthe grisea*. Nuclei of different fungal haplotypes in a heterokaryon, can fuse with one another and generate new haplotypes as a result of mitotic crossing over and haploidization with segregation of whole chromosomes (Anderson and Kohn, 1998). Some samples of *T.subuliformis* appeared to have branches of the thallus tapered toward the main axis instead of the characteristic opposite, where they taper towards the apex. This may have been an indication of two separate thalli growing towards one another and eventually fusing into one thallus resulting in multiple haplotypes within the same thallus. A review by Dyer *et al.* (2001) found that thalli may be composed of more than one fungal haplotype. The presence of multipe haplotypes in one individual has been reported for *Aspergillus nidulans* (Pontecorvo and Kafer, 1958), and the presumed asexual *Magnaporthe grisea* (Leung *et al*. 1988; Levy *et al.* 1991, 1993; Zeigler *et al*. 1995; Nitta *et al*. 1997). Further genetic work, as well as observations of thalli fusing together,

is needed to conclusively state that *Thamnolia subuliformis* reproduces by parasexual processes. In fungi that have no sexual cycle, parasexuality would have a strong influence on a species ability to adapt to changing conditions (Anderson and Kohn, 1998). Relatively few parasexual events would be required to have a large impact on population structure (Dyer *et al*. 2001). The occurrence of parasexuality in *Thamnolia subuliformis* would generate genetic variation thereby allowing the species to adapt to changes in the environment from human intervention or global temperature changes.

Lichens and Global Warming

Low-arctic environments, such as those in northeastern regions of Wapusk National Park, are expected to be the first to experience global temperature increases (Hansen and Lebedoff, 1987). Tundra lichens are continually exposed to direct light as a result of the absence of high vegetative cover in tundra to shade out UV radiation. The light thallus color of some lichens such as the white *Thamnolia subuliformis*, may reflect excess UV radiation for protection against overheating (Kershaw, 1971a) and UV damage. This reflectance may inhibit the mutation effect that UV radiation is known to cause (Walker, 1984; Sankaranarayanan, 1982; Schmaulhausen, 1949). According to Meyers (2005), mutation, which may be minimal in this study area, and recombination are known to be mechanisms that generate genotypic diversity. If *Thamnolia subuliformis* is mixing alleles by parasexuality, this would make the overall population genetically diverse. This would be beneficial in habitats which are known to have harsh environmental conditions, and are anticipated to be the first to experience global temperature increases. Populations of *Buellia frigida* and *Xanthoria elegans* from the

Vestfold Hills in eastern Antarctica were found to contain little or no variation in the ITS region, and it was suggested that they would lack the genetic resources to adapt to environmental change (Dyer and Murtagh, 2001). The fungal biont of *T.subuliformis* in northeastern regions of Wapusk National Park in contrast, may be well suited, based on the 2 different haplotypes generated from RFLP of ITS rDNA[2] and the variation from the ISSRs, for the pressures of climate change and natural selection. Whether the presence of the intron in the SSU rDNA or the variation reported for the ISSR primers is beneficial, has yet to be assessed. However, future work is required to confirm or discredit the parasexuality process. Observations must be made in laboratory conditions to conclusively suggest that *T. subuliformis* is reproducing parasexually. The genetic diversity of the algal biont must also be examined to understand if *Thamnolia subuliformis* has the genetic resources to adapt to change in northeastern regions of Wapusk National Park.

[2] Algal specific primers AL700F and 1780A did not amplify the algae in *Thamnolia subuliformis*. Instead the fungal biont was amplified, which was confirmed by sequencing and doing a BLAST search using Genbank.

Chapter 5

Species Compositions, Diversity and Abiotic Influences in Northeastern Regions of Wapusk National Park

Introduction

Wapusk National Park, covering almost 12,000 km^2, was designated an ecological reserve in 1996 by the federal government of Canada (www.pc.gc.ca). Prior to the establishment of the park, and currently, locals utilized the area for hunting and recreation. Archealogical evidence indicates that Inuit, Cree and Dene tribes have been in this area for 3000 years (Tough, 1997). Because of the use by local people, the arrival of Parks Canada, researchers, and impending tourism, raise concern about potential impacts on the vegetation and wildlife populations in the park. Wapusk National Park is an important landscape because approximately 1,200 polar bears build dens in the park every year. The large concentration of polar bears drives the tourism industry in the town of Churchill. The park is also a haven for bird enthusiasts because of migratory birds such as snow geese, Canadian geese, and the diversity of shorebirds in marine habitats along the coast of Hudson Bay. Researchers at the University of Manitoba have been annually assessing the floral diversity in different regions of the park (Punter *et al*. 2003; Piercey-Normore *et al*. 2004; Ford *et al*. 2005 and Piercey-Normore *et al*. 2006), which generates baseline data that will be useful in future studies aimed at interpreting the effects of land use by local people, Parks Canada, researchers and tourists.

Assessment of diversity of wildlife and vegetation in Wapusk National Park is important for the local economy, and for the overall health of the ecosystem. According to Meyers (2005), diverse populations containing more alleles are more able to adapt to changing conditions. Populations with low diversity contain few alleles and are thought to be able to adapt to fewer changes in the environment (Romeike *et al*. 2002). Knowledge of the present distribution, abiotic influences and genetic variability (see

Chapters 3 and 4) of populations is critical for assessing future changes in populations as an indicator of how well species adapt to the environmental changes. Lichens and bryophytes are prominent in the park (Piercey-Normore, 2005); they are sensitive to environmental changes (Richardson, 1981) and are useful organisms to assess the effects of human intervention and climate change. Changes in climate due to global warming are expected to be pronounced at higher latitudes (Hansen and Lebedoff, 1987; Romeike *et al.* 2002). Exposed tundra in Wapusk National Park may be sensitive to environmental changes and populations may be at risk of alteration or elimination as a result of changes due to global warming. Assessment of the current distribution of vegetation in Wapusk National Park is essential for future conservation efforts and research on global warming.

The objective of the current study was to characterize exposed tundra including beach ridges and fens, with regards to species composition of vascular plants, bryophytes, and lichens in northeastern regions of Wapusk National Park. Information generated from the present study will serve as baseline data for future studies on human intervention and global warming, and may be used for conservation purposes by Parks Canada.

Methods

Sampling of lichens and bryophytes was carried out for six days in May of 2005. Transects (Figure 5.1) were randomly laid out perpendicular to beach ridges. Each transect was 75 meters long with ten 0.5m x 0.5m plots and each transect was separated by a minimum of 0.5 kms. *Thamnolia subuliformis* and *Dicranum elongatum* were collected from every plot in which they were encountered. Representative samples of all vascular plants, bryophytes and lichens were collected and identified to species either in the field or in the lab using Brodo *et al*. (2001), Crum and Anderson (1981) and Johnson *et al*. (1995). Ground cover percentages were assessed for each of the following categories: bare ground (with no stones or pebbles), detritus, wood cover, gravel/rock, vascular plants, bryophytes, grasses/sedges, lichens and lichen fragments. Within every plot, abiotic factors measured were organic matter thickness and aspect of the plot. The pH and soil type were also assessed in plots that were located on ridge tops and in fens for each transect. Soil type was determined using the Manitoba Soil Classification System (www.mb.gov.ca) and pH was determined by dissolving 5-10 grams of soil in distilled water, and testing the subsequent solution (outlined in Chapter 2).

Samples were partially air dried at camp and oven dried at 80°C in the Parks Canada compound near the Northern Studies Centre. After identification, samples were placed in packets, affixed with a descriptive label and vouchers were placed in the cryptogamic division of the University of Manitoba herbarium (WIN).

Data analysis and multivariate assessments Principal co-ordinates analysis (PCoA), Unweighted arithmetic average (UPGMA), and nonmetric multidimensional scaling (NMS) were performed using the program Syntax 5.0 (Podani, 1994) and outputs

were edited using Microsoft Powerpoint®. Diversity indices utilized were the Shannon-Weaver (H), Hill's effective species richness (N1) and the corresponding eveness indices; E1 and E2, respectively. The Shannon-Weaver index was calculated using the following formula (Shannon and Weaver, 1949):

$$H = -\Sigma p_i \, Ln \, p_i$$

(Where p_i is the proportion of species x, and Ln is the natural logarithm.)

Hill's effective species richness (N1) was utilized to calculate an index where rare species receive less weight. It was determined by using the following formula (Hill, 1973):

$$N1 = e^{H}$$

(Where e is a universal constant and H is the value from Shannon-Weaver Index)

Figure 5.1: Transect locations in Wapusk National Park, Manitoba. Nestor I was the location of the camp, between transects 1 and 14 on the beach ridge. Transects are represented by 'T' from 1 to 14. (B=beach ridges, F=fen areas and W=inland lakes and bogs). Hudson Bay is on the right.

Results

Diversity

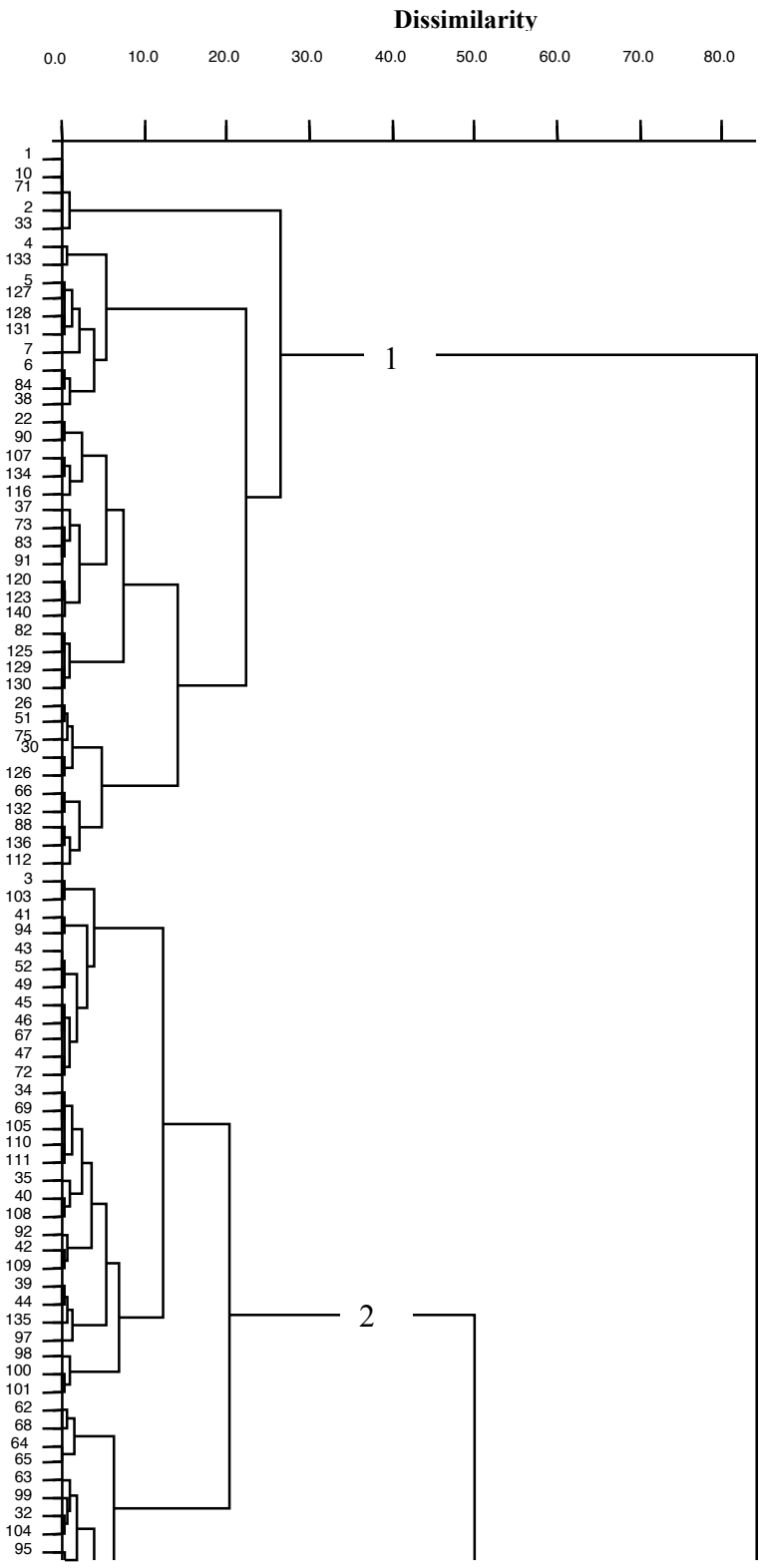
In total, 54 species (40 lichens, 12 mosses, 1 liverwort and 10 vascular plants, see Appendix A) were identified in northeastern regions of Wapusk National Park. The highest overall diversity (Table 5.1) was located in fen areas (H=2.2866, N1= 9.8411), where vascular plants (H=0.793, N1=2.21) and mosses (H=0.993, N1=2.699) were the most diverse and lichens (H=0.6470, N1=1.9098) were the lowest. The diversity of lichens (H=1.2872, N1=3.622) and lichen fragments (H=1.205, N1=3.337) were greatest on ridge tops. The only prominent vegetation on the sides of ridges was found on hummocks. This seems to be reflected by the incease in overall diversity in group 2 (H=1.1248, N1=3.0795).

Species Composition

Gymnosperms, mainly *Picea mariana* (Mill.) BSP (black spruce) and *Larix laricina* (Du Roi) K.Koch (tamarack) were present as clusters approximately 1.5 meters in height. The stems were stunted and branches were more prominent on the leeward side. Shrubs included *Salix* spp. and *Betula glandulosa*, which were prominent. The UPGMA dendrogram (Figure 5.2) shows seperation into three main clusters or species assemblages.

Table 5.1: Diversity measures of all vegetation types in three different habitats. (H=Shannon-Weaver Index, E1=Shannon-Weaver Eveness (0-1), N1=Hill's Effective Species Richness, E2= Hill's Eveness).

	H	E1	N1	E2
Group 3-Ridge top (total)	2.0061	0.6312	7.4340	0.3090
Vas.plants	0.7320	0.3760	2.0790	0.2970
Bryophytes	0.0687	0.0990	1.0710	0.5350
Lichen fragments	1.2050	0.4450	3.3370	0.2220
Lichens	1.2872	0.5590	3.6220	0.3620
Group2 (total)	1.1248	0.3969	3.0795	0.1811
Vas.plants	0.6840	0.3820	1.9820	0.3300
Bryophytes	0.4410	0.2270	1.5540	0.2220
Lichen fragments	0.0002	0.0000	1.0000	0.0769
Lichens	0.9578	0.4150	2.6060	0.2606
Group 1-Fen (total)	2.2866	0.6862	9.8411	0.3515
Vas.plants	0.7930	0.3810	2.2100	0.2760
Bryophytes	0.9930	0.4140	2.6990	0.2450
Lichen fragments	0.5006	0.2278	1.6497	0.1833
Lichens	0.6470	0.2945	1.9098	0.2122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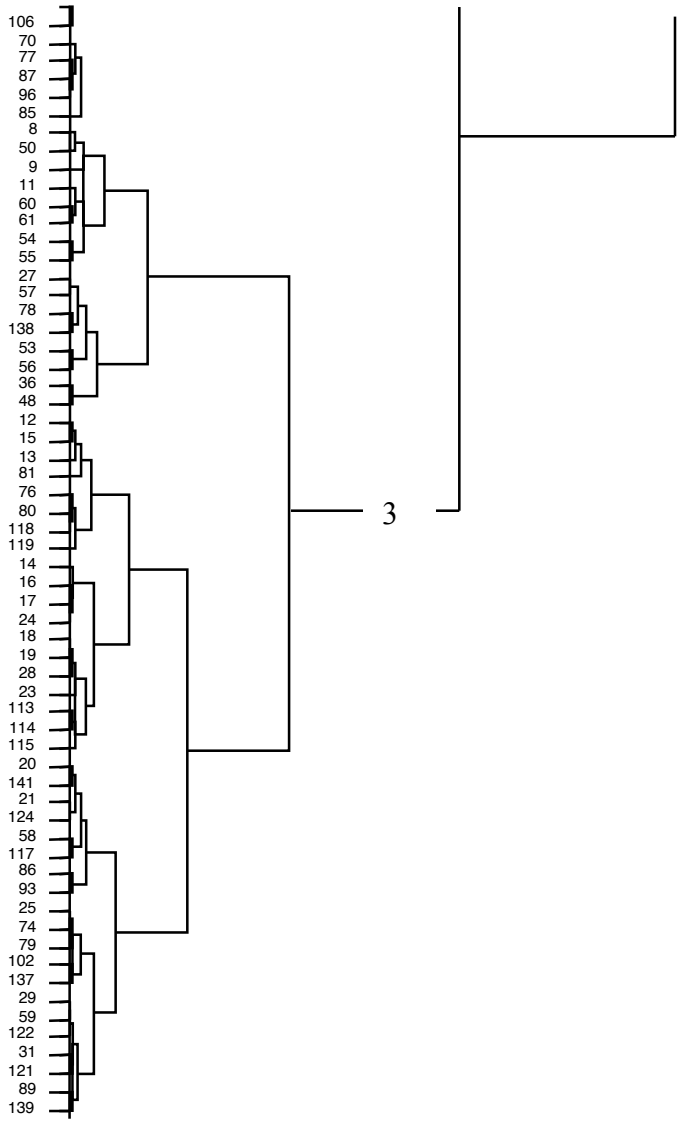

Figure 5.2: UPGMA dendrogram of 141 plots in northeastern regions of Wapusk National Park, based on species frequencies. See figure 5.3 for species compositions of these 3 groups.

The three groups differentiated in the UPGMA dendrogram (figure 5.2) are representative of fen habitats (group 1), beach ridges (group 3) and a distinct group between these elevationally different regions (group 2). There are several species which occur across the elevational gradient in varying frequencies and some that are group specific.

Fens (group 1)

Low lying areas that collect water between beach ridges are called fens (group 1 Figure 5.2; Figure 5.3). In fens, organic matter is thicker (2 cm) than on ridge tops, pH is more acidic (=6.5), and the particle size of the soil is smaller (Figure 5.3). Hummocks are encountered along sides of beach ridges and in fens. Hummocks are mound shaped structures comprised mostly of dead or inactive bryophyte gametophytes with only the top layer being photosynthetically active. Hummock dominated fens create an array of microhabitats. *Dicranum elongatum* is occassionally mixed, in hummocks, with other bryophytes such as *Aulacomium acuminatum*, *A. turgidum*, *A. palustre*, *Hylocomium splendens*, *Pleurozium schreberi, Tomenthypnum nitens*, *Pohlia nutans* on humus, and the less common *Encalypta rhaptocarpa*.

Many vascular plants grow on hummocks. Vascular plant species that are most common on hummocks are *Andromeda polifolia, Arctostaphylos rubra, Ledum decumbens, Pinguicula vulgaris* and *Rhododendron lapponicum*. There is also an increase in the cover of graminoids in fens compared with drier beach ridge areas (Figure 5.3).

Figure 5.3: Schematic showing species compositions and abiotic parameters [pH (averge of all values determined), soil type and organic matter thickness] of a beach ridge and fen habitat of northeastern regions of Wapusk National Park. The yellow line indicates a profile of elevation, and within each habitat, species are listed in order of frequency from top to bottom. Group numbers correspond to those found in Figure 5.2. Vascular plants=red, bryophytes=green, lichens=black, abiotic parameters=black at top of list.

Lichens, such as *Cladonia rangiferina*, present as fragments on beach ridge crests are present as intact thalli on hummocks. *Sphaerophorus fragilis, Cetraria islandica, Flavocetraria nivalis* and *F.cullata* were attached to the hummock and had multiple branches, which was different from the single branch fragments found on the ridge tops. The most abundant fragments found were *Thamnolia subuliformis*, *Bryoria nitidula, Bryocaulon divergens* and *Alectoria ochroleuca*. One species whose abundance and growth form does not change is *Cladonia pocillum* (Ach.) Grognon. Crustose lichens such as *Lecanora circumborealis* and *Ochrolechia upsaliensis*, found on beach ridges are more abundant on hummocks in fen habitats.

Slope of Beach ridge (group 2)

Group 2 (Figure 5.2 & 5.3) is found between beach ridges (group 3) and fens (group 1). Group 2 contains common vascular plants found on beach ridge crests such as *Dryas integrifolia*, *Rhododendron lapponicum*, and *Arctostaphylus rubra* that have decreased in abundance. Other species such as *Andromeda polifolia*, *Ledum decumbens*, and *Empetrum nigrum* have became more prominent. Bryophytes, most abundant in fens, have an increased abundance in these areas, compared to beach ridges. *Dicranum elongatum* had the highest frequency of sampling, followed by *Aulocomium turgidum*, *Hylocomium splendens, Pohlia nutans*, *Encalyptra rhaptocarpa* and *Aulocomium palustre*. Similar lichens are found as fragments (in decreasing abundance: *Thamnolia subuliformis, Flavocetraria nivalis*, *Flavocetraria cuculata*, *Sphaerophorus fragilis*, *Bryocaulon divergens*, *Alectoria ochroleuca* and *Cetraria islandica*) but there is an increase in foliose species occurrence. Foliose species were not sampled in either beach

ridge or fen habitats. Foliose species sampled in group 2 (between fens and ridge crests) were *Pannaria pezizoides*, *Peltigera didactyla*, *P. rufescens*, *Psoroma hypnorum*, *Solorina saccata*, *Umbilicaria hyperborea*, and *Hypogymnia physodes*.

Beach ridge crest (group 3)

The typical beach ridge (group 3 Figure 5.2) substrate (see Figure 5.3) is comprised of sand particles (75%), small pebbles and stones, has a neutral pH (=7.0) and a thin layer of organic matter (0.5 cm). Lichens such as crustose, *Rhizocarpon geographicum* and foliose *Xanthoria elegans* are typical on pebbles and larger stones. There is a high percentage of bare ground with wood debris and detritus, which supports an array of lichen species. On wood and detritus, crustose lichens are *Lecanora epibryon***,** *L. circumborealis***,** *L. symmicta***,** *Ochrolechia frigida*, *O. androgyna***,** *O. upsaliensis***,** *Pertusaria dactylina, Rinodina turfacea***,** fruticose species *Cladonia pocillum***,** and foliose species *Physcia phaea*, and *Pannaria pezizoides***.** In addition, many lichens are present in fragments on top of beach ridges. These include *Alectoria ochroleuca, Bryocaulon divergens***,** *Bryoria nitidula***,** *Cetraria islandica***,** *Flavocetraria nivalis***,** *F. cucullata***,** *Sphaerophorus fragilis***,** *Thamnolia subuliformis* and *T. vermicularis.* Two lichens not found as fragments but as intact thalli are *Physconia muscigena* and *Physcia phaea.* Bryophytes are not common on beach ridge crests, but *Tortula ruralis* is present. It is a small acrocarp, growing as a mat with 5-6 mm of stem exending above the substrate. As wind continually deposits sand and debris, the gametophyte grows apically to remain above the substrate level. As a result, each successive growth period adds length to the previously buried gametophyte. Many bryophytes prefer wet conditions and are not predominant in these habitats. *Dicranum*

elongatum, *Pohlia nutans*, *Aulocomium turgidum* and *Encalyptra rhaptocarpa* were sampled only once or twice on beach ridge crests.

The most common vascular species on beach ridges (group 3, Figure 5.2 & 5.3) is *Dryas integrifolia*, known as the mountain aven. This species has white flowers, which bloom in late June to early July. Growth is close to the substrate and therefore is spreading. Another species, *Rhododendron lapponicum*, which is sparse on beach ridges, increases in abundance towards fens**.** A showy purple inflorescence is typical of this species. Other species that may be encountered on beach ridges are *Arctostaphylus rubra*, *S. oppositifolia* and rarely *Andromeda polifolia*, *Pinguicula vulgaris* and *Saxifraga ceaspitosa*.

Discussion

Three types of habitats were evident in northeastern regions of Wapusk National Park. Beach ridge tops and fen areas were differentiated based on species compositions (Figures 5.2, 5.3), and diversity (Table 5.1). Beach ridges were dominated by lichens and lichen fragments, the latter of which had the greatest diversity amongst all the habitats. High densities of fragments on beach ridge tops may reflect the increased ability of lichen fragments to disperse by wind in these drier habitats that are exposed and at higher elevations than the fens. Vegetation on the tops of beach ridges is restricted to within 5 cm of the ground and may not provide the height required to capture fragments.

The abundance of lichens may also reflect their physiological ability to tolerate unfavourable environmental conditions (Kershaw, 1989), such as dry soils, calcareous substrates, high wind gusts, UV levels and desiccation. These harsh, dry conditions are

not favourable for bryophytes and vascular plants, yet crustose lichens are able to cope with these conditions. The soil on beach ridges contains large pore sizes (Figure 5.3) and a low abundance of vascular plant roots. This may promote water drainage on beach ridge tops, thereby allowing nutrients and water to flow to lower fen areas.

Fen habitats, accordingly, had the greatest overall diversity, with bryophytes and vascular plants being most dominant. The presence of water and nutrients in fen habitats may explain the higher diversity found in these habitats. Bryophytes, preferring wet acidic conditions (Ireland, 2002), have the highest diversity in fens. With increased diversity, organic matter accumulates as a result of increased growth in these areas.

The OM thickness is higher in fen areas (Figure 5.3) and is lower on beach ridges. The low production of organic matter on beach ridge tops may be the result of less plant growth and increased desiccation by wind. Hummocks, in contrast, create a favourable microhabitat for many vascular plant, bryophytes and lichen species. With high species richness on hummocks, biomass increases, explaining the thicker layer of organic matter (Figure 5.3).

Characterizing beach ridges, fens, and ridge slope habitats in northeastern regions of Wapusk National Park will serve as baseline data for conservation applications and human intervention aimed at better understanding the effects of global warming. Species in these regions appear to have specific niches in which they occupy. Knowledge of their current distribution, abiotic preferences and genetic variability (Chapter 3 and 4) is paramount in assessing the adaptability of a species to changing environmental conditions.

Chapter 6

Conclusions

Northeastern regions of Wapusk National Park contained 40 lichens, 12 mosses, 1 liverwort and 10 vascular plants (see Appendix A). This diversity may be a reflection of the ability of these species to adapt to low-arctic tundra conditions. The growing season is short, the annual precipitation is low and wind gusts are strong. Desiccation is common. Beach ridges have neutral soil conditions, large particle sizes and a thin layer of organic matter. Lichens had the highest diversity on beach ridges where crustose forms were found to be growing on pebbles, stones and wood debris. Lichen fragments were most abundant possibly as a result of the reduced vertical structure of the vegetation thereby inhibiting entanglement of lichen thalli. Vascular plants and bryophytes were less diverse than lichens on ridge tops. Many of the species of lichen-forming fungi (see Chapter 5) found as fragments on ridge tops, were also found as intact thalli in fen areas. Fragments may be broken off thalli in fens by either wind, caribou or rodents and then transported by these same vectors along ridge tops. Dispersal of fragments by species such as *Sphaerophorus fragilis*, *Flavocetraria cucullata*, *F. nivalis*, *Bryoria nitidula*, *Bryocaulon divergens*, *Alectoria ochroleuca*, *Thamnolia subuliformis* and *T.vermicularis* appears to be efficient. Beach ridges seem to be dispersal pathways with less resistance than fens or vegetated areas.

The habitat composed of the slope between ridge top and fen was reported based on the varying species assemblages. Diversity increased in this habitat possibly as a result of the presence of hummocks. Hummocks may retain enough water and nutrients on the slope to provide favourable conditions for the growth of vascular plants such as *Rhododenron lapponicum* and *Arctostaphylos rubra*, bryophytes such as *Dicranum*

elongatum, *Aulocomium* spp., and *Tomenthypnum nitens*, and lichens such as *Ochrolechia* spp., *Lecanora* spp. and *Flavocetraria* spp.

The slightly more acidic pH in fens, coupled with the increase in water creates a situation where bryophytes are favored (Ireland, 2002). *Dicranum elongatum* is one of the dominant mosses found growing as hummocks (Ford *et al.* 2002). The high level of genetic variation reported in this study for *D.elongatum* (Chapter 3) was uncommon for other reported haploid mosses (see Chapter 3). With the absence of sporophytes in this region of Wapusk National Park, one explanation for the level of variation is that several colonization events had taken place via long distance dispersal of meiotic spores from populations outside the study area. Locally, asexual reproduction appears to be predominant, which is evident from the distribution of haplotypes over the study area (Figure 4.4). Another explanation for the variation is that the timing for sporophytes did not coincide with the sampling period. This would mean that sexual reproduction is indeed occurring. A third explanation could be that the variation is from historical gene flow or rare sexual reproductive events in unusual seasons. A similar situation is evident for *Thamnolia subuliformis* (Chapter 4), which is dominant appearing as fragments on beach ridges. Being an asexual lichen (Karenfelt and Thell, 1995), the high level of variation reported from the presence of group-I introns in the SSU rDNA, RFLP of ITS rDNA and with the use of ISSR primers, was not expected. However, the level of variation obtained in this study may suggest that this population has lost the ability to sexually reproduce. The population subdivision of *Thamnolia subuliformis* reported was not expected because of its sterile nature.

The vector of dispersal for both species may be wind blown fragments, or as a result of the movement of caribou and rodents. Understanding the method of dispersal and the genetic variability of a species is important for assessing the overall population dynamics within the study area. In conclusion, populations of *D.elongatum* and *T. subuliformis* in northeastern regions of Wapusk National Park are genetically diverse (in regards to other bryophytes and lichens; see Chapters 3 and 4 respectively), adapted to the conditions and may be well suited for anticipated environmental changes.

Species composition data combined with environmental and genetic data provides a more holistic approach to research. A species presence in a habitat is a result of both environmental tolerance and genetic adaptability (Meyers, 2005). The level of genetic variation reported for both *Dicranum elongatum* and *Thamnolia subuliformis* would be beneficial for tolerating environmental changes such as global warming, which are expected to be most prominent at higher latitudes (Hansen and Lebedoff, 1987). Knowledge of the present species distribution and genetic variation are important in assessing the adaptability of a species to change. The information can be utilized to generate reliable conclusions with regards to conservation applications. In addition, this knowledge will serve as baseline data for future studies aimed at assessing the impacts of global warming.

Future Studies

The present study provided insight into the genetic variation of two common species in Wapusk National Park, but it also generated questions. Future researchers may wish to expand the study area in Wapusk National Park and abroad to gain an insight into species variation in a larger geographic region. Both of these species distributions are larger than the present study reported. A comparison of the genetic variation of these two species from similar habitats in Europe would provide additional information on population biology and dispersal. In addition, future studies on tundras should include more variables, such as soil nutrient status, bacterial and fungal assemblages, mycorrhizal associations and temperature variations. Above ground variables, such as air temperature and humidity studied extensively by Kershaw in northwestern Ontario (1971a; 1975; 1985), would be beneficial for assessing their influence on bryophytes and lichens.

Studies assessing the dispersal agents of bryophyte and lichen fragments would also be beneficial. Testing wind velocity on different fragment sizes would generate results on the dispersal ability of fragments and the distance they can disperse. A more time consuming study would be to spray paint some thalli or gametophytes and infer their dispersal by caribou movement over the landscape. In addition, genetic analysis of the photobiont in *T. subuliformis* would generate a more reliable result for the adaptability and dispersal of this lichen. Dispersal by thallus fragmentation allows for the photobiont and mycobiont to be together within a single fragment.

The low level of gene flow reported for both species means that populations are discrete. This implies that the loss of one or two populations, as a result of human or environmental impact, may mean the loss of a significant proportion of one or two haplotypes. The fragment-like podetia of *Thamnolia subuliformis* may be deceptive. Instead these fragments may be young stunted lichens. The low level of gene flow reported for *Dicranum elongatum* may be a result of the harsh environmental conditions, since sporophytes are produced by *D.elongatum* in populations more inland. Finally, the reported species diversity for beach ridge and fen habitats has allowed for an overall appreciation of the roles *D.elongatum* and *T.subuliformis* play in these Low-Arctic tundra environments.

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Appendix A

Lichens	Bryophytes (including Liverworts)	Vascular Plants
Alectoria ochroleuca (Hoffm.) Massal.	*Aulocomium acuminatum* (Lindberg and H. Arnell)	*Andromeda polifolia* L
Alectoria nigricans(Ach.) Nyl.	*A. palustre* (Wahlenberg)	*Arctostaphylos rubra* (Rehd. and Wilson) Fern.
Bryocaulon divergens (Ach.) Karnefelt	*A. turgidum* (Hedwig)	*Betula glandulosa* Michaux, Fl.
Bryoria nitidula (Th. Fr.) Brodo and D. Hawksw.	*Dicranum elongatum* Schleich. ex Schwaegr	*Dryas integrifolia* Vahl.
Cladonia pocillum (Ach.) Grognot	*Drepanocladus revolovens* (Sw.) Warnst.	*Empetrum nigrum* L.
Cladonia chlorophaea(Flörke ex Sommerf.) Sprengel	*Encalyptra rhaptocarpa* Schweagr	*Ledum decumbens* (Ait.) Hulten
Cladonia arbuscula(Wallr.) Hale and Culb.	*Hylocomium splendens* (Hedw.) Schimp. in B.S.G	*Pinguicula vulgaris* L.
Cladonia rangiferina(L.) Nyl.	*Pleurozium schreberi* (Brid.) Mitt	*Rhododendron lapponicum* (L.) Wahlenb
Cladonia gracilis(L.) Willd.	*Pohlia nutans* (Hedw.) Lindb	*Saxifraga caespitosa* L.
Cladonia borealis S. Stenroos	*Polytrichum commune* Hedw.	*S. oppositifolia* L.
Cetraria islandica (L.) Ach.	*Tomenthypnum nitens*, (Schimp.)	
Flavocetraria nivalis (L.) Karnefelt and Thell	*Tortula ruralis* (Hedw.) Gaertn	
F. cucullata (Bellardi) Karnefelt and Thell		
Hypogymnia physodes(L.) Nyl.	*Ptilidium pulcherrimum* (G. Web.) Hampe	
Lecanora epibryon (Ach.) Ach.		
L. circumborealis Brodo and Vitik		
L. symmicta (Ach.) Ach.		
Micarea maleana (Nyl.) Hedl.		
Nephroma arcticum (L.) Torss.		
Ochrolechia frigida (Sw.) Lynge		
O. androgyna (Hoffm.) Arnold		
O. upsaliensis (L.) Massal.		
Pannaria pezizoides (Weber)		
Peltigera didactyla (With.) J. R. Laundon		
Peltigera rufescens (Weiss) Humb.		
Pertusaria dactylina (Ach.) Nyl		
Physcia phaea (Tuck.) J. W. Thomson		
Physcia aipolia (Ehrh. ex Humb.) Fürnr.		
Physconia spp. (Ach.) Poelt		
Psoroma hypnorum (Vahl) Gray		
Rhizocarpon geographicum (L.) DC		
Rinodina turfacea (Wahlenb.) Korber		
Solorina saccata (L.) Ach.		
Sphaerophorus fragilis (L.) Pers**.**		
Stereocaulon rivulorum H. Magn.		
Thamnolia subuliformis (Ehrh.) Culb.		
T. vermicularis (Sw.) Ach. ex Schaerer		
Tuckermaniopsis sepinola (Ehrh.) Hale		
Umbilicaria hyperborea (Ehrh.) Hale		
Xanthoria elegans (Link) Th. Fr.		