Evolution of Life history strategies in Lophoziaceae

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ABSTRACT

This study used data from literature and data from the field to analyse the patterns of variation in life history characters among members of the liverwort family Lophoziaceae. A combination of Principal Component and Cluster analyses was used to analyse data from literature in testing for recurrent suites of life history variation among species of the family. Data from literature were also used to examine the relationship between mode of reproduction and reproductive system (sexuality) and between diaspore (spore or gemma) frequency and sexuality. Data from the field were used to establish diaspore (spore and gemma) sizes and their production per capsule or shoot and to test for relationships between diaspore size and production per shoot/capsule and also between diaspore sizes and proportion of germination.

The study showed the existence of recurrent suites of life history variation in the family. The clusters produced were shown to be independent of the species' phylogeny, but were closely related to the habitat parameters such as nature of substrate, duration of habitat availability and moisture condition. The study also shows dominance of dioicous species in the family, with about 90% of the studied species being dioicous. There is a statistically significant relationship between reproductive system and sporophyte frequency; monoicous species produce sporophytes more frequently than dioicous species.

A negative relationship was shown to generally exist between spore size and number of spores produced per capsule at both species and population levels. However, no relationship could be established between gemma size and the number of gemmae produced per shoot. For two of the studied species, *L. ciliata* and *L. longiflora*, there was a general positive relationship between spore size and proportion of germination, with bigger spores having higher germination proportions. *L ventricosa* showed a negative relationship between spore size and germinability.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Life Histories, life-history traits and life strategies

1.1.1 The life-history concept

The life history of an organism encompasses all the stages through which it passes between its birth and its death. It is the organism's life time of growth, differentiation, storage and especially reproduction (Begon, *et al.* 1996). It reflects the genotype, the environment and the interaction between the two. In life history studies we concentrate on the average or expected life history of an (hypothetical) organism in the population and on the deviations from or variance around these expectations because it becomes easy to compare the patterns found in one population with those found in another, and to search for conditions that may make these patterns adaptive. We seek life history patterns at the population level in order to make comparisons between populations, but more often we are interested in the degree to which individuals conform to the population patterns and the consequences of deviating from these typical patterns. This information gives insight into why the population patterns exist (Wilson, 1983).

1.1.2. Life-history traits

Life history traits are those directly associated with reproduction and survival (Stearns and Hoekstra, 2005). They include such factors as the number of offspring produced (or some stand-in such as number of eggs), the timing of offspring production – when they are produced over a female's life - and survival of individuals from one period to the next (therefore the longevity). All of these traits are related to some degree, and are subject to modification by natural selection. Change in one life history trait often is constrained by trade-offs involving other traits (Stearns, 1976; 1989; Roff, 2000; Stearns and Hoekstra, 2005). The study of an organism's life history also includes a

consideration of its distribution in both time and space and the life history displayed can be genetically based or a result of the environment or an interaction between the two (Stearns and Hoekstra, 2005). A life history may thus be briefly defined as the lifetime schedule of reproduction and mortality of an organism in some defined environmental context.

1.1.3. Life history strategies

A consideration of life history traits will show that they do not evolve independently of one another. The interactions among them, together with the age specific mortality that drives the evolution of some of them, provide some kind of internal structure to the evolution of life histories. Such a structure leads to the impeding of some trait combinations and facilitation of others thus leading to development of predictable, recurring sets of character combinations in response to particular sets of ecological conditions. These trait combinations are usually referred to as life history strategies (During, 1992; Grime, 1979; Mishler, 1988). Mishler (1988) discusses the debate on the use of the word "strategy" in evolutionary biology from a semantic point of view. The word does not imply a conscious decision. A life history strategy can be defined as "a specialised phenotype of correlated traits that has evolved independently in different populations of species exposed to similar selection pressures" (Silvertown and Charlesworth, 2001) or "co-adapted traits fitting generalised environmental constellations" (During, 1992).

1.2. Life History theory

1.2.1. The theory

Life history theory provides a method of analysis in organismal biology, physiology, and evolutionary socio-biology which postulates that many of the physiological traits and behaviours of individuals may be best understood in relation to key maturational and reproductive characteristics that define the life course. Due to the fact that, for an individual, the resources in a particular environment are finite, variation of these characteristics reflects differing

allocations of an individual's resources. This allocation involves trade-offs (Cody, 1966; Stearns, 1976; 1989; 1992; Roff, 1992; Jordan and Howard, 2002;) and strategies (During, 1979; 1992; Longton, 1988; Mishler, 1988; Johansson, 1994) that can be compared between individuals, populations or species. The most obvious, and perhaps best studied, trade-offs are those between size and number of offspring (Smith and Fretwel, 1974; Stuefer *et al.*, 2002; Mabry, 2004; Sakai and Sakai, 2005) and those between reproductive effort and risk of adult mortality (Stearns, 1976; 1977; Roff, 1992). Production of larger offspring leads to reduction in their number (Roff, 1992, Stuefer *et al.*, 2002) while allocation of resources to reproductive effort may reduce longevity.

In the case of dispersive elements like seeds or spores, for example, an evolutionary tension may arise because smaller diaspores are often most effectively wind dispersed (Miles and Longton, 1992) whereas establishment may be favoured by larger propagules with substantial food reserves (Westoby and Leishman, 1994; Westoby *et al.*, 2002; Moles and Westoby, 2004). Models have been developed to try and predict or explain the outcomes of evolution in such cases. The most common example of such a model includes what has been termed the r-k-selection theory (McArthur and Wilson, 1967; Stearns, 1976; 1977; During, 1992; Roff, 1992). In r-selected environments (unstable or unpredictable environments) the optimal strategy is the production of a large number of offspring as early in life as possible whilst in K-selecting environments the optimal strategy is to produce a smaller number of larger offspring (presumably with higher survival chances) later in life. The idea is that the most favourable balance in the various trade-offs will vary in relation to the nature and stability of the habitat, leading to the evolution of adaptive suits of life history features characteristic of organisms occupying particular niches (Roff, 1992; Hedderson and Longton, 1995).

1.2.2. The role of trade-offs

The optimising ability of natural selection should, in theory, lead to the development of life-history traits with the greatest fitness. And if the life-history traits were independent of one another, natural selection would ultimately

produce Richard Law's "Darwinian demon" (Law, 1979), which is an organism that matures at birth, immediately reproducing an infinite number of offspring with the same characteristics and lives for ever (Stearns and Hoekstra, 2005). Such organisms do not exist because there are constraints on the level of fitness of the traits that can be achieved due to existence of trade-offs. Trade-offs are due to the interaction of traits and occur when a change in one trait that increases fitness causes a change in another trait that decreases fitness. Any organism has limited resources of time and energy for growth, maintenance and reproduction at its disposal. These processes compete for the limited resources. Cody (1966) framed an explanation of life history evolution in terms of limited energy resources and trade-offs between competing demands for this energy.

Though life-history trade-offs are often difficult to observe in nature (Stearns, 1989), and the mechanisms that cause them are little understood, evidence of trade-offs is plentiful (Stearns, 1977, 2000) and research, cited in the previous section, has shown their importance as constraints and determinants of the course of life history evolution.

1.2.3. Why Life Histories?

The study of evolution involves the study of changes in the genetic composition of populations. The process of natural selection is a major driving force of evolution. Natural selection is itself a consequence of variation in the lifetime reproductive success (fitness) among organisms in a population (Stearns and Hoekstra, 2005). It acts on individuals but the response to it is recorded (carried) in the information in genes (of the fit or reproductively successful organisms) that are transmitted to the next generation.

Natural selection is made possible by variation in life history traits (as expression of genes plus the environment), which are the principal components of fitness, since survival, maturation and reproduction determine fitness. The study of life histories therefore occupies a central position in the study of evolution.

Life history traits also contribute to key ecological interactions through direct participation in population dynamics, in that they partially determine how many individuals of a species are present at any one time and their distribution (See Sommer and Hommen, 2000; Bennet and Owens, 2002). Such dynamics in turn contribute to the structure of biological communities through the population dynamics of interaction among competitors, predators and prey, parasites and hosts, and symbionts and mutualists. The study of life histories therefore links ecological and evolutionary studies of organisms. According to Le Maitre (1998) ecological theories deal with proximate explanations, those attempting to understand how the traits of an organism operate within its current habitat to enable the species to survive while evolutionary theory seeks ultimate explanations, those attempting to understand how the organism has come to posses its present traits. Life-history theory is important in bridging the separate viewpoints of ecological theory and evolutionary theory by seeking explanations for observed relationships between plant/animal traits and demography under a given set of environmental (biotic and abiotic) constrains.

In population and conservation biology information on life history traits can be used to make quantitative predictions about the future sizes of populations, future reproduction rates and the age distribution of members of the population. These predictions are important for designing management and recovery plans for economically important and endangered species including the management of invasive alien species populations (Lambrinos, 2004; Sans *et al.*, 2004). In behavioural ecology, on the other hand, life history traits are important in understanding the evolutionary "decisions" that organisms make. They can be fundamental to the understanding of behaviour because they may involve trade-offs of one behaviour versus another or trade-offs that lead to one behaviour becoming better than the other.

The work presented in this research is on life histories in the liverwort family Lophoziaceae. It is therefore important to consider characteristics of reproductive biology and life history for bryophytes in general before concentarating on Lophoziaceae. The remaining part of this chapter aims to

introduce peculiarities of the bryophyte life cycle and their possible relevance to life history evolution, as well as summarise some of the existing literature on bryophyte life history evolution.

1.3. Bryophyte Life Histories

1.3.1. Bryophyte Life Cycle

Bryophytes are characterised by a diplohaploid life cycle (Fig. 1.1) (During, 1979; 1992). A haploid spore germinates into usually filamentous protonema on which one or more buds develop. The buds grow into a green plant, the gametophore, which may be either thallose or consist of stems and leaves. Sexual organs of the gametophyte produce gametes that fuse to form a diploid sporophyte. The male and female sexual organs sometimes occur on the same plants, in which case the gametophytes are monoicous and when they occur on different plants the gametophytes are dioicous. Specific terms have been designed for monoicous species (autoicous, synoicous and paroicous) depending on the position of the perigonium relative to the perichaetium. Transport of the spermatozoids was previously thought to be only possible through water. However recent studies (Cronberg *et al*, 2006) showed that in some mosses, springtails and mites may carry moss sperm, thereby enhancing the fertilization proces. The sporophyte remains attached to, and dependent on, the gametophyte. Meiosis takes place in the sporophyte to produce haploid spores. Sometimes the gametophyte produces specialised haploid asexual reproductive structures that germinate to produce protonema that give rise to new gametophytes.

Life cycles of bryophytes can be completed in less than one year in some species. For example, the life cycle of *Funaria hygrometrica* Hedwig may be completed in 4 months (Nakosteen and Hughes, 1978). However, some species can live for many years without reproducing (During, 1979). Life span in bryophytes is often rather plastic and often determined by the environment (During, 1973). In a number of species plants die of stress, but leave tubers or

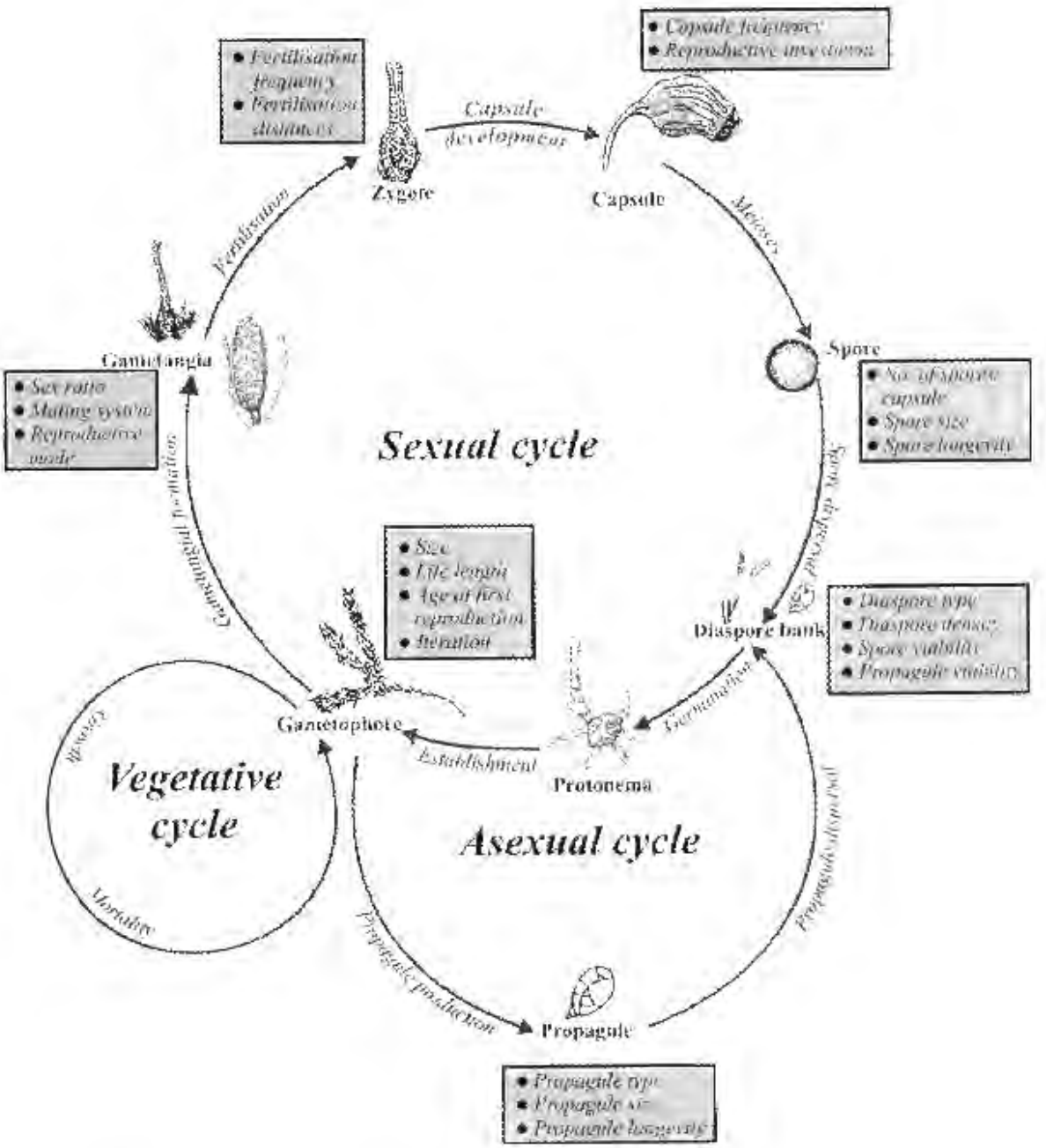

Figure 1.1. An ecological life cycle of a bryophyte. Recognised stages are in bold and their important characteristics are listed in boxes. Processes are in italic along the arrows. (Söderström and Gunnarsson, 2003)

gemmae for the next growing season; therefore the concept of individual becomes problematic. Currently five categories of bryophyte life span are recognised (Joenje and During, 1977; During, 1979). These are;

(i) ephemerals – life cycle generally shorter than one year, events in the life cycle not restricted to a particular season. Mortality is mostly determined by abiotic factors;

(ii) annuals – life cycle normally one year, mostly strongly seasonal with a resting stage in which only spores are alive. Mortality is determined abiotically.;

(iii) pauciennials – life cycles normally one year or a few years, mostly strongly seasonal. Mortality has often partly biotic causes;

(iv) pluriennials – life cycle normally several years, few specimens reaching higher than 5 – 10 years (short lived perennials), seasonality of the gametophyte less distinct. Mortality (indirectly) caused by competition of phanerogams or change of habitat;

(v) long-lived perennials – life cycle many years, age of specimens often much longer than 5 years, seasonality of the gametophyte less distinct. Mortality caused by several factors, all of which play a part, among which are predation by animals and small habitat catastrophes.

1.3.2. Bryophyte diaspores

Diaspores play a significant part in the life cycle of bryophytes. They affect the distribution of bryophytes in both space and time, and are the form in which dispersal takes place. Their ability to disperse, and hence occupy new places, affects their distribution in relationship to the source while their ability or inability to go into a period of dormancy (Laaka-Lindberg & Heino, 2001), and hence contribute to the diaspore bank while being able to germinate later in response to environmental conditions, determines the species' distribution in time. Diaspore dispersal ability is mainly affected by size, with smaller spores being dispersed longer distances (Crum, 1972; Miles and Longton, 1992). It is therefore important to establish how many diaspores are produced and how many of those produced are dispersed. Diaspores are only important to the

future population when they can still germinate. Therefore, how long they can stay viable is also important in the study of bryophyte life history strategies.

The life span of spores also varies with the species and environment, and may range from a few hours to decades (Fulford, 1951; Crum, 1972; Longton and Schuster, 1983; Duckett and Ligrone, 1992; Wiklund and Rydin, 2004). Spores of some species also appear to be very resistant to desiccation and low temperatures. Endurance of spores to harsh environmental conditions such as extreme draught, freezing and ultra violet light is also important and is meaningful in relation to spore size and number. Asexual propagules such as gemmae and tubers have also been shown to survive such adverse conditions significantly longer than the gametophyte shoots (Parihar, 1961; Renzaglia, 1978).

1.3.3. Bryophyte life Strategies

The general concept of life-history strategies for bryophytes was established by During (1979), who defined six categories on the basis of gametophyte longevity, reproductive longevity, reproductive effort, spore size and other attributes in relation to spatial and temporal features of habitat availability. Following During's (1979) general concepts, there have been more studies on bryophyte life strategies with Longton and Schuster (1983) speculating on the evolutionary trends in the characteristics and others considering strategies in particular climatic zones (Longton, 1988; Kürschner, 2004), and communities (Kürschner and Parolly, 1999; Austrheim *et al.*, 2005) and their relationship to rarity (Söderström and During, 2005).

During's (1979) general bryophyte life history strategy system is based on characters such as life form, life span (avoidance versus tolerance), strategy of gametophyte reproduction, age of first reproduction, reproductive effort (sexual reproduction, e.g. regular formation of sporophyte versus asexual reproduction by propagules), size and number of spores, dormancy of spores and dispersal strategy [few, large spores (>25um indicating decreasing long range dispersal) versus many, small spores (<25um providing opportunities for chance dispersal over longer distances].

The six life history strategies identified by During (1979) (summarised in Tables 1.1 and 1.2) are; fugitives, colonists, annual shuttle species, short lived shuttle species, perennial shuttle species and perennial stayers. Frey and Kürschner (1991) (Quoted in Kürschner, 2004) added a seventh strategy, the geophytes defined as those species that survive unfavourable seasons beneath the surface as a small bulb, rhizome, tuber or root bud.

Studies by Hedderson and Longton (1995, 1996) on three moss families have shown clear evidence of life history "strategies" in mosses, and revealed six main groups that correspond at least roughly to the strategies proposed by During (1979). Their studies, which included 357 species, provided evidence for covariation of life history traits across a range of taxonomic levels. They also show that the variation in life histories is strongly influenced by phylogeny with some evidence of adaptive life history variation.

1.3.4 Evolutionary trends in Bryophyte strategies

Longton & Schuster (1983) suggested that the Perennial Stayer strategy is the generalised (presumably they meant plesiomorphic) bryophyte life history strategy, and proposed two evolutionary pathways that lead to more specialised types (Figure 1.2) (See Longton and Schuster, 1983; Longton 1988). Both are considered to be responses to selection along gradients of shorter habitat availability and are marked towards monoicism and high early sexual reproductive effort. Each represents a shift from K-selection towards r-selection.

One pathway leads to colonists and fugitives and the other represents shuttle strategies. There may be evolutionary links between the two with both colonists and shuttle species between occurring within some genera.

Table 1.1. Summary of During's preliminary system of bryophyte life strategies.

Potential Life span (Years)	Spores		Reproductive Effort
	Numerous, Very Light (< 20µm)	Few, Large (>20µm)	
< 1	Fugitive	Annual shuttle	High
Few	Colonists	Short - lived shuttle	Medium
Many	Perennial Stayers	Long – lived shuttle	Low

(Source During 1992)

Table 1.2. A revised system of bryophyte life strategies.

Potential Life span (Years)	Spores		Reproductive Effort
	Numerous, Very Light (< 20µm)	Few, Large (>20µm)	
< 1	Fugitive	Annual shuttle	High
Few	Colonists	Short - lived shuttle	Medium
	Ephemeral colonists	Long – lived shuttle	
	Colonist S S(*senso stricto*) Long – lived shuttle Pioneers		
Many	Perennial stayers	Dominants	Low
	Competitive perennials		
	Stress – tolerant perennials		

(Source During 1992).

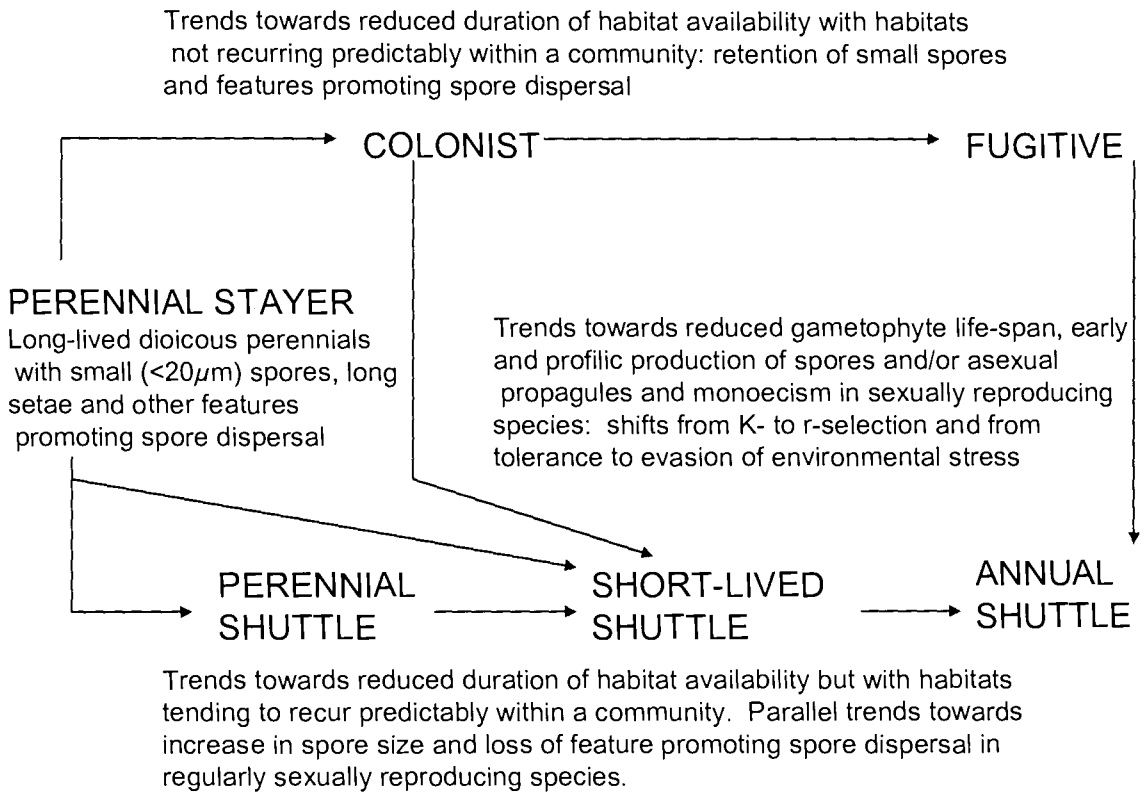

Figure 1.2. Presumed evolutionary trends among bryophytes life histories as proposed by During (1979). The changes are assumed to have occurred independently in several phyletic lines within the Bryophytes.

Source: Longton (1988).

1.4. The family Lophoziaceae

1.4.1. General overview

The family Lophoziaceae (as defined by Grolle and Long, 2000) is a large family of hepatics with 300 to 350 species in about 30 genera. The family has several taxon complexes that are very messy and not clearly defined, and different bryologists working on the family have dealt with them differently. It has variously been treated as a family on its own or as a subfamily of Jungermanniaceae. Recent studies (Schill *et al.*, 2004; Davis, 2004; Yatsentyuk *et al.*, 2004; De Roo *et al.*, 2007.) suggest that Jungermanniaceae is not closely related to Lophoziaceae, and that *Leiocolea* (K. Müll.) Buch and Jamesonielloideae, usually treated as parts of Lophoziaceae, might not belong there. It is also suggested that the family Scapaniaceae could be included in Lophoziaceae.

The plants in the family vary from small to very large (shoots 0.5 – 5 mm wide by 1 – 12 cm long). The stems are erect to prostrate and the cortex opaque, never forming a hyalodermis. There is no stem dimorphism. Branching is chiefly axillary, but occasionally terminal. The leaves are alternate, and fundamentally succubously inserted and oriented. The leaves vary from two to four lobed, in three unequal rows, but usually with a ventral row of leaves strongly reduced and often suppressed. Two to eight or 12 to 50 oil bodies are present per cell and are about 3 to 9 μm long.

Species of Lophoziaceae are terrestrial, growing mainly on soil, rocks or organic substances, and more rarely on mineral soil. Some species are corticolous but none are aquatic. They may be epilithic or epixylic and only very few are epiphytic. They mainly require moist or wet conditions and thus are largely absent from drier regions, or regions with a prolonged dry period (Söderström *et al.*, 2007.).

The species range from narrow endemics or species with very restricted distribution, e.g. *Anastrophyllum tenue* H. Williams occurring in a few localities

in Ontario, Canada, to those that are widespread or occur over large areas (circumboreal) and some species (e.g. *Lophozia excisa* (Dicks.) Dumort. and *Barbilophozia hatcheri* (A. Evans) Loeske) are also bipolar (Söderström and Séneca, In press). They also range from rare species to abundant ones. With the exception of several rather questionably assigned genera, the Lophoziaceae are predominantly developed in the cooler and cold portions of the Northern hemisphere, ranging to the highest portions of the arctic and, to a somewhat lesser extent, in the cooler portions of the antipodes. *Chandonanthus* Mitten and *Anastrophyllum* (Spruce) Steph. are found primarily in montane tropical and temperate rainforest. *Tetralophozia* (R. M. Schust.) Schljakov is holarctic.

Lophoziaceae are dioicous or paroicous, and rarely autoicous. They produce numerous archegonia. The archegonia (and perianth) are always terminal on main or long, normal shoots. The species range from those with frequent sexual reproduction to those unknown with spore production. Asexual reproduction is usually present, normally by means of 1-2 celled gemmae produced on the leaf lobes in branching catenate fascicles and rarely by caducous perianths and never by caducous leaves or shoots. However, some species such as *Mesoptychia sahlbergii* (Lindb. & Am.) Evans (Frye and Clark, 1937) and *Anastrophyllum papillosum* J.J. Engel and Braggins (Engel and Braggins, 1998) still have no known means of reproduction.

1.4.2. Life history evolution in Lophoziaceae

A few recent studies mainly by Laaka-Lindberg and others have considered some aspects of life histories of some members of Lophoziaceae. These studies showed that maturation of archegonia and the proportion of sporophyte-bearing shoots were correlated with mean rainfall and relative humidity in *Lophozia silvicola* (Laaka-Lindberg, 2005). It was also established that the species produces gemmae throughout the growing season, but the gemmae produced later during this period have low germination percentages due to increased dormancy. Biomass allocation to sexual and asexual reproduction and clonal dynamics of the same species was also established

(Laaka-Lindberg, 2001, 2005; Laaka-Lindberg and Henio, 2001). It was also shown that in some species such as *Anastrophylum hellerianum* which reproduce both sexually (spores) and asexually (gemmae), both types of diaspores function in species dispersal (Pohjamo and Laaka, 2003; Pohjamo *et al.* 2006). Apart from these studies, not much of life histories in Lophoziaceae has been published.

1.5. Objectives and thesis outline

It is imperative to have reliable quantitative data on life history traits if any meaningful evaluation and understanding of general life history evolution is to be achieved for any particular group of organisms. Although raw data on hepatic life history traits might exist in the literature, they usually have not been looked at or analysed in that context. Therefore, as highlighted above, life history information on bryophytes is currently very limited. This thesis examines the existence of suites of life history traits in some members of the family Lophoziaceae as a case study in seeking to understand patterns of life history variation in bryophytes. Lophoziaceae provides a good candidate for such a study because it is a large family, whose species range from narrow endemics to those that are wide spread, rare species to very abundant ones, species with frequent sexual reproduction to those unknown with spore production, and species with and without sexual reproduction. Data from the literature as well as original data from field and laboratory work are used to examine relationships among life history traits in the family. The study examines the nature of co variation of life history traits and the evolution of life history strategies including the role of trade-offs at species and population levels.

The work presented here has been organised into a series of interlinked chapters. These are not necessarily independent of each other but are organised sections to help focus on particular aspects of life histories in the family under study. In Chapter 2 data from the literature are used to examine covariation of life history traits and to assess the extent to which either the

phylogeny or the environment is reflected in these patterns of covariation. Chapter 3 deals with a particular methodological aspect of the study – the measurement of spore sizes. It was designed to guide the author in gathering data for subsequent chapters, but the findings are of general significance for anyone undertaking similar studies.

Chapter 4 examines the nature of variation in size and quantity of sexual (spores) and asexual (gemmae) diaspores produced at species and population levels. Also considered is the nature of the relationship between offspring size and number produced per capsule/shoot. Chapter 5 assesses species and population level variation in germinability of diaspores.

The concluding chapter (Chapter 6) attempts to integrate all the information thus compiled. It discusses the role of trade-offs in shaping up bryophyte life histories and the factors which might constrain these trade-offs. It also discusses the relevance of sexual and asexual reproduction in bryophyte life histories.

CHAPTER 2

COVARIATION OF LIFE HISTORY CHARACTERS IN THE FAMILY LOPHOZIACEACE: A MULTIVARIATE ANALYSIS

2.1 Introduction

Life history traits are those features of organisms that are directly related to their reproductive success. The assumption is that life history traits evolve to maximize fitness, here defined as in Stearns (1976; 1982) as genetic representation in the next generation, within constraints imposed by environmental and/or genetic limitations (Partridge and Sibly 1991; Stearns 1976, 1977). Natural selection, acting on the genetic potential of individuals, might be expected to result in organisms displaying a specific combination of life history attributes. However, the actual life history attributes displayed in an organism are a result of trade-offs between conflicting demands associated with maximizing the potential to adapt to changing environments (Cody, 1966; Stearns 1976). Organisms in specific environments, therefore, are expected to display combinations of co-evolved or co-adapted life history traits that are sometimes referred to as tactics or strategies (During, 1979, 1992; Grime, 1979; Mishler, 1988; Longton, 1988; Johansson, 1994; Silvertown and Charlesworth 2001).

The combination of life history traits that an organism displays results from the influence of several intrinsic and extrinsic factors (Hedderson and Longton, 1996). Among these factors are age-specific mortality and spatio-temporal patterns of environmental variability (MacArthur, 1962; Harper 1967; Stearns, 1976), ecological factors of herbivory, competition etc. (Hutchings and Morris, 1985; Hendrix, 1988; Murphy, 1989) and physiological, developmental and genetic factors. The genealogy and history of the organism are also important (Lauder, 1982; Hedderson and Longton, 1996; Bell, 1989)

Co-variation of life history characters has been demonstrated in many animals and tracheophytes. (References in Hedderson and Longton, 1995). During (1997, 1992) provided the first classification of life histories in bryophytes, in which he proposed six life history strategies based on reproductive effort, life span and mortality and on adaptations to periods of severe stress (avoidance versus tolerance) (See Chapter 1). Hedderson and Longton, (1995, 1996) used a combination of ordination and clustering techniques to test for the occurrence of recurrent suites of life history variation among species in the three moss orders Pottiales, Funariales, and Polytrichales. These authors recovered six main clusters corresponding roughly to During's categories and showing a primary gradient between species producing few large spores to those producing many small spores. Also shown was a gradient from long lived species occurring in permanent habitats producing many small spores in sporophytes with well-developed dispersal mechanisms to species with a short life span occupying habitats that undergo long periods of stress producing large spores needing high levels of parental investment. Hedderson and Longton, (1995; 1996) also suggested a strong influence of phylogenetic history on life history variation in mosses. The existence of recurrent suites of life history traits in hepatics has not yet been examined.

The relationship between sexuality (i.e. dioicous versus monoicous) and mode of reproduction (sexual, asexual or mixed) is another important feature in bryophytes. Fertilisation in this group is dependent on water, needed to disperse the small, mobile spermatozoids, and this may limit fertilization distance to a few centimetres (Anderson and Lemmon 1974; Andersson 2002). This would make sexual reproduction easier in monoicous plants that can self fertilise than in dioicous plants which might suffer from "unequal sex-distribution and the absence of one sex" Sundberg (2002). A positive association between monoicy and high frequency of sporophyte production has been shown to exist in a wide range of bryophytes (Longton and Miles 1982; Hedderson and Longton, 1995, 1996; Longton, 1997; Laaka-Lindberg *et al.*, 2000). Rarity or absence of sporophytes in both mosses and liverworts is strongly associated with dioicy, although substantial numbers of dioicous

species can fruit freely (Longton 1997; Laaka-Lindberg *et. al.*, 2000). In addition, the production of asexual propagules has been shown to be much more likely in dioicous than monoicous species for British mosses (Longton 1997).

The significance of asexual versus sexual reproduction in bryophyte life histories has also been a subject of debate. Some doubt has been cast on the importance of spores in perennial bryophytes, even in species that produce spores frequently (Sundberg, 2000 and references therein). It is often argued that asexual reproduction is more important than spores in bryophyte reproduction, at least at local scales, because asexual propagules establish more readily and are produced early in the life cycle (Kimmerer, 1991b). Thus they function in maintainance and expansion of local populations. Spores are thus seen as more important for dispersal and colonization, helping establishing new populations on new habitats e.g. on fresh logs or re-establishing populations following a disturbance (Kimmerer, 1991b; Sundberg, 2000, Hassel, 2003, Longton, 2006)). However, recent studies on dispersal in *Anastrophyllum hellerianum* (Nees) R.M.Schust. has shown that gemmae also function in distance dispersal (Pohjamo *et al.*, 2006).

Although some aspects of hepatic life histories have been studied (e.g. the relationship between sporophyte production and sexuality: Longton and Schuster, 1983; Laaka-Lindberg and Heino, 2001; Laaka-Lindberg, 2005), such studies are relatively rare. This original aim of this study was to provide a broad overview of relationships among some critical life history traits in the family Lophoziaceae. However, due to limitation on the available data for the species, the analyses were perfomed on a limited number of species (maximum 32) from seven genera (See Appendix 1). Ordination and clustering techniques are employed to test for recurrent suites of life history variation in among 32 species from seven genera. In addition I analyse the relationship(s) between sexuality, reproductive mode and sporophyte/gemma frequency among members of the group. The specific questions it aims to answer are (i) Do members of the 32 species form coherent groups based on specific combinations of life history traits; in other words, are there recurrent

suites of life history traits among the species? (ii) Are such groups associated with phylogeny or with ecology? (iii) Do these groups conform or fit with During's (1979; 1992) groups? and (iv) Is there any relationship between sexuality or mode of reproduction and frequency of sporophyte or gemma production among the studied species?

2.2 Materials and Methods

Most of the methods used here are basically modifications of the protocol used for mosses by Hedderson and Longton (1995).

2.2.3 Data Collection

The analysis was based on the species of Lophoziaceae for which data could be compiled from literature on mode of reproduction, sexuality, spore size, sporophyte frequency, gemma size, and gemma frequency. The species used in the analeyes and the sources of data are indicated in Appendix 1. Differences between regions or differences between the literature sources were not considered. Table 2.1 shows a summary of the characters that were used in the analysis. Spore size was represented as the median diameter using the minimum and maximum obtained across all literature sources. Gemma size was represented by the mid range of a spheroid volume calculated using a) the minimum width and minimum length and b) maximum width and the maximum length, in each case obtained from literature.

2.2.3 Statistical analysis

All the analyses presented here were performed using procedures in STATISTICA version 7 (Statsoft, 2003). Principal Component Analysis was used to summarise patterns of correlation in the life history parameters. The idea behind Principal Component Analysis is to represent the main structural features of a multivariate data set in terms of a smaller number of variables so

that they may be better understood and displayed graphically while the features of the data that may be lost consist of irrelevant "noise" (Krzanowski and Marriot, 1994). Thirty-two species had complete data for the five variables (Table 2.1) and therefore were used in Principal Component and Cluster Analyses based on correlations.

Scores on the components were retained and used as variables in subsequent cluster analysis to identify groups of species exhibiting similar degrees of covariation in the life history traits. Ward's minimum variance criterion, where cluster membership is assessed by calculating the total sum of squared deviations from the mean of a cluster, and Euclidian distance were used to construct the cluster diagram. The number of clusters recognised was determined by identifying the major inflection point on the line graph of number of groups versus distance (Figure 2.2).

In order to asses the phylogenetic effect on the clusters produced by Cluster Analysis, cluster membership for each of the species used in Cluster Analysis were superimposed on a modified phylogenetic tree based on molecular data (De Roo *et al.*, in press). The phylogenetic tree was pruned to leave only those species that were used in this analysis. Using MacClade the Rescaled Consistency Index (RCI) of the character members was obtained before an 100 randomised versions of the group characters in which the proportions of states are held constant were generated. A list of the RCI scores for the random data was calculated. From the frequency distribution of RCI for random characters, it can then be seen whether the RCI obtained from the "real" character is significantly larger than the RCI of the membership character. In other words, how often would one get that value from a data set that is random with respect to phylogeny. If the RCI of the character members was better than random, this would imply some degree of phylogenetic structure: otherwise no such conclusion can be made.

Table 2.1. *Description and scoring of traits used in the analysis.*

Characters (abbreviations)	**Descriptions**
Sexuality (RSY)	1-synoicous, 2-paroicous, 3-autoicious, 4-heteroicous. 5-dioicous
Sporophyte Frequency (SFR)	1-never, 2-very rare, 3-rare, 4-occassionally, 5-frequent, 6-always.
Spore Size (SPS)	Median diameter in μm.
Gemmae Size (GSZ)	Median estimate of volume
Gemmae Frequency (GFR)	1-lacking, 2-very rare, 3-rare, 4-occassionally, 5-frequent, 6-always.

2.2.4 Relationship between sexuality and diaspore production

Sporophyte and gemma frequency were initially scored from the literature on a decreasing scale of common, frequent, occasional, rare, very rare, and sterile (for sporophyte frequency) or lacking (for gemmae frequency). The sexuality was scored as dioicous or monoicous. In interpretation of diaspore frequency, plants producing diaspores occasionally, frequently and always were said to produce them freely while those producing them rarely or very rarely were said to produce them rarely. Only those species for which information on both the sexuality and diaspore frequency could be obtained from literature were included in the analyses. The different types of monoicy (autoicous, synoicous, and paroicous) were combined because of the very low numbers involved. The G-statistic was used to examine the relationship between reproductive mode and sexuality and the relationship between sexuality and both gemmae and sporophyte frequency. The G-statistic tests the null hypothesis that the relative proportions of one variable (in this case sporophyte or gemmae frequency) are independent of the second variable (reproductive mode); in other words, the proportions of one variable are the same for different values of the second variable (Sokal and Rohlf 1995).

2.3 Results

2.3.1 The life history groups

Three Principal Components, accounting for 76.1% of the total variation among the species used (Table 2.2), were retained and used in the cluster analysis. Under Ward's criterion, 6 clusters are recognised (Figure 2.2 and Table 2.5). Table 2.5 shows the membership of the groups and their main characteristics.

The first Principal Component accounts for 37.9% of variation. Variables with high loadings on this Component are sexuality (positive), spore size (negative) and gemma size (negative). It also loads negatively for both

Table 2.2. Loadings of the five life history traits on the first three Principal Components.

TRAITS	PRINCIPAL COMPONENTS		
	1	2	3
Reproductive system (RSY)	0.74	0.21	0.04
Sporophyte frequency (SFR)	-0.49	-0.61	-0.53
Gemmae frequency (GFR)	-0.29	0.80	-0.40
Log SPS1	-0.71	-0.05	0.53
Log GSZ	-0.72	0.35	0.05
Eigenvalue	1.90	1.18	0.72
% Total Variance	37.92	23.68	14.44
Cumulative Eigenvalue	1.90	3.08	3.80
Cumulative %	37.9	61.6	76.05

Table 2.3. Pairwise correlations of the life history traits

	RSY	SFR	GFR	Log SPS	Log GSZ
Reproductive system	1.00				
Sporophyte frequency	-0.31	1.00			
Gemmae frequency	-0.06	-0.10	1.00		
Log SPS	-0.35	0.21	0.09	1.00	
Log GSZ	-0.34	0.13	0.29	0.35	1.00

Table 2.4. Summary of each variable for each Cluster. Spore size is expressed as mean spore diameter in μm and gemma size is expressed as mean gemma volume in μm^3.

Cluster	Reproductive mode	Sexuality	Sporophyte frequency	spore size	Gemmae frequency	gemma size
1	Both	Paroicous	Frequent	16	Frequent	48581.7
2	Both	dioicous	Very rare	13.5	Frequent	13870.5
3	Both	Dioicous/paroicous	Occasionally/rare	13.7	Frequent	18788.0
4	Both/spores only	Dioicous/heteroicous	rare	12.9	Rare/lacking	7078.7
5	Both	dioicous	Frequent	9.75	Always/ rare	5999.2
6	Both	dioicous	Rare/never	10.6	Frequent	8202.4

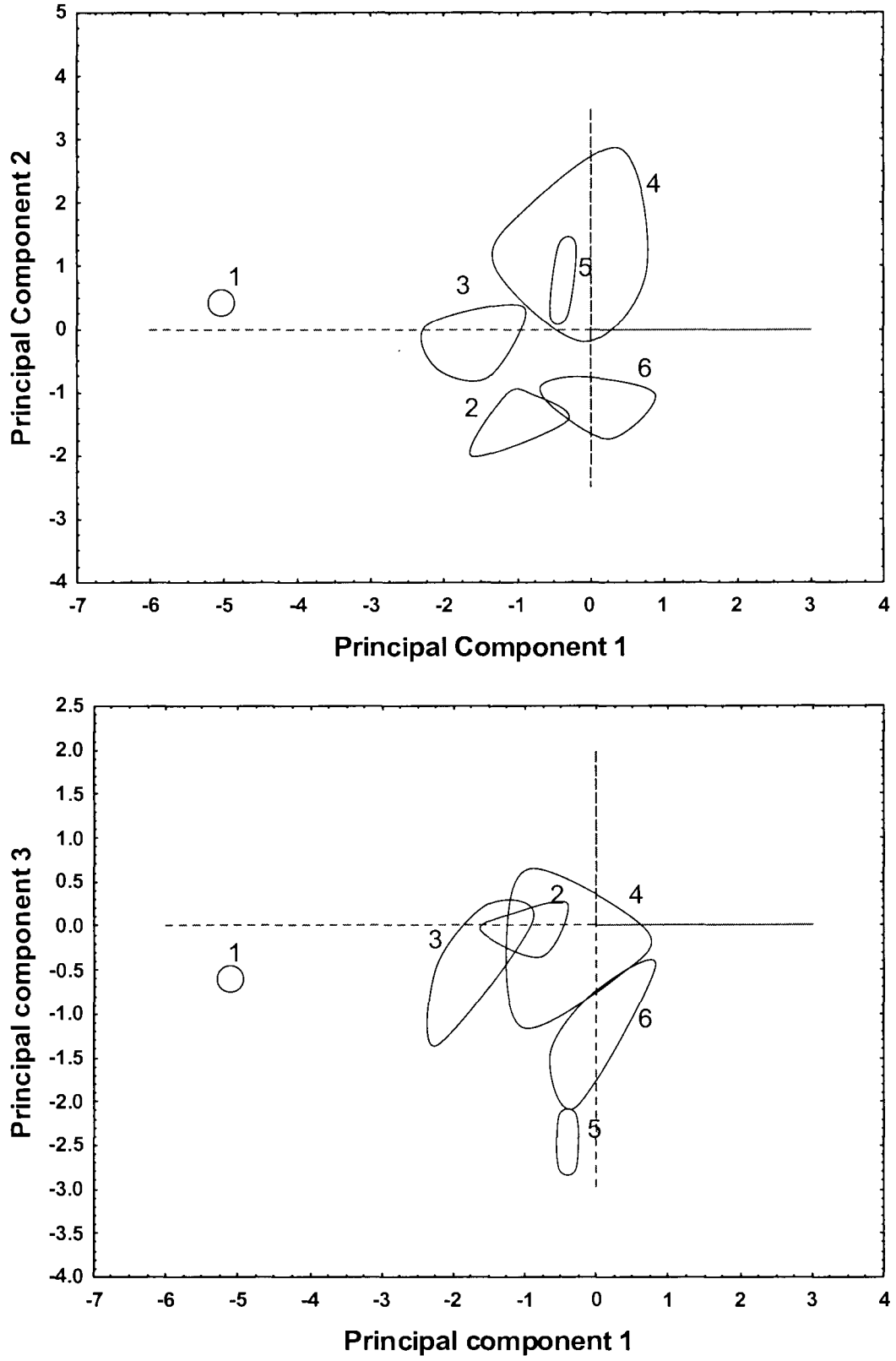

Figure 2.1. Dispersion of the six life history clusters on the first three Principal components. The bivariate range of each cluster is outlined.

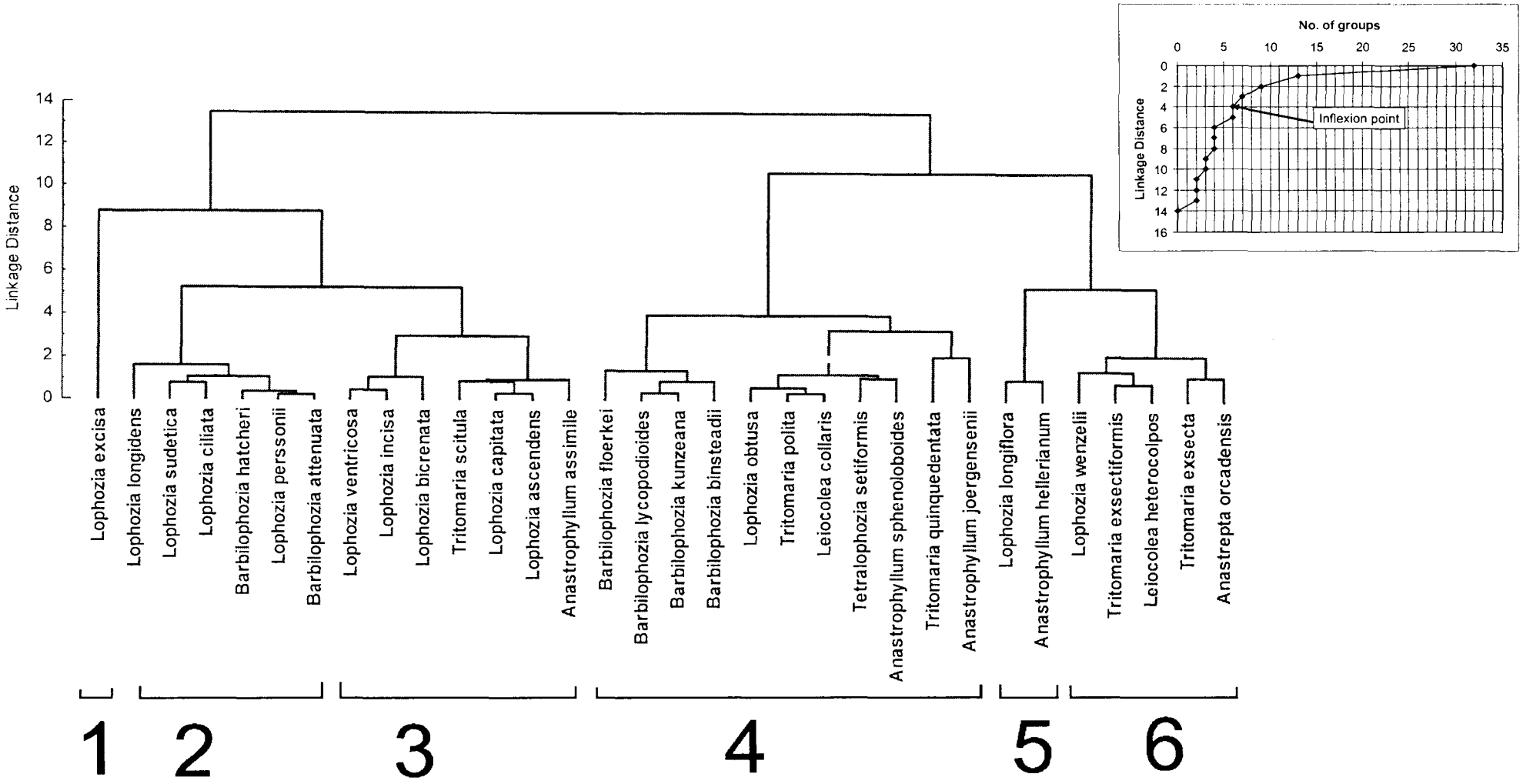

Figure 2.2. Cluster diagram for the species using Ward's minimum criterion method and Euclidean distances. The insert shows how the number of groups was decided.

Table 2.5. Composition of the six clusters identified by cluster analysis

Cluster	Species included	Major characteristics
1	*Lophozia excisa*	Paroicous, sporophytes frequent, spores large, gemmae large.
2	*Lophozia longidens, Lophozia sudetica, Lophozia ciliata, Barbilophozia hatcheri, Barbilophozia perssonii, Barbilophozia attenuata*	Dioicous, sporophytes rare, spores large, gemmae large and frequent.
3	*Lophozia ventricosa, Lophozia incisa. Lophozia bicrenata, Tritomaria scitula, Lophozia capitata, Lophozia ascendens, Anastrophyllum assimile,*	Mostly dioicous, sporophytes mainly rare but sometimes occasional, spores large, gemmae frequent.
4	*Barbilophozia floerkei, Barbilophozia lycopodioides, Barbilophozia kunzeana, Barbilophozia binsteadii, Lophozia obtuse, Tritomaria polita, Leiocolea collaris, Tetralophozia setiformis, Anastrophyllum sphenoloboides, Tritomaria quinquedentata, Anastrophyllum joergensonii.*	Dioicous, heteroicous, sporophytes rare, spores small spores, gemmae small and rare or lacking
5	*Lophozia longiflora, Anastrophyllum hellerianum,*	Dioicous, sporophyte frequent, spores small, gemmae large and rare or small and frequent
6	*Lophozia wenzelii, Tritomaria exsectiformis, Leiocolea heterocolpos, Tritomaria exsecta, Anastrepta orchadensis.*	Dioicous, sporophytes rare or never produced, spores where produced small, gemmae of varying sizes but frequent.

gemma and sporophyte frequency. This variate thus describes an axis along which dioicous species e.g. *Barbilophozia floerkei* that produce small diaspores rarely are contrasted with monoicous species that produce large diaspores frequently e.g. *Lophozia excisa*. In terms of the clusters defined in Figure 2.2, there is a continuum from the mainly paroicous species of cluster 1 through a combination of both paroicious and dioicous species in clusters 3 and 4 to dioicous species in clusters 5 and 6 (Figure 2.1. The second component, which accounts for 23.7% of the overall variation, is mostly correlated with sporophyte frequency (negative) and gemmae frequency (positive). It also loads positively for gemmae size. It describes species that rarely produce sporophytes but produce large gemmae frequently e.g. *Lophozia longidens* and *Leiocolea heterocolpos* as contrasted to species that produce sporophytes frequently and produce small gemmae rarely e.g.*Tritomaria quinquedentata* and *Lophozia longiflora*. This mainly separates clusters 2 and 6 from clusters 4 and 5 (Figure 2.1). The third component represents about 14.4% of overall variation, and loads highly on sporophyte frequency (negative) and spore size (positive). It also loads negatively for gemmae size. Thus it describes an axis along which species e.g. *Anastrophyllum hellerianum* (cluster 5) that produce small spores frequently and gemmae rarely are contrasted with those that produce big spores rarely e.g. *Barbilophozia hatcheri* (cluster 2) and *Barbilophozia binsteadii* (Cluster 4) but gemmae more frequently. It separates cluster 5 from cluster 4 and cluster 2 from cluster 6 (Figure 2.1).

2.3.2 Do the life history clusters fit the phylogenetic clusters?

Figure 2.3 compares the life history clusters to a phylogenetic tree based on molecular evidence. I have not performed a formal statistical test, but the distribution of cluster membership across the phylogeny clearly shows that the clusters are not linked to the phylogeny. The Rescaled Consistency Index (RCI) of the membership character on the molecular phylogenetic tree was 0.09.The value fitted well in the normal distribution of the RCIs of the randomly selected data were the highest frequency was for the group 0.08-

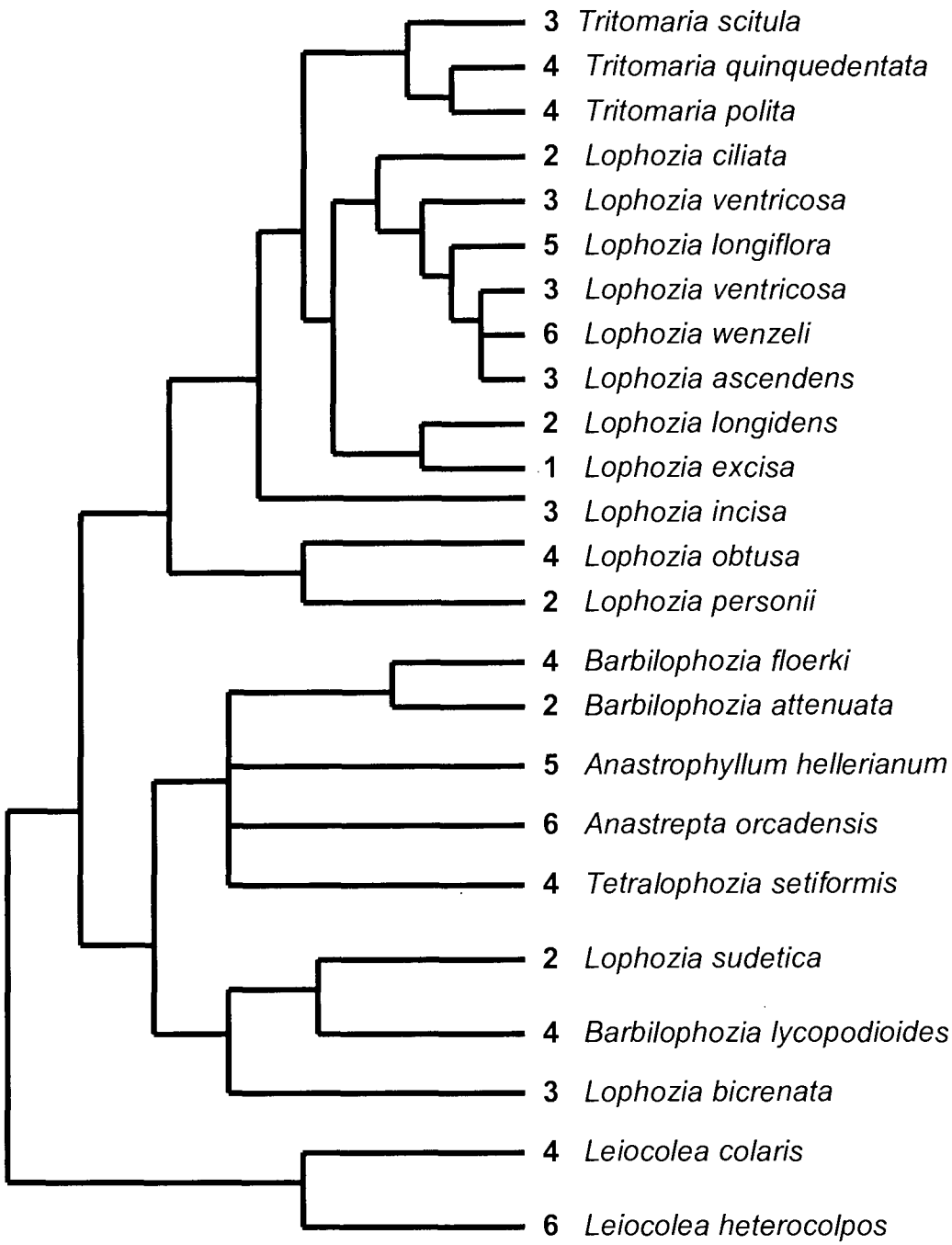

Fig 2.3. The phylogenetic tree of the species used in cluster analysis. The tree has been modified from De Roo *et. al.* (in press) phylogenetic tree to include only those species used in cluster analysis. The numbers before the species name indicate the cluster of the species from cluster analysis.

0.12, which also suggest no close relationship between the clusters and phylogeny.

2.3.3 Sexuality and mode of reproduction

The majority of the species studied (64% of 70) are capable of reproducing both sexually (through spores) and asexually (through gemmae). A further 24% reproduce only sexually whilst 11% are known to reproduce only asexually through gemmae (Table 2.6). Out of the total set, 89% of the species were dioicous (Tables 2.6 and 2.7.

Sixty six (66) percent of the dioicous species are capable of reproducing through both spores and gemmae while 21% produce spores only and 13% produce gemmae only. Fifty percent of the 8 monoicous species studied are capable of reproducing through both spores and gemmae and the other half produce only through spore. None of the studied monoicous species produce gemmae only. The null hypothesis of independence between mode of reproduction and sexuality therefore cannot be rejected ($G = 4.207$, $p = 0.12$).

2.3.4 Sexuality and frequency of diaspore production

Of the 68 species studied (Table 2.7), 25% produce sporophytes freely, 72% produce them rarely, and 3% are known to be sterile. Of the 62 dioicous species, 19% produce sporophytes freely, 77% produce them rarely, and 3% are known to be sterile. All of the six monoicous species studied are known to produce sporophytes with 5 of them (83%) producing sporophytes freely and only one (the only heteroicous species) produces sporophytes rarely. From the G-test we can reject the null hypothesis of independence between sporophyte production and sexuality ($G = 10.227$, $p = 0.006$).

Forty two (42) percent of the 65 species studied (Table 2.8) produce gemmae freely while 29% produce gemmae rarely. The remaining 29% are not known with gemmae. Forty three (43) percent of the 58 dioicous species produce gemmae freely, 31% produce gemmae rarely, while 26% are not known with

Table 2.6. Two-way frequency distributions of sexuality and reproductive mode

Sexuality	Reproductive mode			
	Spore and Gemmae	Spores only	Gemmae only	Total
Dioicous	41	13	8	62
Monoicous	4	4	0	8
Total	45	17	8	70

Table 2.7. Two-way frequency distributions of sexuality and sporophyte frequency

Sexuality	Sporophyte frequency			
	Common, Frequent or Occasional	Rare or Very rare	Sterile	Total
Dioicous	12	48	2	62
Monoicous	5	1	0	6
Total	17	49	2	68

Table 2.8 Two-way frequency distributions of sexuality and gemmae frequency

Sexuality	Gemma frequency			
	Common, Frequent or Occasional	Rare or Very Rare	Lacking	Total
Dioicous	25	18	15	58
Monoicous	2	1	4	7
Total	27	19	19	65

***Table* 2.9.** Two-way frequency distributions of sexuality and sporophyte frequency for species capable of reproducing both sexually and asexually

Sexuality	Sporophyte frequency		
	Common, Frequent or Occasional	Rare or Very Rare	Total
Dioicous	10	29	39
Monoicous	2	1	3
Total	12	30	42

Table 2.10. Two-way frequency distributions of sexuality and gemmae frequency for species capable of reproducing through both spores and gemmae

Sexuality	Gemmae frequency		
	Common, Frequent or Occasional	Rare or Very Rare	Total
Dioicous	20	14	34
Monoicous	2	1	3
Total	22	15	37

Table 2.11. Two-way frequency distributions of sexuality and sporophyte frequency for species that produce spores only and not gemmae.

Sexuality	Sporophyte frequency		
	Common, Frequent or Occasional	Rare or Very Rare	Total
Dioicous	4	7	11
Monoicous	3	0	3
Total	7	7	14

Table 2.12. Two-way frequency distributions of sexuality and gemmae frequency for species that reproduce asexually only

Sexuality	Gemmae frequency		
	Common, Frequent or Occasional	Rare or Very Rare	Total
Dioicous	4	2	6
Monoicous	0	0	0
Total	4	2	6

gemmae. Gemmae frequency therefore appears to be independent of sexuality ($G = 2.765$, $p = 0.251$).

Seventy four (74) percent of the 39 dioicous species that are capable of reproducing through both spores and gemmae rarely produce sporophytes (Table 2.9). Of the three monoicous species, two (both paroicous) produce spores frequently. The one that rarely produce spores is heteroicous. Seventy nine (79) percent (of 34) of the dioicous species that are capable of reproducing through both spores and gemmae produce gemmae frequently (Table 2.10). Two out of the three monoicous species (both paroicous) produce gemma frequently. Generally, a higher proportion (59% of 37) of species that are capable of reproducing both sexually and asexually produce gemmae freely (Table 2.10) while a low proportion (29% of 42) produce sporophytes freely (Table 2.9). However the G-test suggests independence of sexuality and sporophyte frequency ($G = 2.033$, $p = 0.154$) and between sexuality and gemmae frequency ($G = 0.072$, $p = 0.789$) for species that can reproduce both ways. The power of the test may be limited by the small sample size for monoicous species in this case.

Four of the eleven dioicous species that produce spores and no gemmae produce sporophytes freely while all three of their monoicous counterparts produce sporophytes freely (Table 2.11). From the G-test ($G = 4.98$, $p = 0{,}026$), we can reject the null hypothesis of independence between sporophyte production and sexuality for this group. No monoicous species among the species studied are known to reproduce only asexually. Of the six (6) dioicous species that are known to reproduce only asexually (Table 2.12), four (4) produce gemmae commonly, frequently or occasionally while two (2) produce gemmae rarely.

2.4 DISCUSSION

The results presented above are consistent with the existence of recurrent suites of life history traits in the family Lophoziaceae. The clusters observed do not suggest obvious relationship to the species' phylogeny (Figure 2.3). The species studied, though few when considering the number of species in the family, resolve, as discussed below, into more or less coherent groups that are associated with identifiable ecological conditions, especially habitat. There is evidence of a relationship between sexuality and sporophyte frequency, whilst gemma frequency appears to be independent of sexuality.

2.4.1 Associations with habitat

Table 2.13 summarises the ecological conditions that can be associated with each of the clusters. Generally clusters 1, 2 and 3 contain those species from damp places, where there is abundant water for longer periods. Clusters 4, 5 and 6 occur in less wet, or at least better drained, conditions.

Cluster 1 contains only one species - *Lophozia excisa*. The species occurs in damp sites. It produces relatively large sexual and asexual diaspores frequently. The habitat is available for relatively long periods and the plants grow mainly on nutrient rich soils. It is possible that larger diaspores might have a germination, establishment and survival advantage (During 1997, Sundberg, 2000). Since the species is a pioneer, longer survival of the diaspores in the diaspore bank would be necessary since the new habitats might take long to present themselves. The species is paroicous, more likely as selection for close proximity of gametangia. The occurance of male and female gametangia on separate branches of an individual is said to promote sporophyte production because of the short distances the spermatozoids travel to reach the eggs while maximising chances of cross fertilisation (Longton, 2006).

Table 2.13. Ecological conditions for each of the species cluster.

Cluster	Ecological conditions
1	Occur on damp, sandy, loamy and calcareous soils mainly in mires.
2	Occur on moist to damp, mostly acidic or chalk soils. Also on tree trunks, decaying logs & stumps and on surfaces of rocks and boulders. The habitats include forest floors, below water falls, river banks and mountain slopes above tree line.
3	Occur on humid to damp, poorly drained and leached loamy soils. Also on rock wall and rock crevices and ledges, decaying logs and thin soil layer over rocks and exposed peat. Mainly associated with siliceous rocks and slate. Habitats include shaded places, seepage coverings, on banks of springs, near lakes, on the boarders of swamps, near falls and along brooks and in ditches.
4	Occur on wet, well drained soils and on organic (spruce & fir needles, humus, decaying logs) less peaty ground. Also associated with siliceous or calcareous soils as well as sediments or sedimentary rocks. In mires they are only found on logs. Habitats include rich ferns, soil on edge of ledge, grassy mountain slopes above tree line, shaded rock walls and north-facing and west-facing walls of ravines and boulders(Scandinavia
5	Occur on decaying logs and rarely on peat. Habitats include shaded site but also rarely in mires (only on logs).
6	Occur on moist to wet, peaty, calcareous and sandy soils. Also on soil cover over cliffs or rocks. Habitats include wet depressions, vertical and horizontal cliffs and rock intrusions at higher elevations.

Species in cluster 2 mainly occur on damp to wet habitats. They are mainly dioicous and sporophytes are rare. Dioicous species are known to exihibit relatively low levels of sexual reproduction because of infrequent sex expression, male rarity, spatial separation of sexes and sporophyte abortion (Longton and greene, 1969; Wyatt, 1977, 1994; Bowker et al., 2000; Stark et al., 2000). Cluster 3 species also occur on damp habitats with shallow soil layers or leached soils. The expected longevity of the habitat might be medium to long term. Most of the species are dioicous though some are paroicous and this might also explain why sporophyte frequency is occasional to rare. The spores, when they are produced, are also relatively large. Bigger spores are more likely to remain viable in the diaspore banks (Jonsson, 1993; During, 1997) and this might help ensure species future existence even when no spore production occurs. The gemmae are also big, probably to give the species germination and establishment advantage.

Cluster 4 species occur in wet, well-drained habitats such as mountain slopes and on calcareous rocks and humus. The suitable habitat conditions seem to be short-lived with the habitats drying off early in the season. Under such conditions, moisture availability is likely to restrict the period during which active metabolism can occur. This would mean that diaspore development has to occur in the short periods before the gametophytes dry out, during which time only limited resources are exploited for diaspore development. Thus both gemmae and spores when produced are small. Most of the species are dioicous and hence sporophyte production is rare.

Species in Clusters 5 and 6 occur on less moist habitats, such as decaying wood, peat, and calcareous sandy soils. These habitats are available for shorter periods and sometimes scattered. The spores produced are small, perhaps as a consequence of selection for easy dispersal to new scattered habitats. The two species in Cluster 5 show evidence of a trade-off in gemmae size and frequency. *Lophozia longiflora* rarely produces large gemmae, while *Anastrophyllum hellerianum* always produces small gemmae. Species in Cluster 6 produce sporophytes rarely most likely due to separation

of sexes. However, the gemmae are produced more frequently, more likely as a cheaper alternative means of reproduction.

2.4.2 Comparison to previous Classifications

It is very difficult to compare the current groupings to previous classifications (During, 1979, 1992; Longton, 1988; Hedderson and Longton 1995, 1996) mainly because the current study did not consider some of the generally considered life history traits such as gametophyte longevity, age at first reproduction, number of diaspores produced and reproductive effort. These were not easily available in the literature. The other contribution is that the family seems to have little diversity in terms of their ecological preferences and dispersal mechanisms.

Considering the traits that have been used in this analysis, there are some common trait combinations that are similar to those identified in previous classifications of bryophytes. For example, *Lophozia excisa* in cluster 1 would fit very well in During's (1979) colonist strategy. It is a pioneer with high frequency of both asexual and sexual diaspore production. It also has big gemmae, and although the spores are the largest among the studied species, they are below 20μm. Species in cluster 4 in producing both sexual and asexual diaspores rarely and having small spores would compare easily with During's ephemerals.

2.4.3 Correlation patterns among traits – evidence for trade-offs

The principal component analysis suggests trade-offs among life history traits in the family (Table 2.3) with dioicous species producing small diaspores rarely and monoicous species producing large diaspores frequently. Dioicous species are less likely to have successful fertilisation compared to monoicous species because of greater distances between the male and female gametangia: hence the less frequent production of diaspores. Species that rarely produce sporophytes produce large gemmae frequently while species that produce sporophytes frequently tend to produce small gemmae rarely. If frequency of diaspore production could reflect the quantity of diaspores

produced i.e if frequent diaspore production means more diaspores are produced, then for those species that produce sporophytes rarely to ensure continued existence they would select for frequent production of gemmae, which in that sense would translate to more gemmae. Such species would therefore be expected to be less mobile in space compared to those that produce sporophytes frequently. However they are more likely to be able to maintain their local populations since asexual propagules offer the advantages of germinating and establishing quickly in habitats that already favour the genetic make up. They are likely to select for small spores to favour dispersal and compernsate for the low numbers. However in species that produce large spores rarely, gemmae become necessary for maintainance of local populations during the periods when the spores are not produced. Some studies have also shown that gemmae also fuction in longer-distance dispersal (Pohjamo *et al.*, 2006), hence their small sizes might be an adaptation for this.

2.4.4 Relationships among sexuality, reproductive mode and sporophyte and gemmae frequency.

The results presented here agree with earlier findings (Longton and Schuster, 1983, Longton 1992; Hedderson and Longton, 1995) in that a majority of dioicous species produce sporophytes rarely while the majority of monoicous species produce sporophytes freely, albeit monoicous species are very poorly represented in the data set. This seems to support the idea that proximity of the male and female gametangia is a determining factor for successful, frequent sporophyte production. Dioicous species that are capable of reproducing through both spores and gemmae mostly produce sporophytes rarely but they produce gemmae more freely while their monoicous counterparts produce sporophytes freely. The same reasons stated above of low sex expression, segregation of sexes, and sporophyte abortions can explain rarity of sporophyte among dioicous species. Although the current study showed no correlation between gemmae frequency and reproductive system, from a selectionist perspective, a dioicous species that don't reproduce sexually and that can't make gemmae (or some other form of asexual propagule) have a high probability of extinction. Over time therefore

we should see a correlation between being dioicous and having gemmae, at least for those that only produce spores rarely,

For successful fertilisation to occur, dioicous species need, all else being equal, to produce far more spermatozoids than monoicous species because the chances of more spermatozoids dying before they fertilise increases with distance between the male and the female gametangia, which is likely to be higher when they are on separate individual plants than when they are on the same plant. In such a case it would be expected for sexual reproduction to increase from heteroicous to synoicous among the monoicous species. This could not be tested in this research since the small number of monoicous species available precluded any statistical tests. Of the three monoicous species studied that reproduce both sexually and asexually (Table 2.9), the two that produce sporophytes freely are paroicous and one that produces sporophytes rarely is heteroicous. This suggests the same trend but has no statistical value.

None of the monoicous species studied reproduce solely by gemmae. The evolutionary trend of bryophyte life histories in shot-lived habitats is thought to be following a trend towards monoicy, no production of asexual propagules and high early sexual reproduction (Longton and Schuster, 1983; Longton 1988). Gemmae production may thus be seen as an alternative way to ensure continuity of the species only when sexual reproduction fails. However, failure of sexual reproduction is much more likely in dioicous than in monoicous species, as long as there are no self incompatibility problems. Most monoicious liverwort species are said to be autoicous or synoicous (Longton and Schuster, 1983) an arrangement which is thought to promote sporophyte production (Longton, 2006). This arrangement of gametangia in close proximity together with the facts that monoicous species express sex (producing gametangia) frequently ensures high chances of successful fertilisation resulting in frequent sporophyte production. This is further upheld by the fact that none of the studied monoicous species are recorded as either sterile or their sporophytes production recorded as unknown

Both dioicous and monoicous species that reproduce through both spores and gemmae produce gemmae freely. If sexual reproduction was more expensive than asexual reproduction, those monoicous species that are capable of reproducing both ways would be expected to produce gemmae freely and sporophytes rarely. Instead, they also produce sporophytes freely. However, such trade-off could be in numbers of each type of diaspore produced and not in the frequency at which they are produced.

The studies here show existence of suites of life history traits in the family Lophoziaceae which conform to a greater degree to the previous described life history groups (During 1979, 1992; Hedderson and Longton, 1995,1996). These coherent groups are more associated with ecological conditions. No obvious relationship to the phylogeny could be displayed. The studies also show that monoicous species are more likely to produce sporophytes than dioicous. However no relationship could be detected between sexual system and gemmae production. With the trend of life history evolution in bryophytes selecting towards sexual reproduction (Longton and Schuster, 1983; Longton, 2006) it can be predicted that in future, due to difficulties of sporophyte production there is likely to be a correlation between gemmae production and dioicy.

CHAPTER 3

SPORE SIZE MEASUREMENTS IN LIVERWORTS: EFFECTS OF DRYING AND REHYDRATION TIME

3.1 Introduction

Because of its taxonomic utility (e.g. Tallis, 1962; Cao and Vitt, 1986), spore size is one of the most commonly documented bryophyte life history traits. Given its central role in reproductive ecology, it has also been considered a key trait in most life history studies. A number of studies on ecology and reproductive life histories of bryophytes have therefore considered spore size and its relationship to capsule size, spore production, dispersal ability and possible dispersal distances (Hedderson and Longton, 1995; Sundberg and Rydin, 1998; Longton and Miles, 1982; Miles and Longton, 1992; Bateman, 1947; McClymont, 1954). Spore size has also been studied in relation to germination and to post germination establishment, survival and development of the plant (Hedderson and Longton, 1995; During, 1997; Sundberg, 2000).

The preceding chapter has considered spore size in relation to a number of other traits, and later chapters involve measurement of spore sizes. It was evident (see chapter 2), just as Mogensen (1981) noted, that the literature available on spore morphology and size presents many contradictions. Some of the ranges of spore diameters for the same species from different literature sources do not even overlap. For example, ranges for *Lophozia longiflora* (Nees) Schiffn. in Damsholt (2002), and Paton (1999) are 8 - 10 μm while Schuster (1969) and Frye and Clark (1937) indicate 14 - 16 μm. Estimates of spore size for many bryophyte species are easily available in the literature, but if such variance exists the question becomes whether such data can be trusted to serve their intended purposes, in this case whether the spore size can be taken as a reliable phylogenetic, taxonomic or identification character or life history parameter.

There are a number of possible explanations as to why such disparities can arise:1) the spore sizes measured really differ within species, being affected mainly by, for example, environmental conditions, 2) errors of measurement result in different sizes being recorded, 3) at least one of the specimens measured was a different (misidentified) species with different spore sizes, and 4) freshly collected spores might have significantly different sizes compared with dried material, and thus the time between collection and spore measurement might have caused the difference. According to Clarke (1979), it is important to examine spores in a comparable state. Mature spores in a moist environment where their cytoplasm is fully turgid take on a different shape, and most likely size as well, from spores that have dried out and shrivelled, even when the latter are rehydrated (Clarke, 1979)

Sundberg and Rydin (1998) point out that the accuracy and precision of spore size measurement and spore counts depend critically on techniques and sampling procedures employed. As a general rule, literature containing spore sizes usually does not include information on the methods used in obtaining these sizes. However, it is likely that most have been done on dried material collected for different purposes and using different techniques (e.g. time of rehydration). Whilst ideally the measurements would have been done on freshly collected, turgid spores, some have been taken on spores from capsules in herbaria whose period of storage and maturity state at the time of collection would not have been considered. Such differences might also contribute towards the disparities seen in data from the literature.

The aim of this study is to investigate the effects of drying and subsequent rehydration times on measurements of spore size. Key questions are (i) Is there any significant difference in size (as measured on immediate rehydration) between freshly collected and dried spores? (ii) What is the time course of any such effects? iii) Do measured sizes increase with rehydration time? (iv) If so, how long a rehydration period is needed to regain the original fresh size when rehydrated from the dry state?

3.2 Materials and Methods

3.2.1 The study Species

The nature of this study required a species that could be readily collected in some abundance and measured without delay. *Fossombronia leucoxantha* (Lehm.) Lehm. & Lindenb was therefore used instead of a member of the Lophoziaceae because it is common and fruits prolifically on the University of Cape Town Campus where the study was undertaken.

3.2.2 Spore collection and Measurement of spore sizes

Fresh capsules were collected from the field and each placed in a separate micro-tube. The tubes were opened to let the capsules dehisce and then stored until measurement. The spores were measured at the following time intervals after collection; immediately after dehiscing, 6 hours, 12 hours, 24 hours, 48 hours, 96 hours and 1 week (168 hours). Seven randomly selected tubes (thus 7 capsules) were used at each time interval and 20 randomly selected spores were measured from each tube. One capsule was measured at a time with measurements taken immediately after addition of water.

Just prior to measurement water was added to the tube along with a drop of detergent to reduce surface tension. The suspension was shaken to homogenise the spore distribution before drawing a portion of the suspension with a pipette and placing it in a Neubauer improved Counting chamber. A normal thin cover-slip was used instead of the thick haemocytometer cover-slip. Measurements were made with a calibrated eyepiece graticule at 1000X.

To evaluate the effects of rehydration time, spores that had been dried and kept were measured at the following intervals after water was added, in addition to the immediate measurements: 10 minutes, 30 minutes, 1 hour, 6 hours, 12 hours, 24 hours, 48 hours and 96 hours.

3.2.3 Statistical Analysis

All the analyses presented here were performed using procedures in STATISTICA version 7 (Statsoft, 2003). One Way Analysis of Variance (ANOVA) was used to test for significant difference in mean spore diameters between time intervals. Post hoc analysis, employing multiple range tests, was used to determine the time periods at which the spore sizes would be significantly different from the other. Regression analysis was used to determine the rate of change in spore size with time.

3.3 Results

3.3.1 Effects of drying on sizes spores.

The diameter of spores as measured on initial suspension in water, decreased significantly with drying time (regression coefficient (b) = -0.37, $p < 0.0001$) (Table 3.1 and Figure 3.1). Significant differences are apparent between the different drying intervals (ANOVA, $F_{(1, 6)} = 43$, $p < 0.00$. However, most of the change in observed size occurred within the first 24 hours (Tab 3.1, Figure 3.1), and the post hoc analysis shows that the spore sizes after one hour are significantly larger than those from all the other drying times. Further change is much slower and diameters for the 6 and 12 hour intervals are only significantly larger than those after 96 hours and one week (168 hours).Put in another way, of the total 8% decrease in measured diameter between initial and final measurements, 50% of this loss (i.e. a 4% decrease) occurs in the first six hours.

Table 3.1. Variation in spore size measurements with storage time for *Fossombronia leucoxantha*. Values are based on measurements of 20 spores for each of seven capsules at each time interval. SD is the sample standard deviation

Time	Spore size (diameter) μm			
Hrs/Wks	Minimum	Maximum	Mean	SD
Immediately	45.5	58.5	50.43	1.04
6	42.9	54.6	48.46	1.31
12	42.9	53.3	48.59	0.76
24	40.3	53.3	48.05	0.95
48	39	52	47.72	0.70
96	39	52.6	46.62	1.07
1 Week	40.3	52	46.36	0.88

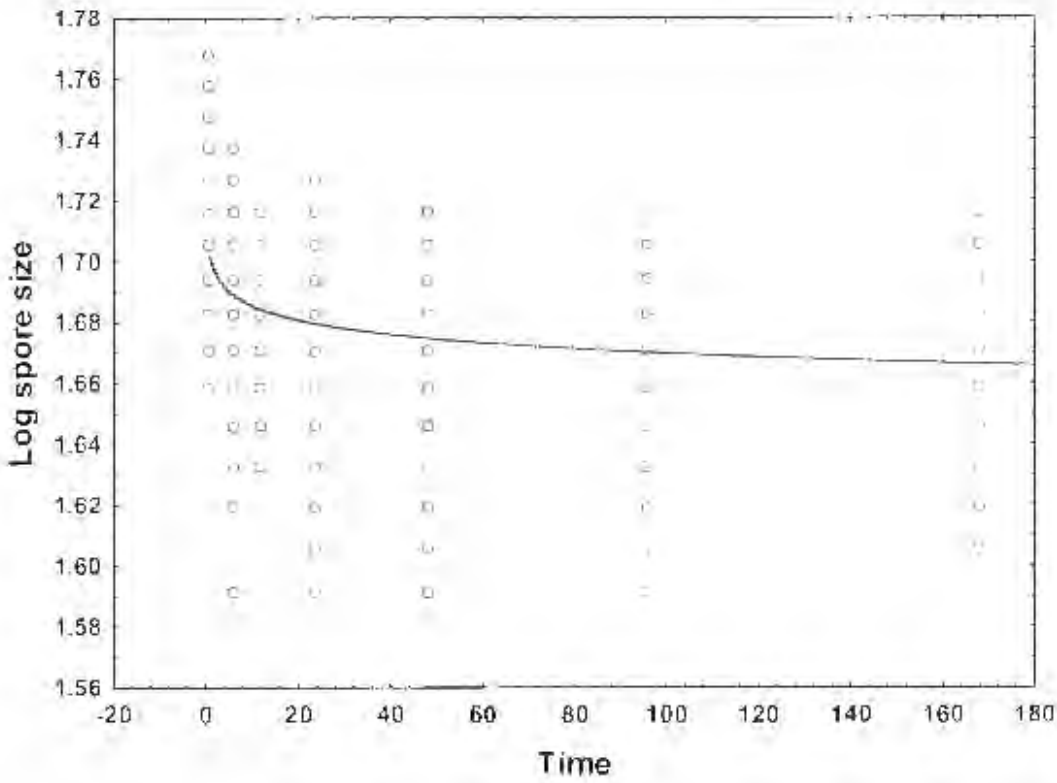

Figure 3.1. Variation in spore size with drying time measured after different time periods after collection.

3.3.2 Effects of soaking on spore size.

Mean spore size of dried spores increased with rehydration time with a significant regression coefficient of r = 0.33, (p <0.0001). As with drying time, most of the change occurs in the first 6 hours (Figure 3.2). Figure 3.2 summarises the pattern of increase in spore size over the duration of measurement in comparison to the mean fresh spore size. The increase in size of rehydrated spores did not manage to get them back to the mean size of freshly collected spores (Figure 3.2)

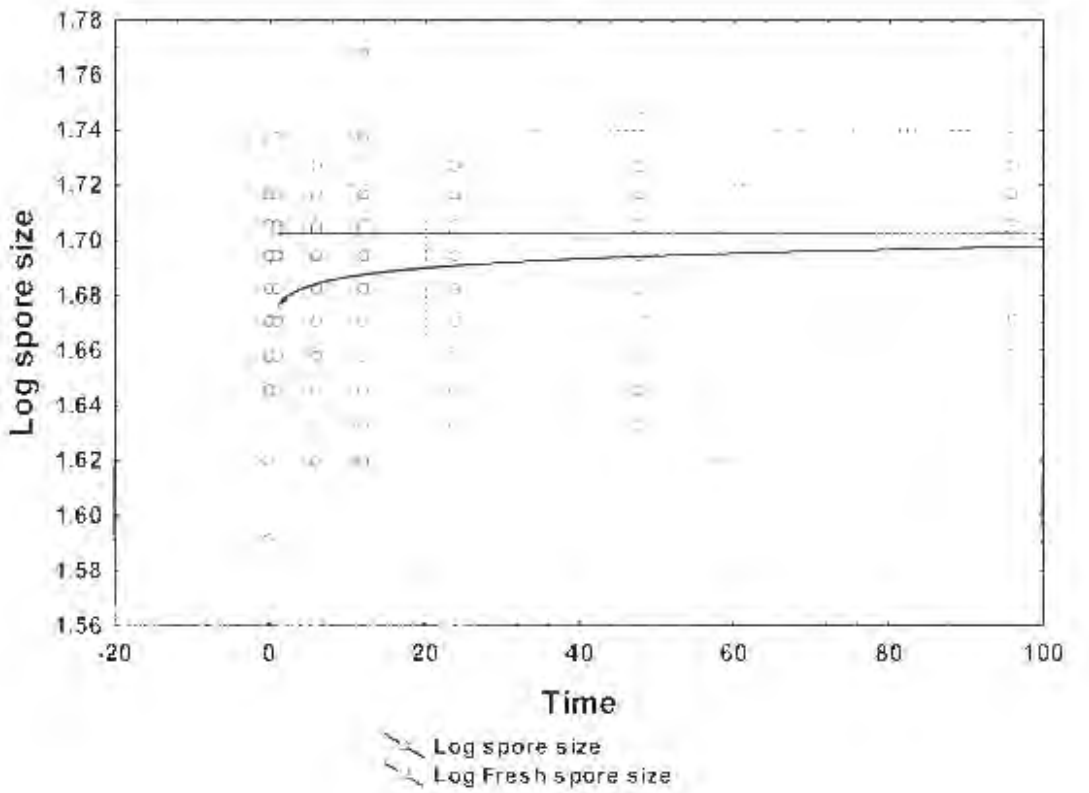

Figure 3.2. The spore sizes measured after different time periods of being immersed in water.

3.4 Discussion

Two main conclusions follow from the results presented here: 1) Increased drying time significantly decreases the apparent size of spores measured on immediate rehydration. 2) Rehydration times of at least 48 hours are necessary before dried spores begin to approach fresh spore measurements.

These findings underscore the importance of ensuring that spores be measured under comparable conditions (Clarke 1979). In many contexts, for example in the study of population differentiation, one is seeking to show quite small differences in average spore diameters. In the present case an 8% difference in mean spore diameter translates to 22% difference in volume for spherical spores. Considering that a 4% change in diameter which occurred within the first 6 hours translates to 11.5% change in volume, it shows that a delay of that long will be of great significance to the final results. However, the actual time it will take for the difference in size to be significant will depend on the conditions under which the spores are drying. Under very humid conditions, where they are expected to dry slowly, it would be expected to take longer than under dry conditions where they would dry at a faster rate.

The results here also suggest that when spores dry, they decrease size only to a certain minimum size. It would therefore be encouraged to measure spores either immediately after collection or wait until they have dried and avoid the period when they are still changing their size as they dry. Wherever possible, estimates of both would more informative.

A number of substances can be used as medium for spore suspension when the spore sizes are measured (Mogensen, 1981). However, water is commonly used as well. The results here suggest that dry spores may significantly increase in size when they rehydrate., However there is no evidence that the change in size will get the spores back to their fresh sizes. After 96 hours in water the change in spore size had still not reached the fresh (immediate) spore size. I however think that after a longer period the spores

would increase further as a result of swelling prior to germination. Such results do suggest that when water is used as a medium for measuring spores, the spores can be kept in water for up to 96 hours without achieving the fresh sizes. When spores are soaked in water, they are expected to undergo symmetrical swelling which is a process of remoistening then asymmetrical swelling which is more of a germination stage (Mogensen, 1978; Olesen and Mogensen, 1978). The stage of interest in this research is the symmetrical swelling stage and it seems not easy to tell the exact stage at which symmetrical swelling stops and asymmetrical swelling begins. The results here suggest that if one has only dry spores at their disposal for obtaining size measurements, it might not be possible to rehydrate them and expect them to get back to their fresh size. Although the spores will increase in size when hydrated, they will not be able to regain their fresh state sizes through symmetrical swelling. In such a case it then would be advisable to report the dry spore size.

Although the results will have limited support since only one species was used in the tests, they still contribute to the need for reporting the state in which spores were measured. The important outcome here is that fresh bryophyte spore sizes are significantly different from their dry sizes and that change can occur within such a short period as 6 hours. Also important is the fact that dry bryophyte spores increase size at a significant rate when they are hydrated.

CHAPTER 4

SEXUAL AND ASEXUAL REPRODUCTIVE LIFE HISTORY STRATEGIES IN LOPHOZIACEAE

4.1 Introduction

The study of life history strategies in combination with habitat structure is important for understanding how species survive locally and disperse to new localities (Söderström & Herben, 1997). A life history strategy consists of several inter-related components including reproductive effort, fecundity, reproductive life span, age at first reproduction, and juvenile and adult mortality schedules (Wilbur *et al.* 1974). In bryophytes, the study of life strategies normally centres on reproduction, which, in this group, is closely allied to dispersal. It has been suggested that asexual reproduction often plays the more important role in bryophyte life histories compared with sexual reproduction (During, 1979).

Sore size is expected to be strongly correlated with dispersal ability, with smaller spores expected to disperse over longer distances (Miles and Longton, 1992; Kürschner 2004), thereby increasing chances of colonizing new habitat patches. Crum (1972) suggested that 25μm was a critical threshold, and Hedderson (1992) has provided some support for this in showing a marked influence of diaspore size on local range size. However, during dispersal smaller spores may stay longer in the harsh environments of the upper atmospheric layers which might eventually reduce their ability to germinate and successfully establish upon being deposited on new habitats. Nontheless, all else being equal, smaller spore size is expected to be associated with greater dispersal capacity. Spore size is said to be usually relatively constant within species (Vitt, 1968 in During, 1979.) although in a few, spore size is known to vary under the influence of environment (Sundberg, 2002) Thus depending on spore sizes, different species will have

different abilities to move across habitats and in the few whose spore sizes depend on the environment their mobility across habitats might vary according to the environment.

Bryophyte diaspore number, just like seed number in higher plants, can also be viewed as a measure of dispersal capacity. Species producing fewer seeds (in the case of bryophytes fewer spores) are thought, all else being equal, to be less mobile across the landscape compared to species with greater output (Rees, 1995; Jakobsson and Eriksson, 2000; Aarssen and Jordan, 2001; Murray *et. al.,* 2002). Limited seed dispersal has often been implicated in the limited colonisation ability of some species, particularly in fragmented landscapes and in secondary woods (Dzwonko and Loster, 1992; Matlack, 1994; Mabry, 2004). In bryophytes, spores are often the main dispersive agent and spore productivity is therefore expected to be a key component of dispersal ability. Spore number per sporophyte varies enormously, depending on the species (Miles and Longton, 1992; Sundberg, 2002), ranging for example from one to thirty two in *Archidium* spp. to about sixty million in *Dawsonia* spp. Within a species the number is thought to be variable but within the same order of magnitude (During, 1979). Thus for example, Miles and Longton, (1992) estimated that *Atrichum undulatum* produced 309 000 $\pm$104 000 spores per capsule while *Bryum argenteum* produced 155 000 $\pm$44 000 spores per capsules.

On the other hand, increased propagule size is expected to be associated with higher survival and juvenile growth rates in bryophytes and many other organisms (Hedderson & Longton, 1996; During 1997, Sundberg, 2000)

Given that resources available to any organism are limited, spore number and spore size probably cannot both be increased simultaneously; one would expect them to be traded off against each other (Roff, 1992; Stearns, 1992). Thus bryophytes, like virtually all other organisms studied, are likely to exhibit size-number compromises (Cody, 1966; Mabry, 2004). Several studies have shown that diaspore size and number are negatively correlated in bryophytes, both within and among species (Hedderson & Longton, 1995, 1996; Sundberg

& Rydin, 1998). Environmental conditions might also be expected to affect such trade-offs within species i.e. in some circumstances there might be stronger selection for increased dispersal ability which should lead to smaller, more numerous spores. The consequence of this may be lower survival ability and lower juvenile growth rates.. The study of life history strategies of bryophytes, therefore, needs to correlate reproduction with fluctuations in environment and their predictability in time and space. This will help explain their abundance and distribution in time and space.

As noted by a number of authors (During 1979, 1992; Hedderson & Longton, 1995, 1996; Sundberg & Rydin, 1998) the study of broad patterns of life history variation in bryophytes is hampered by the lack of basic data on individual traits such as numbers of diaspores produced. In addition, further work depends on knowledge of the extent and spatial structuring of variation in such traits within and among species. Absence of such basic information is perhaps particularly acute in liverworts. Thus one of the primary aims of this Chapter is to provide such information for some of the species in the family Lophoziaceae as well as to estimate the inter- and intra-specific variation in diaspore size and the number of diaspores produced per capsule/shoot by the studied species. At the same time, relationship between number of diaspores per capsule/shoot and the size of the diaspores is investigated and the possible existence of tradeoffs evaluated. I also attempt to interpret the trade offs in terms of likely important selective features of the environment.

The questions asked are: 1) How many spores/gemmae are produced per capsule/shoot by each of the studied species and what are their sizes? 2) Do the sizes and numbers of spores/gemmae produced per capsule/shoot vary significantly among the species and among the different geographical localities for each species? 3) Is there any correlation that suggests a trade-off between the size of spore/gemmae and the number of spores/gemmae produced per capsule/shoot?

4.2 Materials and Methods

4.2.1 Field Collection

Species were studied at previously known localities in Finland, Norway, Sweden and Spain between June and September 2003 and between July and September 2004. Tables 4.1 and 4.2 show the localities from which the different samples studied were collected. A maximum of 10 capsules per colony was collected whenever the availability permitted. Ripe but undehisced capsules were removed from gametophytes with forceps and placed in individual micro-tubes. The tubes were closed immediately. The tubes were later opened in a room with stable air conditions, where spores would not be blown away, to allow the capsules to dehisce as well as to dry the spores. After this the tube was closed to prevent loss of spores.

To estimate the average number of gemmae produced per shoot, newly matured gemmiparous shoots were picked randomly and placed each in individual micro-tubes. Same procedure as for spores was used to dry the gemmae in the microtubes.

4.2.2. Estimation of diaspore production and size measurements

To measure spore sizes and estimate spore numbers each tube was weighed before pouring in half-strength Knop's solution (Nehira, 1988) to suspend the spores. Knop's solution was used instead of water because the remainder of the spores had to be incubated later for germination tests (See Chapter 5). The tube was reweighed to determine the volume of Knop's solution in which the spores were suspended. The contents were shaken to ensure equal distribution of spores in the solution before sub-samples were withdrawn from the middle of the tube using a micropipette. The sub-samples were placed on a Neubauer improved counting chamber and spores counted on the grid under a light microscope. The number of spores in each capsule was estimated by counting four sub-samples.

To measure gemma sizes and estimate gemma numbers each tube was weighed before the shoots were placed individually in a drop of water and the gemmae removed with a thin insect pin under a dissecting microscope and rinsed in a drop of water. The solution was poured back into the tube and a drop of mild detergent was added to reduce surface tension. The tube was reweighed to determine the volume of water in which all the gemmae from the shoot were suspended. The suspension was shaken before four counts per solution were made with a Neubauer improved counting chamber and the mean number of gemmae per shoot was calculated.

Measurement of spore and gemma sizes was done after each spore/gemma count. The heavy counting chamber cover slip was replaced by an ordinary, thinner, cover-slip. Diameter measurements of 20 randomly selected spores were made under a light microscope at 1000X magnification using an eyepiece graticule. For gemmae, width and length, triangular base or diameter were measured, depending on the gemma's shape. Size was calculated as the estimated surface area of each gemma taking into consideration its shape. The spores that remained in the tubes were incubated for germination tests (See chapter 5).

Table 4.1. Localities from which the capsules for study were collected.

Species	Locality			Habitat Classification	No. of colonies	No. of Capsule
	Country	Name	GPS position			
B. barbata	Finland	Kotinen	61°14'39"N 25°04'07"E	Boreal forest	1	3
B. quadriloba	Sweden	Låktatjåkka	61°03'19"N 25°02'44"E	Boreal forest	1	6
L. bantriensis	Norway	Unndalen	62°25'34"N 09°53'00"E	Boreal forest	1	6
L. collaris	Sweden	Låktatjåkka	61°03'19"N 25°02'44"E	Boreal forest	1	1
L. heterocolpos	Norway	Estenstadmarka	63°23'23"N 10°29'11"E	Boreal forest	1	3
L. bicranata	Finland	Kotinen	61°14'39"N 25°03'43"E	Boreal forest	1	10
L. ciliata	Norway	Urvatnet	63°07'47"N 09°46'47"E	Boreal forest	3	24
	Sweden	Låitavare	64°28'63"N 15°48'08"E	Boreal forest	1	3
		Rödberget	69°16'28"N 17°03'07"E	Boreal forest	1	3
		Vändåtberget	63°48'51"N 18°18'18"E	Boreal forest	7	51
L. excisa	Norway	Unndalen	62°25'34"N 9°53'00"E	Boreal forest	1	4
L. grandirietis	Sweden	Låktatjåkka	61°03'19"N 25°02'44"E	Boreal forest	1	4
L. longiflora	Sweden	Låitavare	64°28'63"N 15°48'08"E	Boreal forest	10	60
		Rödberget	69°16'28"N 17°03'07"E	Boreal forest	1	8
		Vändåtberget	63°48'51"N 18°18'18"E	Boreal forest	8	29
L sudetica	Norway	Unndalen	62°25'34"N 9°53'00"E	Boreal forest	1	7
L ventricosa	Norway	Lidarende	63°20'45"N 10°32'01"E	Boreal forest	1	5
		Unndalen	62°25'34"N 09°53'00"E	Boreal forest	1	4
	Sweden	Vändåtberget	63°48'51"N 18°18'18"E	Boreal forest	7	29
		Låitavare	64°28'63"N 15°48'08"E	Boreal forest	4	14

Table 4.2. Localities from which the gemmae for study were collected

Species	Locality			Ecological classification	No. of colonies	No. of shoots
	Country	Name	GPS position			
B. hatcheri	Spain	Lérida	42°38'57"N 00°58'54"E	Alpine Forest	6	18
		Lézidan	42°37'21"N 01°23'04"E	Alpine Forest	4	12
L. longidens	Norway	Gråkallen	63°25'32"E 10°11'44"E	Boreal forest	8	24
		Lidarende	63°20'45"N 10°32'01"E	Boreal forest	3	9
		Urvatnet	63°07'47"N 09°46'47"E	Boreal forest	4	12
L. ventricosa	Norway	Gråkallen	63°25'32"E 10°11'44"E	Boreal forest	6	18
		Urvatnet	63°07'47"N 09°46'47"E	Boreal forest	5	15
	Spain	Huesta	42°36'44"N 00°37'46"E	Alpine Forest	4	12

4.2.3 Statistical Analysis

All analyses were performed in STATISTICA version 7 (StatSoft, 2003). One Way Analysis of Variance (ANOVA) was performed to test for significant differences in the means of the number of spores per capsule, size of spores, number of gemmae per shoot and size of gemmae among the species and among the localities for each species. A post-hoc HSD test for unequal sample sizes (Statsoft, 2003) was used to identify significantly different species pairs. Detailed analyses of spatial variation were performed on species for which adequate collections could be made from more than one locality. Three species (*Lophozia ciliata*, *Lophozia longiflora* and *Lophozia ventricosa*) were analysed in this way for spore characters and three species (*Barbilophozia barbata*, *Lophozia longidens* and *Lophozia ventricosa*) were analysed for gemma variation.

Nested ANOVA (Sokal and Rohlf, 1981) was used to analyse the spatial hierarchy of variation in spores per capsule, gemmae per shoot, spore sizes and gemma sizes. The hierarchy used for spore characters was species, localities within species, colonies within localities, capsules within colonies, and spores within capsule. For gemmae characters, the hierarchy was species, localities in species, colonies within localities, shoots within colonies, and gemmae within shoots. The contribution of each level to the total variation was also calculated. Since preliminary examination revealed positive skewness, all four variables were log-transformed prior to analysis.

Correlation tests and regression analyses were used to evaluate relationships between spore size and number of spores produced per capsule as well as between gemma size and number of gemmae produced per shoot. The correlations were evaluated at the among-species levels as well as among localities and among individuals within species.

4.3 Results

4.3.1 Spore size and number

4.3.1.1 Variation among species

Table 4.3 summarises the spore numbers and sizes for the different species studied. Mean spore sizes range from ca. 12.2µm in *Lophozia grandiretis* to 17.2µm in *Leiocolea bantriensis*. The mean number of spores produced per capsule ranges from ca. 20, 600 in *B. quadriloba* to ca. 203, 700 in *Leiocolea heterocolpos*. The patterns of variation in spore size and number of spores per capsule are represented in figures 4.1 and 4.2, respectively.

The ANOVA results show significant differences in both spore size ($F_{(1,10)}$ = 39.6, $p < 0.001$) and the number of spores produced per capsule ($F_{(1, 10)}$ = 11.65, $p < 0.001$) among species, and species membership accounts for 76% of the total variation in spore size and 39% of the total variation in the number of spores produced per capsule.

Post hoc analysis shows that *L. bantriensis, L. ventricosa, L. excisa* and *B. barbata* do not differ significantly from each other in mean spore size. *L. heterocolpos, L. ciliata L. longiflora* and *L. grandiretis* also do not differ significantly from each other, but have significantly smaller spores than the previous group of species. *B. quadriloba*, *L. sudetica* and *L. bicrenata* do not differ significantly from each other or from most of the other species, but they have significantly smaller spores than *L. bantriensis*.

The *post hoc* analysis of spores per capsule shows that *Lophozia longiflora, Lophozia ciliata* and *Lophozia ventricosa* differ significantly from each other in the number of spores per capsule. *Lophozia excisa* has significantly fewer spores than *Barbilophozia barbata*, *Lophozia heterocolpos, Lophozia longiflora* and *Lophozia sudetica*.

Table 4.3. Summary of Spore sizes and spore number for each of the studied species. SD is the sample standard deviation, and n is the total number of capsules from which counts and sizes were obtained. Number of spores per capsule is expressed in Thousands.

Species	Spores per capsule				Spore diameter um				n
	Min	Max	Mean	SD	Min	Max	Mean	SD	
B. barbata	95.75	152.17	121.71	28.24	14.6	19.4	16.0	0.4	3
B. quadriloba	20.63	108.28	20.63	30.55	13	14.6	15.1	1.1	6
L. sudetica	45.05	145.84	83.10	32.97	12.3	15.0	14.1	0.9	7
L. bantriensis	39.15	99.27	63.11	28.87	14.3	21.9	17.2	0.9	6
L. collaris	53.04	53.04	53.04	-	11.7	14.3	13.5	-	1
L. heterocolpos	75.44	203.68	115.39	76.58	10.4	15.6	12.7	0.9	3
L. bicrenata	26.95	123.22	65.16	33.36	12.2	19.4	13.8	0.7	10
L. ciliata	13.84	123.40	44.30	33.00	10.4	16.9	13.2	0.9	80
L. excisa	10.81	23.93	15.60	5.98	13	19.5	15.6	0.8	4
L. grandirietis	24.11	111.56	70.77	38.59	10.4	14.3	12.2	0.3	4
L. longiflora	24.89	261.66	89.69	36.48	10.4	14.3	13.0	0.8	97
L. ventricosa	28.70	152.02	62.12	34.69	10.4	19.5	15.7	1.5	56

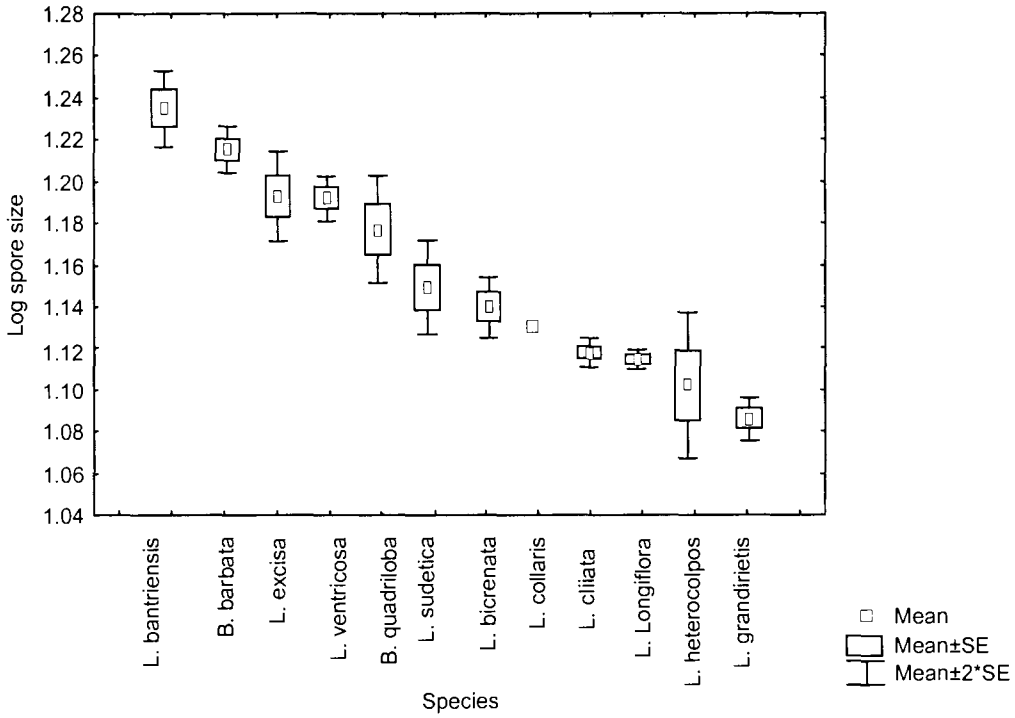

Figure 4.1. Box and whisker plots for spore sizes of the different species arranged by descending values of the mean

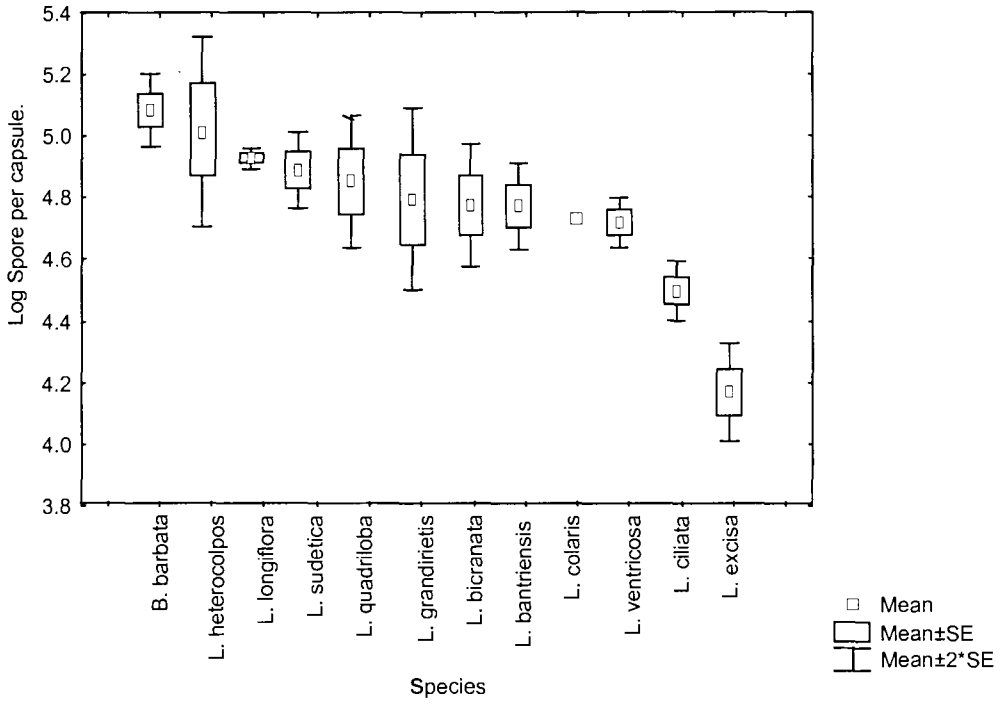

Figure 4.2. Box and Whisker plots for number of spores per capsule in different species.

4.3.1.2 Spatial variation

Appendix 2 summaries spore numbers and sizes for the different localities of each of the three species studied in detail (*L. ciliata*, *L. longiflora* and *L. ventricosa*). For each species, the variation in both the number of spores produced per capsule and the spore sizes were statistically significant at locality, colony and capsule levels (Table 4.5). For spore size, most of the variation occurs among the localities, with this level accounting for 61 to 71% of the total variation. However, the species differ somewhat in the importance of this level in accounting for variation in spore numbers. In *L. ciliata* and *L. ventricosa* locality level differences account for 84% and 73% respectively while in *L. longiflora* only 43% of the total variation occurs at this level. Variation among colonies within localities accounts for 19 to 32% of total variation in spore size. However, species also differ in the amount of variation that colonies contribute to total variation in number of spores produced per capsule. In *L. longiflora* colony level variation contributes 39%, in *L. ventricosa* it accounts for 22% and in *L. ciliata* it explains 14% of total variation. Differences among capsules within colonies contribute between 3% and 8% of the total variation in spore size. Residual variation, (variation among spores within capsules for spore sizes and among capsules within colonies for number of spores per capsules) contributes a relatively tiny proportion of the variation.

As an example, to show the pattern of variation in spore size and number of spores produced per capsule among localities, detailed results are shown for *L. ciliata* (Table 4.4). This species shows great variation (more than an order of magnitude) in spore production between localities with averages from ca. 6 654 spores per capsule for Urvatnet to ca. 67 998 spores per capsule for Rödberget.

The post hoc analysis shows that in *L ciliata,* the mean number of spores per capsule produced by plants from Urvatnet is significantly smaller than that of plants from Vändåtberget nature reserve, Låitavare nature reserve and those from Rödberget. Plants from Rödberget also produce significantly more spores per capsules than plants from Låitavare nature reserve.

Table 4.4. Summary of spore size and number variation among study localities for *L. ciliata*. SD is the sample standard deviation, and n is the total number of capsules from which counts and sizes were obtained. Number of spores per capsule is expressed in Thousands.

Locality	Spore per capsule				Spore sizes				n
	Min.	Max	Mean	SD	Min.	Max.	Mean	SD	
Vändåtberget	7.83	123.40	57.69	30.26	10.4	16.9	13.196	0.6613	51
Låitavare	5.07	26.94	19.23	12.28	10.4	15.6	12.068	2.16	3
Rödberget	37.83	94.42	68.00	28.48	9.1	11.7	10.898	0.19	3
Urvatnet	4.63	34.76	6.65	7.85	11.7	15.6	13.536	0.47	24

Table 4.5. Summary of Nested Analysis of Variance on spore size and number of spores produced per capsule on three species. Numbers indicate the percentage of total variance attributable to each source. The residual term represents variation among spores within capsules for spore size and variation among capsules within collections for spore numbers. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

Species	Spore size				No. of spores per capsule		
	Localities	Colonies	Capsules	Residual	Localities	Colonies	Residual
L. ciliata	70.6***	19.8***	2.9***	0.3	83.8***	14.4***	0.7
L. longiflora	61.3***	29.2***	7.5***	0.9	43.8**	39.3***	6.7
L. ventricosa	64.1***	32.1***	3.1***	0.3	72.5***	22.1***	2.6

In *L. longiflora,* all the 3 studied localities (Vändåtberget nature reserve, Låitavare nature reserve, and Rödberget) differed significantly from each other in the mean number of spores produced per capsule. In *L. ventricosa* the mean number of spore produced per capsule by plants from Vändåtberget Nature reserve differ significantly from those produced by plants from Lidarende and those from Unndalen. There was no significant difference among the other localities.

4.3.2 Gemma size and numbers of gemmae per shoot.

4.3.2.1 Variation among species

Table 4.6 summarises gemma production and gemma sizes for the three species studied (*L. ventricosa*, *L. longidens* and *B. hatcheri*). *Barbilophozia hatcheri* produces the highest number of gemmae per shoot (mean ca. 3 036), but the smallest gemmae (mean size ca.346. μm^2) while *Lophozia longidens* produces the least number of gemmae per shoot (mean ca. 1 481) and has the biggest gemmae (mean size ca. 619 μm^2). *Lophozia ventricosa* is intermediate for both.

The species show significant differences from each other in both gemma size ($F_{(1, 2)} = 2096$, $p < 0.001$) and the number of gemmae produced per shoot ($F_{(1, 2)} = 7.33$, $P < 0.0001$). Nested ANOVA shows that variation among species is significantly greater than that among localities within species, and accounts for 94.4% of the total variation in gemma size and for 59% of total variation in the number of gemmae per shoot.

4.3.2.2 Spatial variation

Appendix 3 summarises gemma production and gemma sizes for the different localities of each species. For each of the species, most of the variation in gemma size occurs among localities, with this spatial scale accounting for 84 to 88% of the total variation (Table 4.7). Variation amongst colonies within localities accounted for 7 to 11% of the total variation. The differences among

shoots within localities accounted for 7.2% of total variation in *B. hatcheri*, while in *L. longidens* and *L. ventricosa* it accounted for 1.1% and 0.9% of the total variation respectively. The patterns of variation in gemma sizes among localities for *L. longidens* and *L. ventricosa* are represented in figures 4.3 and 4.4, respectively. *Post* hoc analysis shows significant differences between each pair of the three localities (Urvatnet, Gråkallen and Lidarende) for *L. longidens*. For *L. ventricosa* there is no significant difference in mean gemma size between the two Norwegian localities (Urvatnet and Gråkallen) but Spanish locality (Huesta) had a significantly smaller mean gemmae size than and each of the Norwegian localities.

There is no significant variation in the mean number of gemmae produced per shoot among localities for *B. hatcheri* and for *L. longidens.* In *L. ventricosa* variation in gemma production is significant only among colonies, with this level contributing about 40% of total variation.

Table 4.6. Summary of species gemma parameters. SD is the sample standard deviation, and n is the total number of shoots from which counts and sizes were obtained.

Species	No. gemmae per shoot				Gemma size (area) μ^2				n
	Mean	Min	Max	SD	Mean	Min	Max	SD	
B. hatcheri	3036.42	481	9542	2135.13	346.4	143	558	57.863	30
L. longidens	1480.69	289	3981	698.904	619	373	1217	115.428	45
L. ventricosa	1847.46	261	5421	1347.84	395.7	203	811	77.196	45

Table 4.7. Summary of Nested Analysis of Variance in gemma size and number of gemmae produced per shoot on three species. Numbers indicate the percentage of total variance attributable to each source. Residual term represents variation among gemmae within Shoots for gemma size and variation among shoots within collection for gemmae produced per shoot. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

Species	Gemma size				No. of gemmae per shoot		
	Localities	Colonies	Shoots	Residual	Localities	Colonies	Residual
B. hatcheri	84.7***	7.0***	7.2***	0.5	6.7	68.3	5.1
L. longidens	88***	10.1***	1.1**	0.4	50.2*	31*	11.8
L. ventricosa	87.9***	10.5***	0.9**	0.3	30.7	40.3**	17.4

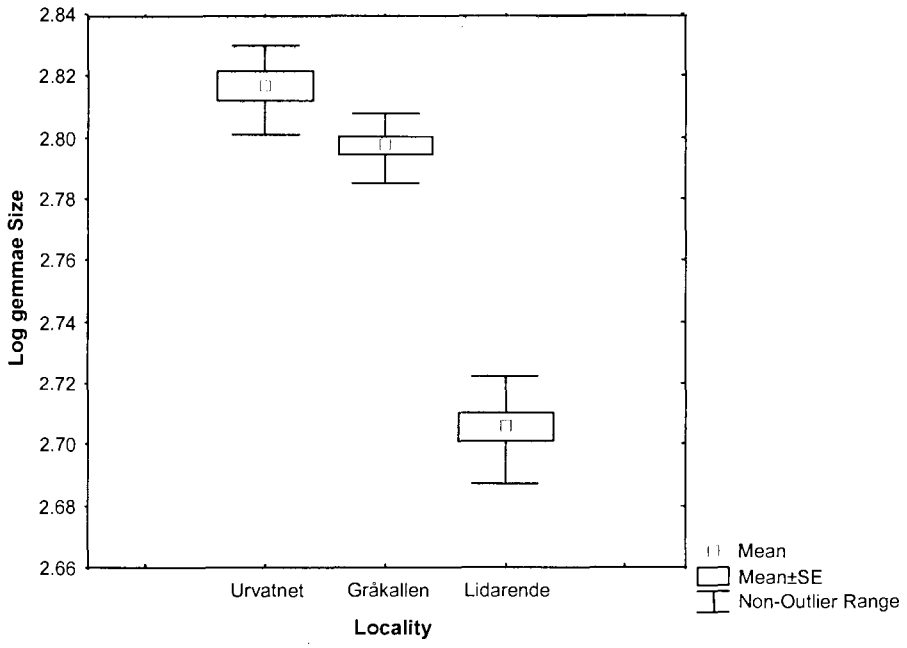

Figure 4.3. Box and whisker plot for gemma size in *L. longidens* for the three localities

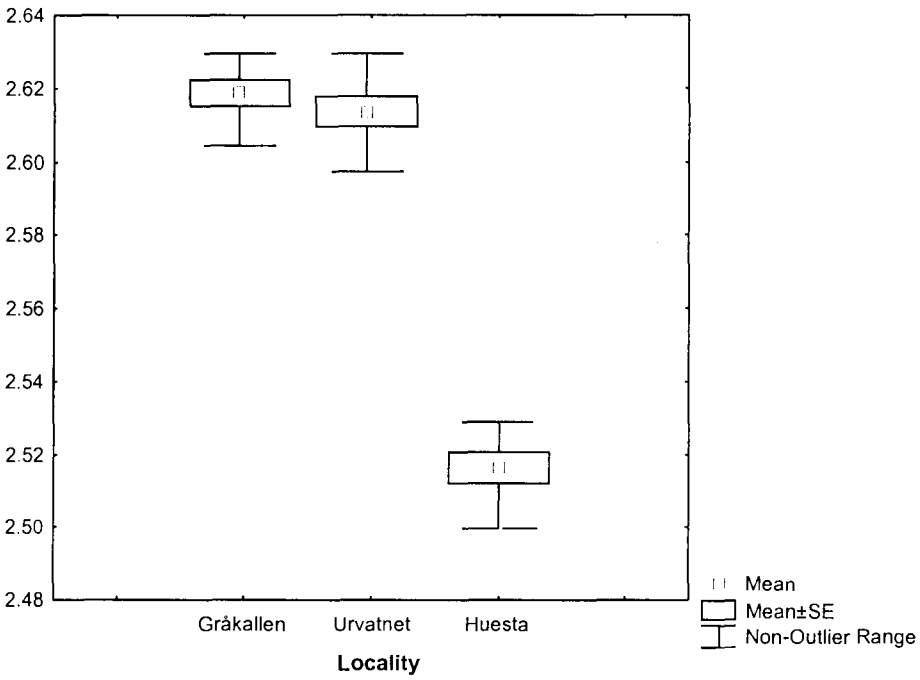

Figure 4.4 Box and whisker plots for mean gemma size in *L. ventricosa* for the three localities

4.3.3 Correlation between spore size and number of spores per capsule

The correlation between mean spore size and mean number of spores per capsule when analysed across species (Figure 4.5), though negative, is not statistically significant ($r = -0.38$, $b = -2.48$, $p = 0.2197$, $n = 12$).

In each of the three species studied (*Lophozia ciliata, Lophozia longiflora* and *Lophozia ventricosa)* the correlation between spore size and spore number is in the expected (negative) direction both when all samples are considered together as well as within localities (Figures 4.6 – 4.8). In most cases the observed correlations are significantly different from zero, although the bivariate plots exhibit considerable scatter. However, for both *Lophozia longiflora* and *Lophozia ventricosa* the correlations are not statistically significant for the Vändåtberget locality

4.3.4 Correlation between gemma size and number of gemmae per shoot

Most of the correlations between gemmae size and the number of gemmae produced per shoot are not significant. The directions of relationships are highly variable; some are positive and others negative for the same species, e.g. for *L. longidens* (Figure 4.10). Some of the ones that approach significance ($p = 0.05$ for *L. ventricosa*, Gråkallen locality or 0.06 for *L. longidens*, Urvatnet locality) are in the positive direction. However, at the two Spanish localities for *Lophozia ventricosa* the two are quite strongly and negatively associated.

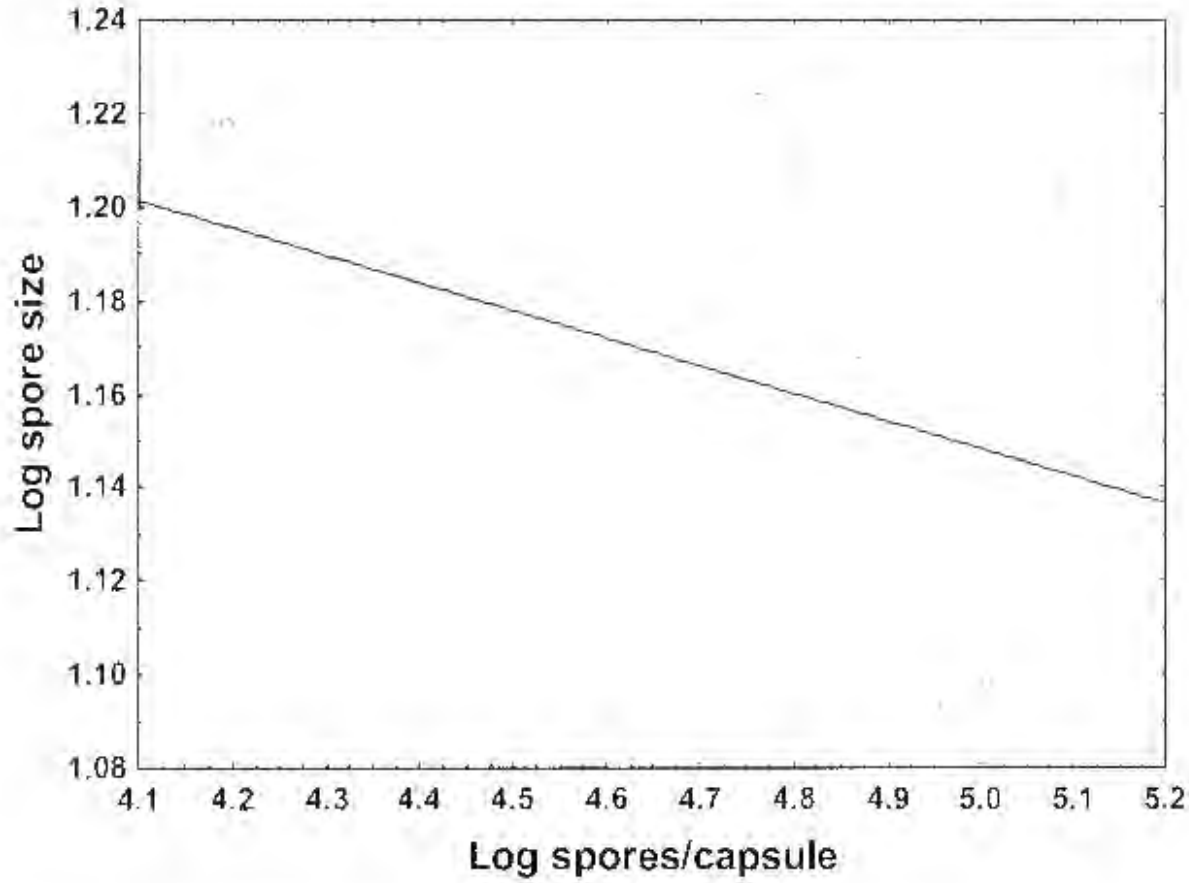

Figure 4.5. Scatter plots of number of spores per capsule versus spore size for the species studied. Correlation coefficient (r) = -0.38, regression coefficient (b) = -2.48, p = 0.220, n = 12.

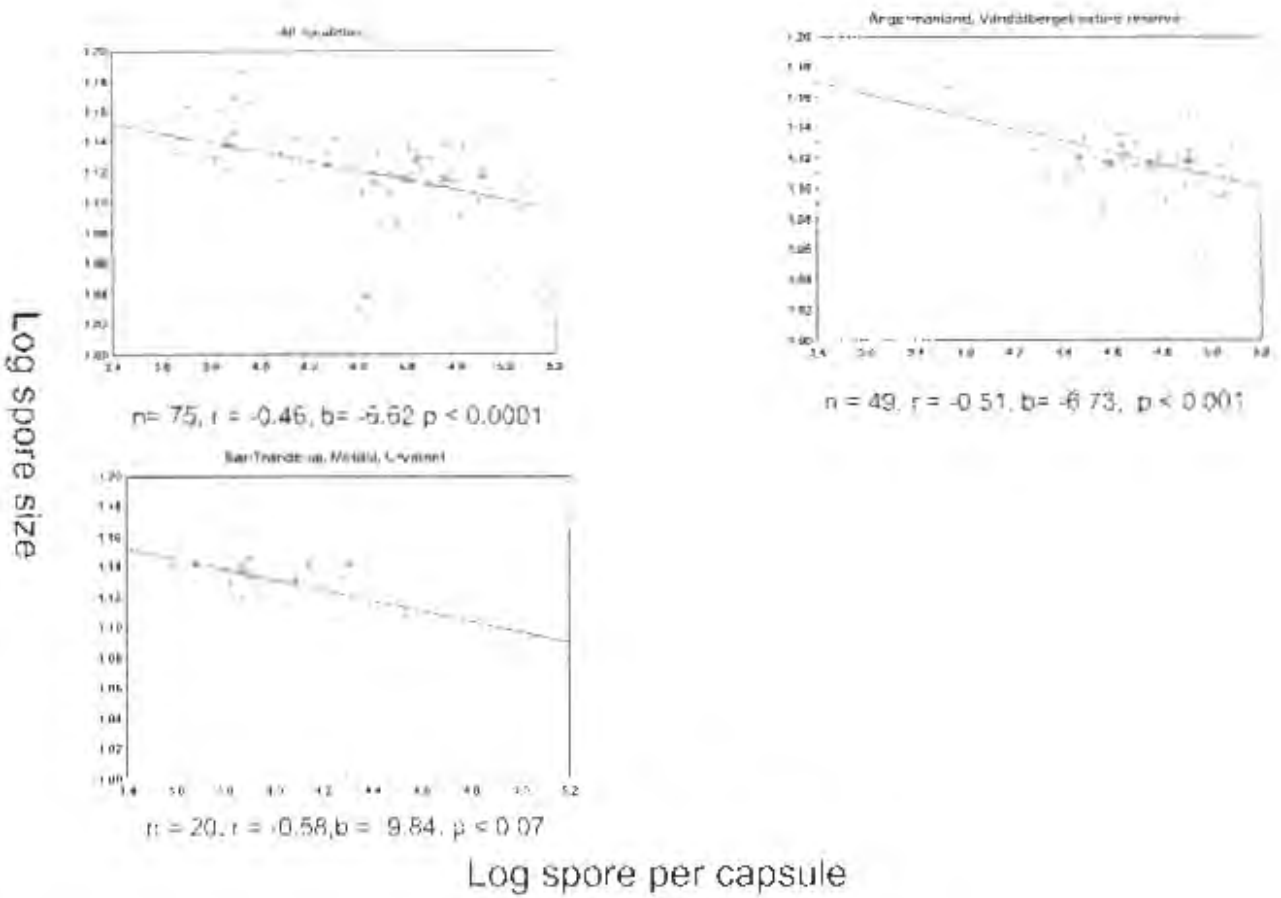

Figure 4.6. Scatter plots of number of spores per capsule versus spore size in *Lophozia ciliata* for all the localities combined, and then for separate localities.

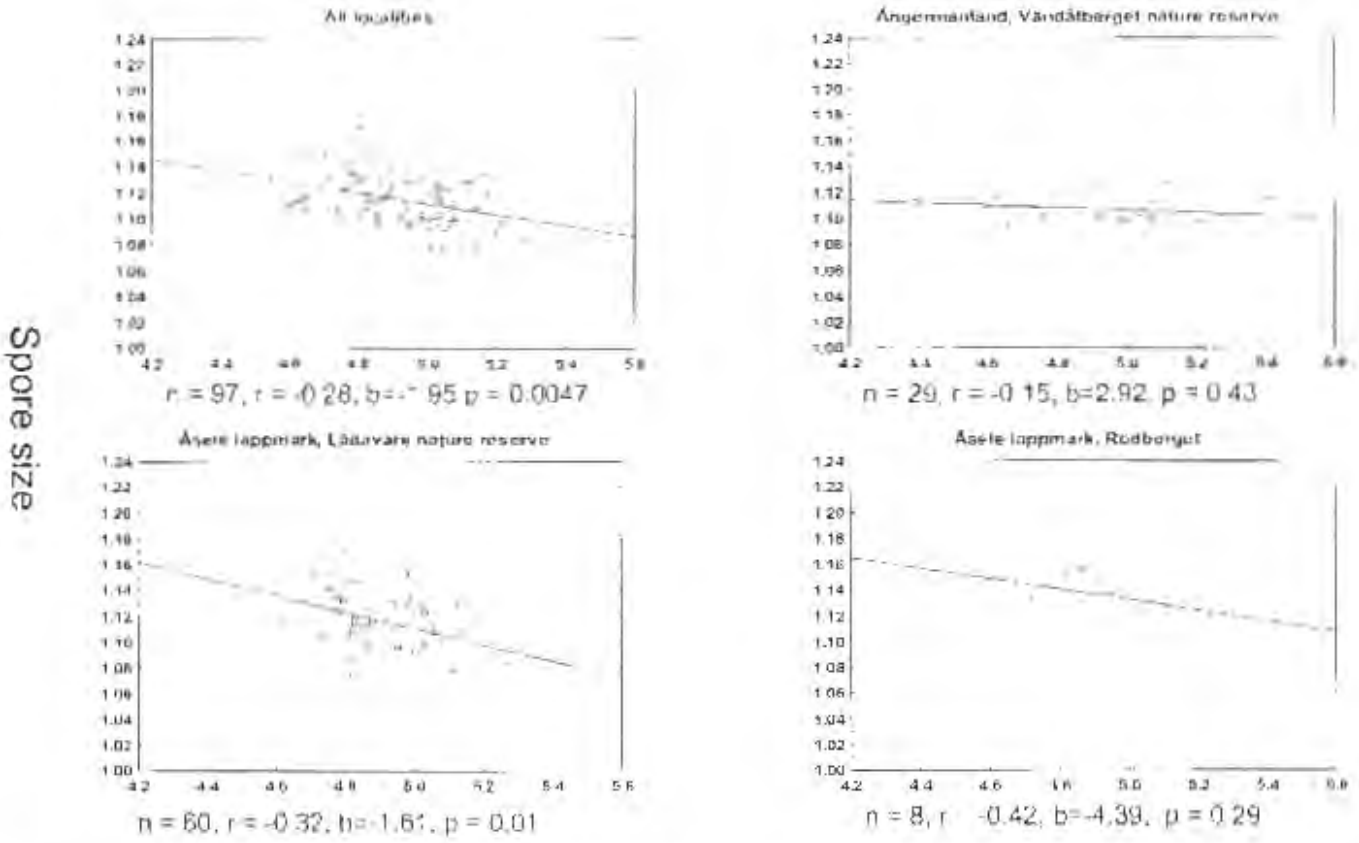

Figure 4.7. Scatter plots of number of spore per capsule versus spore size in *Lophozia longiflora* for all the localities combined, and then for separate localities.

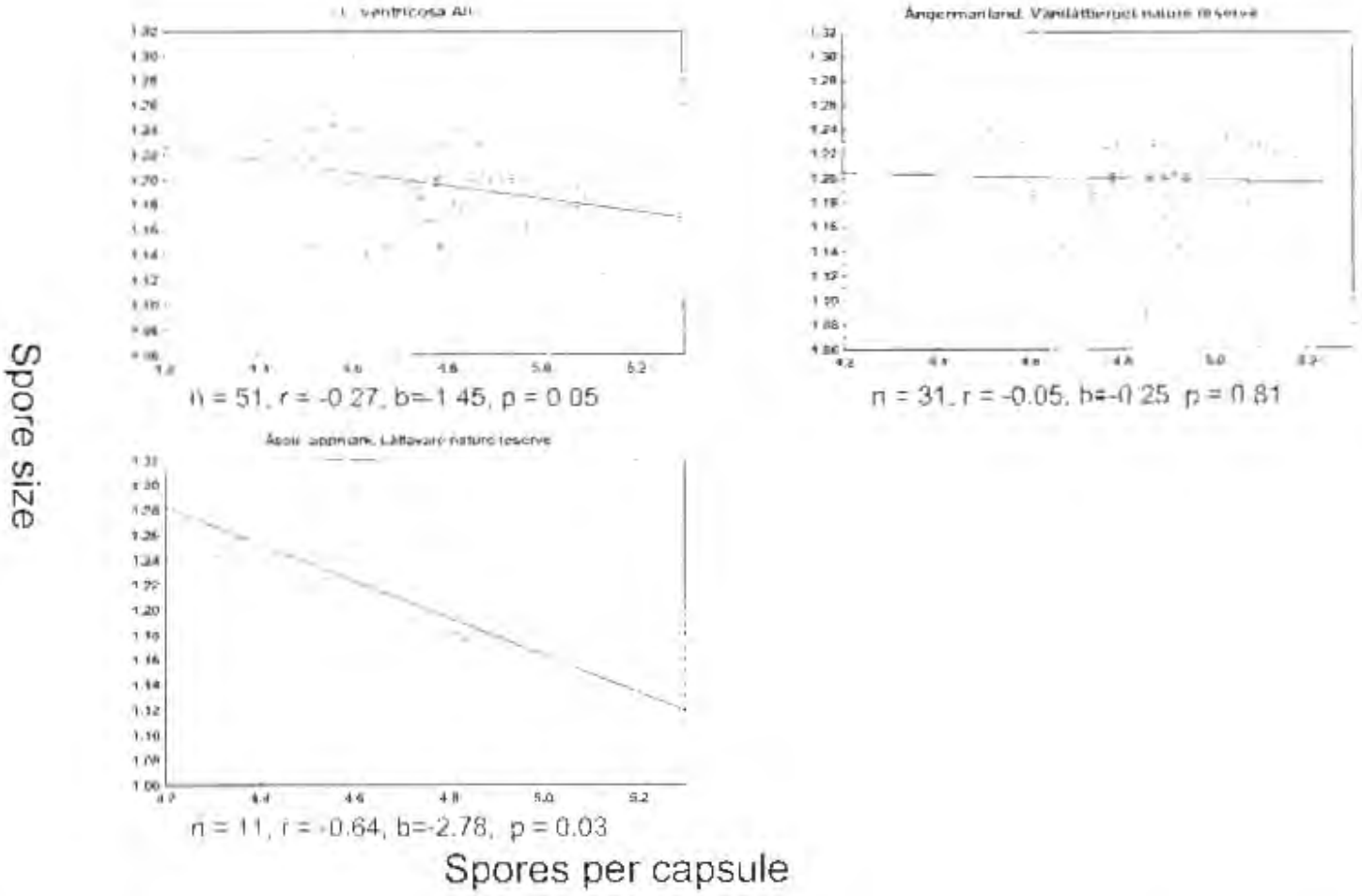

Figure 4.8. Scatter plots of number of spore per capsule versus spore size in *Lophozia ventricosa* for all the localities combined, and then for separate localities.

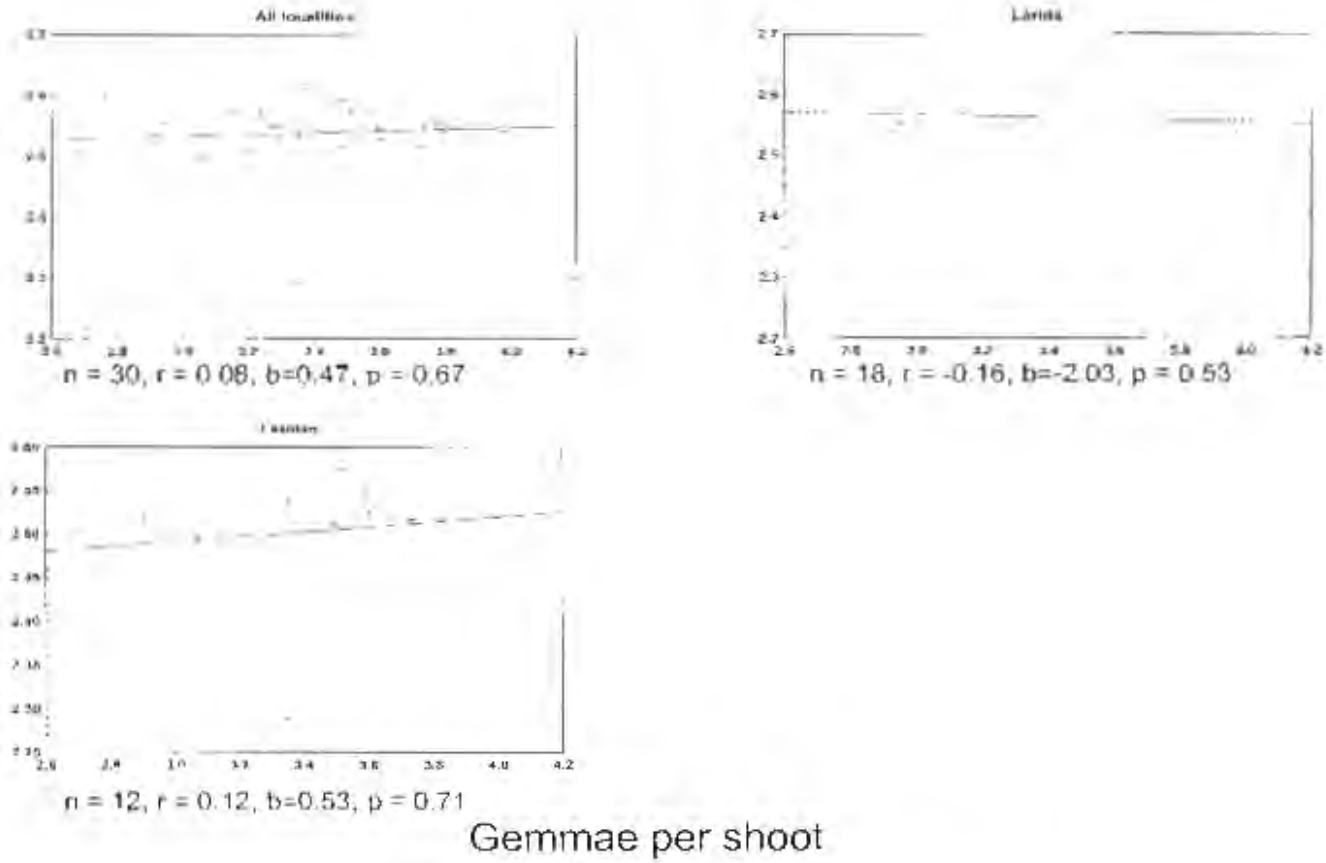

Figure 4.9. Scatter plots of number of gemmae per shoot versus gemma size in *Barbilophozia hatcheri* for all the localities combined, and for separate localities.

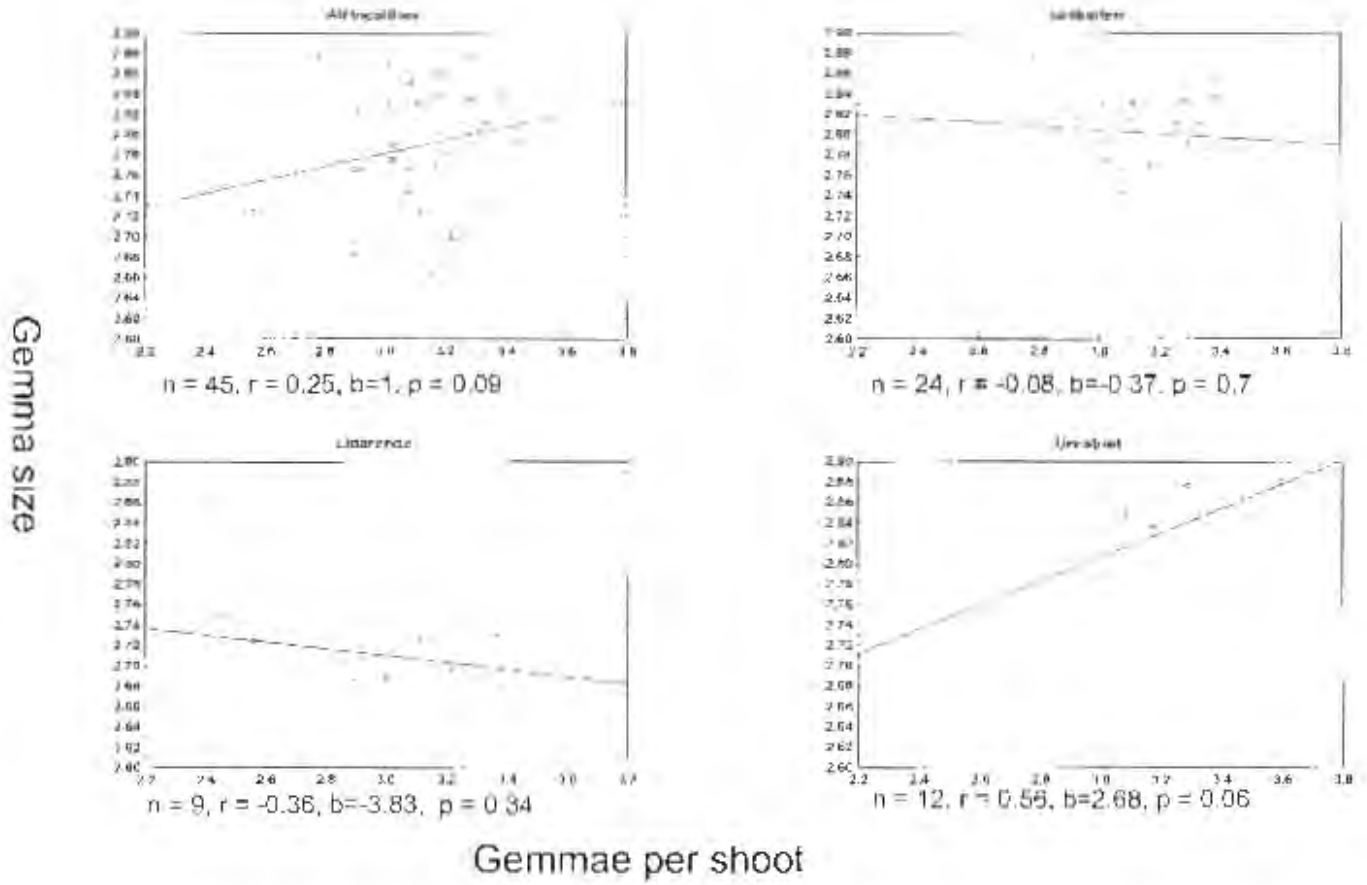

Figure 4.10. Scatter plots of number of gemmae per shoot versus gemma size in *Lophozia longidens* for all the localities combined, and for separate localities.

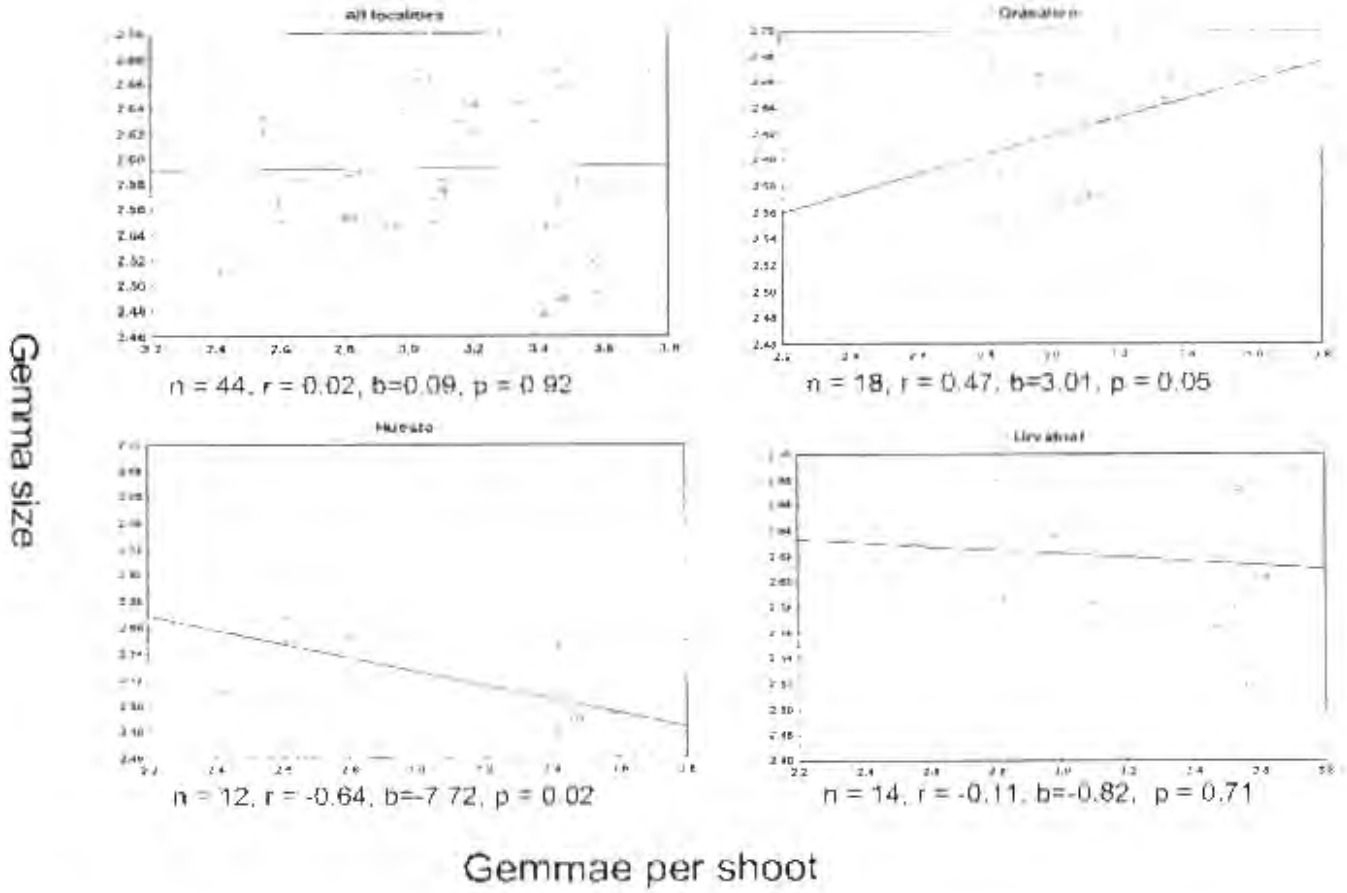

Figure 4.11. Scatter plots of number of gemmae per shoot versus gemma size in *Lophozia ventricosa* for all the localities combined, and for separate localities.

4.4 Discussion

The results presented above demonstrate substantial variation in diaspore size and number traits among Lophoziaceae. Spore size, often a conserved trait in bryophyte groups, varies from 12.2-17.2 um in diamter, translating to almost three-fold difference in volume. Within at least some species, this trait varies over two-fold among populations. Similarly gemmae volume varies by over two-fold. For some species it varies by over one and a half-fold among populations.

4.4.1 Spore production and spore sizes

The sizes of spores obtained for the different species in this study are compatible with ranges given in literature (See Appendix 2). Not much information on spore production exists to be able to compare with the results from this study. Both the spore size and the number of spores per capsule differ significantly among species. This is consistent with the idea that spore size and spore numbers vary more between than within species. However, in contrast to the idea that they vary within the same order of magnitude within species (Vitt, 1968 in During, 1979), in the present case some of the spore numbers for the same species also vary across orders of magnitude, as in *L. ciliata*.

Of greater interest from an evolutionary perspective is the extent to which variation among species can be understood in terms of likely selective forces. A number of factors are thought to influence propagule size in tracheophytes. These include environmental conditions under which the diaspores are expected to germinate, method of dispersal, size of the plants and the stage of succession with which the species is associated (Salisbury, 1974; Foster and Janson 1985; Hammond and Brown 1995; Westoby *et al.*, 2002). For bryophytes, the duration for which the habitat is available or suitable may be important (During 1979). Plants in drought conditions have larger seeds than plants in wetter conditions. This does not seem to be the case for liverworts, maybe because many of the species occur in situations where they are

unlikely to experience drought. What therefore would affect liverworts is the duration the habitat remains suitable for their occupation. Species like *L. bantriensis* and *B. barbata* which occur on damp places like springs and mires where the habitat is available longer seem to select for bigger spores that have faster germination and establishment advantage which would make more competitive and help them maintain their local populations. Species like *L. heterocolpos* and *L. grandiretis* which occur in less wet, exposed habitats, where the habitats are suitable for plant metabolic activities for shorter periods and those plants occupying disturbed habitats that are available for shorter periods would produce many diaspores as a selection for greater dispersability. The large diaspore numbers are likely to cater for the losses that occur during dispersal and before successful establishment (During, 1979, Hedderson and Longton 1995, 1996; Hanski, 2001), and due to trade-offs they are bound to be associated with small size.

Though not statistically tested, evidence of significant variation in spore sizes among different localities for the same species seem to exist in the literature (References used for Chapter 2; Appendix 1). This study shows that inter-specific variation contributes more to total variation than variation within species. This would be expected since spore size is used as a taxonomic character. The results nevertheless, show that variation among localities contributes significantly to the study of life history evolution within species.

Most of the common explanations for such variation include variations in physical factors such as amount of rainfall, water table and temperature. It is difficult from the nature of the study to tell which of either environment or genetic effects contributed more to the differences between localities. Results of post hoc analysis for *L. ciliata* and *L. ventricosa* show that the further localities are from each other the more likely they are to be significantly different from each other. Environmental conditions commonly differ more when two localities are further away from each other. For example, the L. ventricosa spore sizes for Spaninish alpine locality (Huesta) are significantly smaller than both Norwegian boreal localities (Urvatnet and Gråkallen). Hassel (2003) studying *Pogonatum dentatum* found significant differences in

spore sizes between lowlwnd populations and mountain populations, but he found little genetic difference between the two populations.

The study also shows significant difference in spore size among colonies collected from the same localities. Taking into consideration the patchy nature of bryophyte habitats and how the physical conditions experienced by each small patch can vary significantly within the same locality, it is not surprising to find significant difference in spore size among colonies within the same locality. Most of the physical factors may affect diaspore size through their effect on the whole colony and not at capsule level and hence the difference is significant more between colonies than within colonies.

4.4.2 Spore size-number trade-offs

The results are consistent with the existence of a trade-off between spore size and the number of spores produced per capsule. Spore size generally decreases with increasing number of spores per capsule. This trade-off is displayed at all levels tested among species, within each species and within each of the localities although it is not significant at the among species levels (Figure 4.5 and Figures 4.6 – 4.8). Although the correlation is still insignificant after removal of outliers, the trend is in the expected direction. Given high levels of inherent scatter, the sample size available is not likely to provide sufficient statistical power for the test. The nature of such a trade-off is expected to result in species/populations/individuals that produce few large spores at one end and those that produce many small spores at the other end. Species such as *L. excisa* produce some of the largest spores and in the least numbers while species like *L. heterocolpos* produce small spores in large numbers. However, there also exist some species like *L. ciliata* that produce few small spores as well as species like *B. barbata* that produce large spores in large numbers. This might be related to habitat productivity and some plants in a relatively well-moistened and nutrient rich habitat might be able to produce both bigger and more numerous spores while those in less-moistened and nutrient deficient plants produce small fewer spores.

The spore size–number trade-off among species might not have been significant because of the small sample size used in the analysis. Only 12 species were used in the analysis, the majority of which had fewer than 10 capsules, sometimes from only one colony. Such data do not give the best representation of species means. However, trade-offs in bryophyte reproduction do occur at different levels which might influence the diaspore size-number relationships. Trade-offs might occur between sexual reproduction (through spore production) and asexual reproduction (e.g. through gemmae). It is more likely that, in species that are capable of producing both types of diaspores, the production of one type of diaspore will reduce the energy available for the production of the other type of diaspore. In such cases, when only spores are considered, the diaspore size-number relationships for the species would be different from the one that exists when such a species produces only one type of diaspores. Such trade-offs might not exist in those species that reproduce through one type of diaspore.

L. excisa produces both spores and gemmae commonly (Damsholt, 2002). It occupies damp places, therefore is likely to live longer because mortality risk to adult shoots is lower since they dry out less frequently. Diaspores therefore contribute to diaspore banks and hence they are big to survive longer. The longer life might also help the spores grow bigger. *L. heterocolpos* develops gemmae frequently but spores rarely (Damsholt, 2002). Spores are in this case likely to be more important for occupation of new habitats in which case both their small sizes and their large numbers are important characteristics. *L. ciliata* habitats may last very short periods, since logs dry easily, and may be spaced. This would mean that the conditions favourable for development of diaspores are short lived. There is selection for early growth and reproduction thus production of small spores. The small size also helps dispersal to new habitats. It would be expected for *L. ciliata* to produce a large number of spores per capsule. The results presented here do not suggest that. *B. barbata* produces both spores and gemmae rarely (Damsholt, 2002). It could be that *B. barbata* produces spores only when certain favourable conditions are met and when it does so the spores are in large numbers. However, because it could take long before it reproduces sexually again the spores are

big in order to be able to stay alive (viable) longer in the diaspore bank. Though the sizes are not favourable, the large numbers could also promote dispersal.

4.4.3 Gemma size and gemma production

Both gemma size and number of gemmae per shoot show significant variation among species. These results suggest the idea that gemma size is also a species character. Gemma size also shows significant differences among localities and among colonies, but not among gemmae within colonies while the number of gemmae per shoot shows no significant spatial variation, other than among individuals. The results here are consistent with the idea that gemma size is under tight genetic control. Just like the gametophyte, it might be that gemma size is also affected by environmental factors that affect the whole colony other than individual shoots. The number of gemmae produced per shoot, on the other hand, is likely to be more influenced by the environmental conditions. The number of leaves on one shoot that will produce gemmae is likely to be "decided" by how much food or resources are available. Individual shoots within colonies also show significant differences in gemma size, suggesting that gemma size might also be influenced by resource allocation to individual shoots within the colony. However, the colony might include several genetic individuals, all with different allocation patterns. In seed plants questions have been raised on the influence of plant size on the on seed size, i.e. whether bigger plants have bigger seeds. This is also likely to be applicable to bryophytes in which case plants that have bigger gametophytes will produce bigger gemmae.

4.4.4 Gemma size and number trade-offs

Generally no relationship seems to exist between gemma size and number of gemmae produced per shoot. A trade-off might be expected with the result showing a decrease in the number of gemmae produced as the size increases. Such a relationship might not always be apparent for a number of reasons. The production of gemmae occurs throughout the growing season. Therefore the number of gemmae measured in this study was the number that

was present at the time of collection and not the total number of gemmae produced by the shoot. Since gemmae are produced throughout the season (Laaka-Lindberg, 2001, 2005), the sizes of gemmae measured might also include gemmae that are not fully grown. These factors might affect the apparent relationship between size and number even when a trade-off between the two traits exists. Another reason might also be the extent to which gemmae are dependent on gametophyte. Gemmae do have chloroplast and seem to be capable of making their own food. This thus makes them less dependent on the gametophyte hence limiting the effect of resources on size number trade-off, if this would be compared with spores. In an earlier study, Pohjamo and Laaka-Lindberg (2003) showed that no trade-off exists between shoot mortality and gemmae production because gemma production does not involve high investment on the part of a shoot. It is also possible that gemmae are produced to certain sizes for a particular species regardless of number. In such cases the resources would only limit the number produced. In a study on seed production in a crop of *Mucuna andreana* Micheli, Janzen (1977) showed that there was no relationship between the seed size (seed mass) and the number of seeds in the pods.

From the results here, it can be concluded that the number of spores that are produced per capsule in Lophoziaceae is a species determined character. However, environmental conditions under which they are produced also influence their size through selection for either dispersal or maintainance of current populations. Gemma sizes and numbers also seem to be influenced by the species' lineage. There is evidence of trade-off between spore size and number at both species and population levels. Species and populations either produce few big spores or they produce many small spores. Such evidence is not clear for gemmae.

CHAPTER 5

DIASPORE GERMINATION IN LOPHOZIACEAE AND ITS RELATIONSHIP TO NUMBER/SIZE COMPROMISES

5.1 Introduction

Spores and gemmae in bryophytes, like seeds in higher plants, are the means by which new individuals are produced and are a key means by which new populations are founded. These diaspores, either separately or together, are the main dormant or resting stages in the life cycle of bryophytes. They are also usually the stages at which most dispersal and colonisation of new localities occur. Germination rates and requirements of gemmae and spores are therefore key elements in the population biology of bryophyte species, influencing as they do both distribution and abundance.

In higher plants many studies have examined seed germination including the influence of seed size and morphology, the types and importance of seed dormancy and the effects of physical factors such as water availability, pH, soil conditions etc. (Eiken & Springer, 1995 and references therein; Gómez, 2003; Baraloto, *et al.* 2005; von Mölken *et al.*, 2005). It has been shown that germination is positively related to seed size i.e. species with larger seeds have higher germination proportions and germinate faster (e.g. Schaal, 1980; Weis, 1982; Hendrix, 1984; Zhang and Maun, 1990; Gómez, 2003). Three types of seed dormancy are recognised (Harper, 1957). Innate dormancy is caused by endogenous factors such as immaturity of the embryo or presence of inhibitors and can be overcome by a period of after-ripening or some seasonal stimuli such as photoperiod. Induced dormancy is due to an adverse factor acting upon the seed and producing a suspended animation that continues after the causal factor has ceased to act. Enforced dormancy is imposed by an exogenous factor such as the absence of normal requirements

of growth (e.g. water, appropriate temperature or oxygen) or the presence of inhibiting elements (e.g. high concentrations of carbon dioxide) and lasts only as long as the factor acts upon the seed. Seeds that do not germinate, due to dormancy, have been shown to form persistent seed banks (Baskin and Baskin, 1989; Rice, 1989; Simpson, et al., 1989; Degreef *et al.*, 2002).

Considerably less research has been undertaken on germination of bryophyte diaspores, possibly because their fate in natural conditions is hard to follow. Spores, gemmae and protonemata are hard to detect on natural substrates. A number of laboratory studies, however, have examined bryophyte spore germination, germination of protonema and bryophyte tissue cultures in general (Kimmerer, 1991a; Bates, 2000; Wiklund, 2003 and some references in Hohe and Reski, 2005). Some studies have also reported the existence of dormancy of one type or another on both spores and asexual diaspores of bryophytes (Miles and Longton, 1992; Duckett & Renzaglia, 1993; McLetchie, 1999; Laaka-Lindberg and Heino, 2001; Hock, *et. al.*, 2004). Laaka-Lindberg and Heino (2001) showed that germination percentages in *Lophozia silvicola* in Finland declined with the growing season. This they attributed to increased dormancy as the growing season progressed. Hohe and Reski, (2005) reviewed the development and application of *in vitro* techniques in bryophytes and highlight a number of studies that have been done on conditions for bryophyte germination including use of different culture media. As noted by Wiklund and Rydin (2004), the challenge of such laboratory work is to mimic the range of conditions the species may encounter in the field.

It is important to consider germination as part of the plant life cycle. The timing and regulation of the stages of the life cycle is an important aspect of plant life history. In bryophytes reproduction can either be sexual, through the production of spores, or asexual, by means of gemmae or through some segments of the gametophyte (Newton and Mishler, 1994). The life cycle of sexually reproducing species includes spores, protonema, buds, full-grown gametophytes, gametangia and sporophytes. The asexual cycle includes diaspores, protonema (usually but not necessarily) and full-grown gametophytes (See Chapter 1). Within these gross stages, a finer subdivision

is possible (Mogensen, 1978). The transition from each stage to the next is mostly governed by the interplay of environmental fluctuations and physiological traits of the plant. For most transitions the exact conditions are not known and it is difficult to asses their importance for life strategies (During, 1979). For germination it is possible, at least in vitro, to determine and asses the importance of most of the necessary conditions.

In most mosses, spore germination is thought to be inhibited as long as they remain in the operculate theca (Buch, 1920; Oppenheimer, 1922; Both quoted in During 1979); not much is known for liverworts. Once shed, spores of many species can germinate immediately, while others first have to pass through an innate dormancy stage (Howe and Underwood, 1903; Thompson, 1941; Miles and Longton, 1992; Duckett and Renzaglia, 1993; McLetchie, 1999; Hock *et. al.*, 2004). Dormancy of propagules is often a strategy to by-pass periods of unfavourable conditions. Secondary dormancy, which occurs when propagules are exposed to certain environmental conditions and continues after the environment changes to prevents germination at the wrong time of year, has also been demonstrated (McLetchie, 1999; Laaka-Lindberg & Heino, 2001; Hock *et al.*, 2004). In others, mature spores need rest but immature spores germinate immediately (Gaibsberg, 1921 in During, 1979). Spores from different sporophytes of the same species might also have different germination capacities. Dormancy of asexual propagules has been even less studied (Duckett & Renzaglia, 1993; Laaka-Lindberg & Heino, 2001).

In addition to how long it will take diaspores to germinate, what proportion of the produced and/or dispersed diaspores will germinate is an important factor in the species' survival in a locality and its ability successfully to colonise new suitable localities. A number of studies, mainly in mosses, have estimated spore viability, in some instances in relation to spore longevity and environmental conditions (Fulford, 1951; Hoffman, 1970; Newton, 1972; Egunyomi, 1978; Longton, 1979; Dalen and Söderström, 1999). The timing of germination, especially in response to environmental factors, has been identified in several studies as a key factor for successful establishment.

Wiklund and Rydin (2004), working on *Buxabaunia viridis* and *Neckera pennata*, showed that the interaction between pH and water potential are important for spore germination, affecting both the lag time before germination and the final germination percentage. How long gemmae/spores are able to remain viable in the storage system (diaspore banks) is also important for the temporal distribution of the species. The longevity of spores varies greatly with species and environmental conditions, from dying within one hour of desiccation to surviving about 11 years, though the germination percentages decrease greatly (Fulford, 1951; Longton and Schuster 1983; Duckett and Ligrone, 1992; Wiklund and Rydin, 2004). Although considered very important in seed plants (see above), possible associations between germination rates and propagule size have not been explored for any bryophyte.

The aim of this chapter is to estimate germination proportions for spores and gemmae from a range of Lophoziaceae species. It also aims to investigate the relationship between germination rates and both diaspore size and production levels for each of the studied species. The specific questions posed are; 1) What are the average proportions of spore/gemmae germinations for each of the species? 2) Is there any significant difference in the mean proportions of diaspore germination among species? 3) Is there any significant difference in average proportions of germination of the diaspores of the same species between different geographical areas? and 4) Are spores and gemmae germination levels related to either their size or the numbers produced per capsule/shoot?

5.2 Methods and Materials

5.2.1 Spore and gemmae collection

The spores and gemmae used were collected, treated and stored in the same way as described in Chapter 4.

5.2.2 Germination tests

To test for the rate of spore germination, the remainder of the spore suspension used for spore counts (Chapter 4) was incubated at 22 °C under 12 hours light and 12 hours darkness for 50 days. For a few collections, the germination tests were done by incubating the spore suspension on agar prepared using the method of Nehira (1988).

For gemmae germination tests, mature gemmiparous shoots, collected and stored as described in Chapter 4, were used. The gemmae were scraped off the leaves as described above but were rinsed in Knop's solution (Nehira 1988) instead of in water and no detergent was used. The suspension was poured into a micro-tube and placed in incubation chambers at a temperature of 22 °C under 12 hours of difuse light and 12 hours darkness for 50 days. Since the spores and gemmae were collected in Europe, and the germination tests done in a lab in Cape Town, they were kept for periods of upto five months before they were germinated.

Germination was regularly checked and recorded. Germination was recorded as positive when the spore or gemmae cell walls were swollen and/or a short protonemal tube had emerged. It was expressed as a proportion (between 0 and 1) of the germinated spores or gemmae to total number of spores or gemmae counted, which was aimed to be at least 100 wherever possible. Four counts per solution were made with a Neubauer Counting chamber.

5.2.3 Statistical Analysis

All the analyses were performed in STATISTICA version 7 (StatSoft, 2004). The rate of germination was log arcsine transformed while the spore sizes

and numbers were log transformed for this analysis (Sokal and Rohlf 1995). One Way Analysis of Variance (ANOVA) was performed to test for significant differences in the average proportions of spores and gemmae that germinate among the species and among the localities for each species.

Two aspects of germination were measured. First, the proportion of individuals showing at least one germinating propagule ("Positives") was determined. Secondly, the proportion of propagules germinating for each individual capsule was recorded. In comparisons of means at the various spatial scales used, average proportions germinating were compared both with and without "zero germination" individuals included. For spore germination the species tested on agar were analysed separately from the species tested in suspension. One Way ANOVA was used to test for significant difference in the mean spore sizes between the capsules that showed germination and those where no germination was recorded.

Nested ANOVA was used to test for significant differences in the average proportions of germination of spores and gemmae among species, localities in species, colonies in localities and among capsules/shoots within colonies. The contribution of each level of variation to the total variation was also calculated. Since species collected from only one locality obviously could not show differences among localities only three species (*L. ciliata, L. ventricosa and L. longiflora*) were analysed at these spatial scales.

Correlation tests and regression analyses were performed to test for relationships between germination proportions and both diaspore numbers and diaspore size. Correlations between spore germination proportions and spore sizes were tested at both species and locality levels. Because the gemmae used for estimating size and production were from different shoots to those used in germination tests, the means used in the correlation test were the averages of colonies. This produced very low numbers for comparison of localities of each species. Therefore for gemmae, the correlations and regression analyses were done at species level only.

One Way ANOVA was also used to test for significant difference in mean spore size between the capsules that showed some germination (the Positives) and those that showed no germination at all for each species.

5.3 Results

5.3.1 Variation in spore germination

5.3.1.1 Variation among species

The proportions of spore germination for the different species studied are summarised in tables 5.1a and 5.1b below. *L. ventricosa* had the highest percentage of positives (67%), but among the positives, *L ciliata* had the highest average proportion of spore germination (0.166) while. *L. longiflora* had the lowest proportion of capsules showing some germination (14.4% of 97 capsules). It also had the lowest average rates of germination per capsule.

There are significant differences in the average proportion of spores that germinated among species. This result is true for both the spores germinated on agar and those germinated in suspension. The differences are significant whether the mean of all capsules is used or whether the analysis is restricted to positives only. Species variation accounts for 76.7 % of the total variation among all the capsules tested and for 68.76 % among the positives.

5.3.1.2 Spatial variation

Table 5.2 summarises the patterns of variation in spore germination proportions for the different localities of each species. None of the *L. ventricosa* capsules collected from Lidarende showed any germination. Species that were collected from only one locality are not included in Table 5.2.

When all the capsules that were tested were considered, all three species showed significant difference in the proportion of spores that germinated

Table 5.1. Summary table showing the mean and standard deviation of spore germination proportions for each of the species tested. **a** are those tested on agar and **b** those tested in solution A dash (-) indicates species where either no germination at all occurred or only one capsule showed some germination. Positives are germination results based only on sporangia where at least some germination occurred

a.

Species	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
B. quadriloba	0.093	0.186	0.371	-	4	25
L. bantriensis	0.989	0.009	0.988	0.009	4	100
L. collaris	0.971	-	0.971	-	1	100
L. heterocolpos	0.303	0.525	0.909	-	3	33.3
L. grandiretis	0.491	0.567	0.982	0.006	4	50
L. ventricosa	0.459	0.093	0.459	0.093	2	100

b.

Species	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
B. barbata	0	0	-	-	3	0
L. bicrenata	0.634	0.217	0.634	0.217	10	100
L. ciliata	0.166	0.282	0.323	0.323	72	51.4
L. longiflora	0.008	0.031	0.056	0.066	97	14.4
L. ventricosa	0.160	0.022	0.218	0.218	51	67

Table 5.2. Summary tables showing the mean and standard deviation of the proportions of spore germination for the different localities of each of the species. A dash (-) indicates species where either no germination occurred at all or where only one capsules recorded some germination.

a. *Lophozia ciliata*

Locality	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
Urvatnet	0.343	0.360	0.362	0.305	19	94.7
Vändåtberget	0.058	0.173	0.211	0.320	47	27.7
Rödberget	0.312	0.099	0.312	0.099	3	100
Låitavare	0.583	0.292	0.583	0.292	3	100

b. *Lophozia longiflora*

Locality	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
Vändåtberget	0.005	0.013	0.034	0.411	29	13.8
Rödberget	0.049	0.094	0.195	0.115	8	25
Låitavare	0.004	0.015	0.033	0.330	60	13.3

c. *Lophozia ventricosa*

Locality	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
Lidarende	0	0	-	-	4	0
Vändåtberget	0.194	0.216	0.250	0.023	31	77
Låitavare	0.136	0.236	0.218	0.270	16	69

among localities. *L. ciliata* and *L. ventricosa* also showed significant difference among colonies within localities but not among capsules within colonies. *L. longiflora* showed no significant difference among colonies or among capsules.

For *L. ciliata* the *post hoc* analysis shows that the mean spore germination proportions of plants from Vändåtberget nature reserve are significantly lower than those from Låitavare, Rödberget and Urvatnet. In *L. longiflora,* Rödberget has significantly higher germination than the other two localities, which did not differ from each other.

For the positives only, *L. ciliata* and *L. ventricosa* showed no significant difference in the proportion of spores that germinated among localities or among capsules within colonies. They did, however, show significant differences among colonies within localities. *L. longiflora* showed significant variation among localities, but not among colonies and among capsules. Table 5.3 summarises the contribution of each of the levels of variation to the total variation in proportion of spore germination. These results suggest that variation in proportion of germination is mostly between localities. However, once that is ignored, variation is strongly a function of colony for *L. ciliata* and *L. ventricosa*.

5.3.2. Correlation between spore size and spore germination

L. ciliata and *L. longiflora* show positive correlation between spore size and the rate of spore germination (Figures 5.1 and 5.2). However, for *L. ciliata* the relationship is significant among colonies for the plants from Vändåtberget only after the outliers were excluded. For *L. longiflora* the relationship is significant at both species and locality levels, except for Vändåtberget, when considering all the capsules tested, but is not significant when only the positives are considered even after excluding the outliers. This suggests that non-positive capsules have smaller spores than the positive ones (see below). *L. ventricosa* generally shows a negative relationship between spore size and rate of spore germination (Figure 5.3). This relationship is statistically significant at species and at all locality levels for the positives only.

5.3.3 Variation in spore size between the positives and the negatives

For all the three species, the average spore size for the positive capsules was higher then the average spore sizes for the negatives. However, One Way ANOVA showed a significant difference in mean spore size between the positives and the negatives in *L. longiflora* ($F = 11.9$, $p < 0.001$) only, with the observed differences in *L. cilliata* ($F = 0.59$, $p = 0.44$) and *L. ventricosa* ($F = 0.77$, $p = 0.39$) not reaching statistical significance.

Table 5.3. Summary of Nested ANOVA on mean proportion of spore germination per capsule for three *Lophozia* species. Numbers indicate the percentage of total variance attributable to each source. Residual tern represents variation among capsules within collection. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

Species	Total			Positives		
	Localities	**Colonies**	**residual**	**Localities**	**Colonies**	**residual**
L. ciliata	67.57***	22.53**	4.21	21.14	59.39**	12.58
L. longiflora	76.80**	3.68	10.47	68.22**	2.11	8.16
L. ventricosa	25.09**	67.56***	4.49	3.6	80.48**	9.18

5.3.4 Correlation between spore production and spore germination

Figures 5.4 to 5.6 summarise the patterns of correlation between the numbers of spores produced per capsule and the proportions of germination. *L. ciliata* and *L. ventricosa* generally show negative correlations within both species and localities. For *L. ciliata* this is significant at species level and only for Vändåtberget at locality level while for *L. ventricosa* it is significant at all levels for the positives but is negative when outliers were excluded except for Vändåtberget. It is also positive for Låitavare only when considering all capsules tested. *L. longiflora* shows significant negative correlation only for specimens from Vändåtberget even when outliers were excluded.

5.3.5 Gemma germination

5.3.5.1 Variation among species

Table 5.4 summarises the average proportion of gemma germination per species for the three species that were studied. All three species show average proportions of gemma germination of less than 15%, with *L. longidens* showing the highest proportion (14.9%). The mean proportions of germination do not differ much between all shoots measured and the positives. *L. longidens* shows the highest percentage of positives (86.6% of 45) and it also has the highest average proportion of germination. *L. ventricosa* shows the lowest value for both while *B. hatcheri* falls between the two for both, the percentage of positives and average proportion of gemmae germination.

One Way ANOVA shows significant differences in the mean rate of gemmae germination among species for both all shoots tested ($F_{(1, 2)} = 16.44$, $p < 0.001$) and for the positive shoots only ($F_{(1, 2)} = 8.9$, $p < 0.001$). For all the shoots tested, the average proportion of gemmae germination *in L. longidens* varies significantly with the mean rates for both *B. hatcheri* and *L. ventricosa*. However the mean rates of gemma germination between *B. hatcheri* and *L. ventricosa*, dot not significantly differ. For the positives the mean rate of

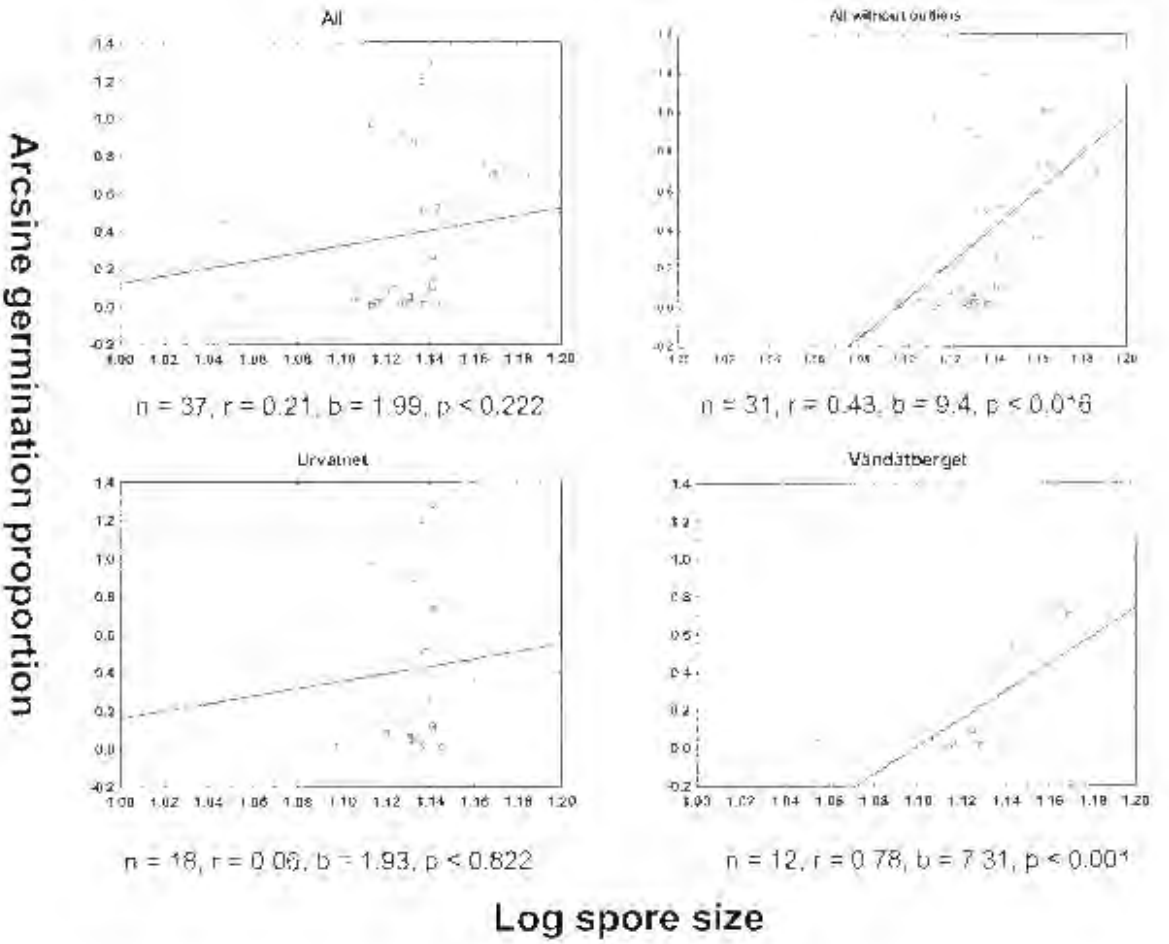

Figure 5.1 Scatter plots of proportion of germination versus spore size in *Lophozia ciliata* for all the localities combined, and then for separate localities. Included below each plot are sample size (n), correlation coeffient (r), regression coefficient (b) and p-values from the regression analysis.

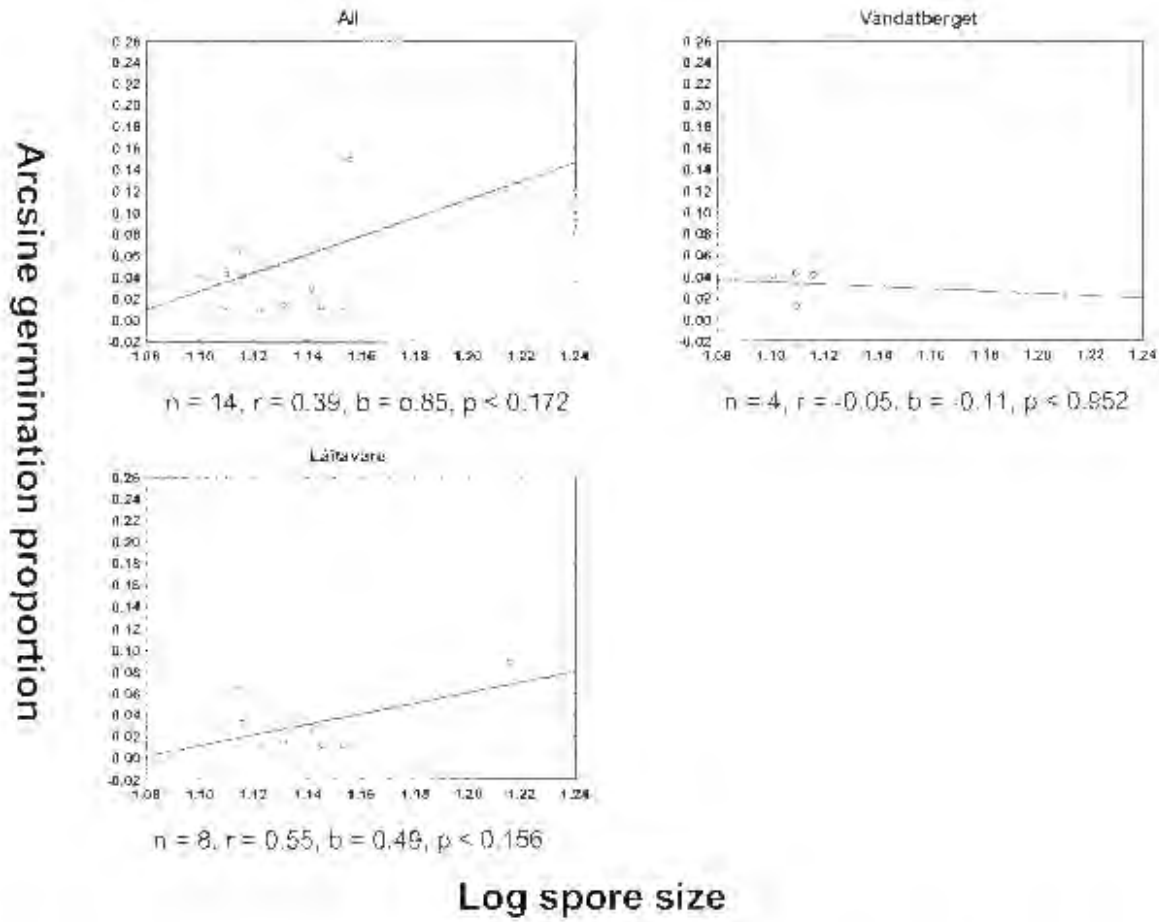

Figure 5.2 Scatter plots of proportion of germination versus spore size in *Lophozia longiflora* for all the localities combined, and then for separate localities. Included below each plot are sample size (n), correlation coeffient (r), regression coefficient (b) and p-values from the regression analysis.

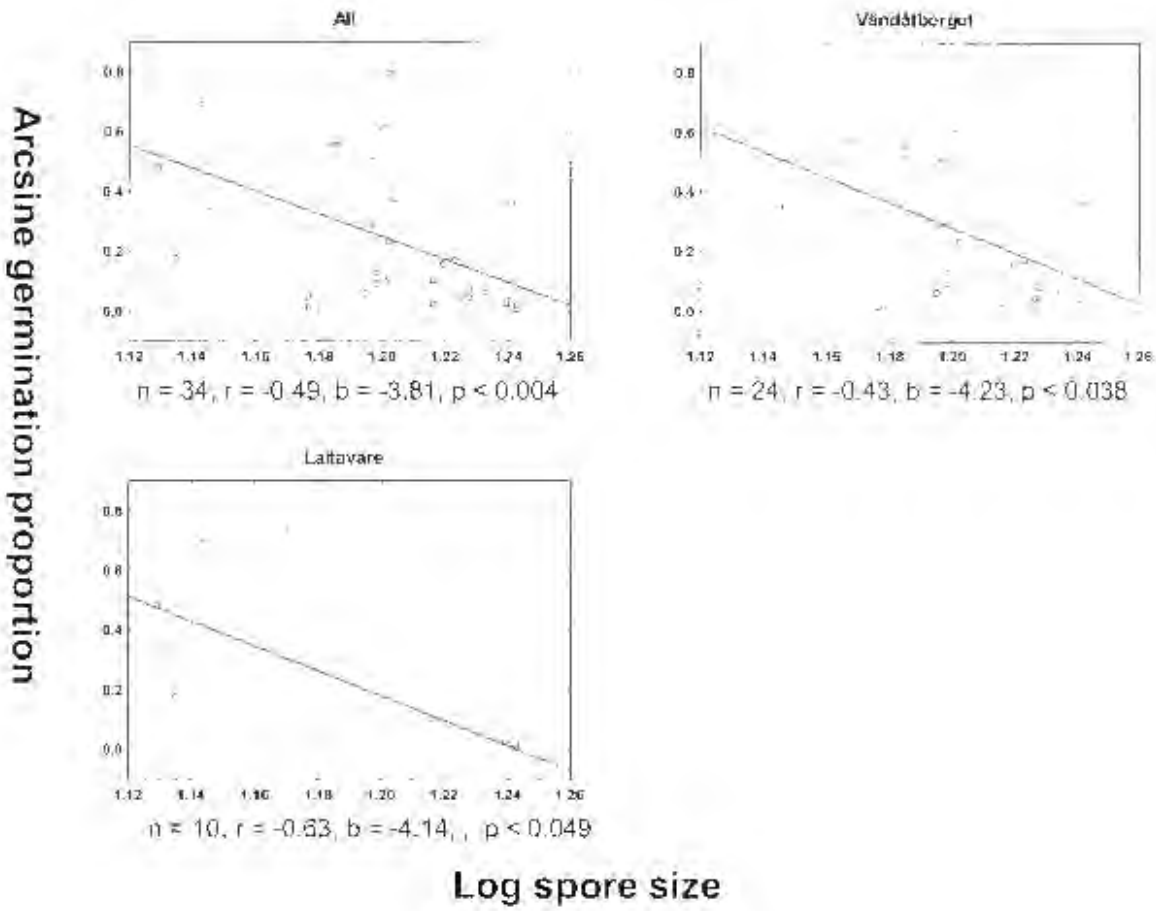

Figure 5.3 Scatter plots of proportion of germination versus spore size in *Lophoria ventricosa* for all the localities combined, and then for separate localities. Included below each plot are sample size (n), correlation coeffient (r), regression coefficient (b) and p-values from the regression analysis.

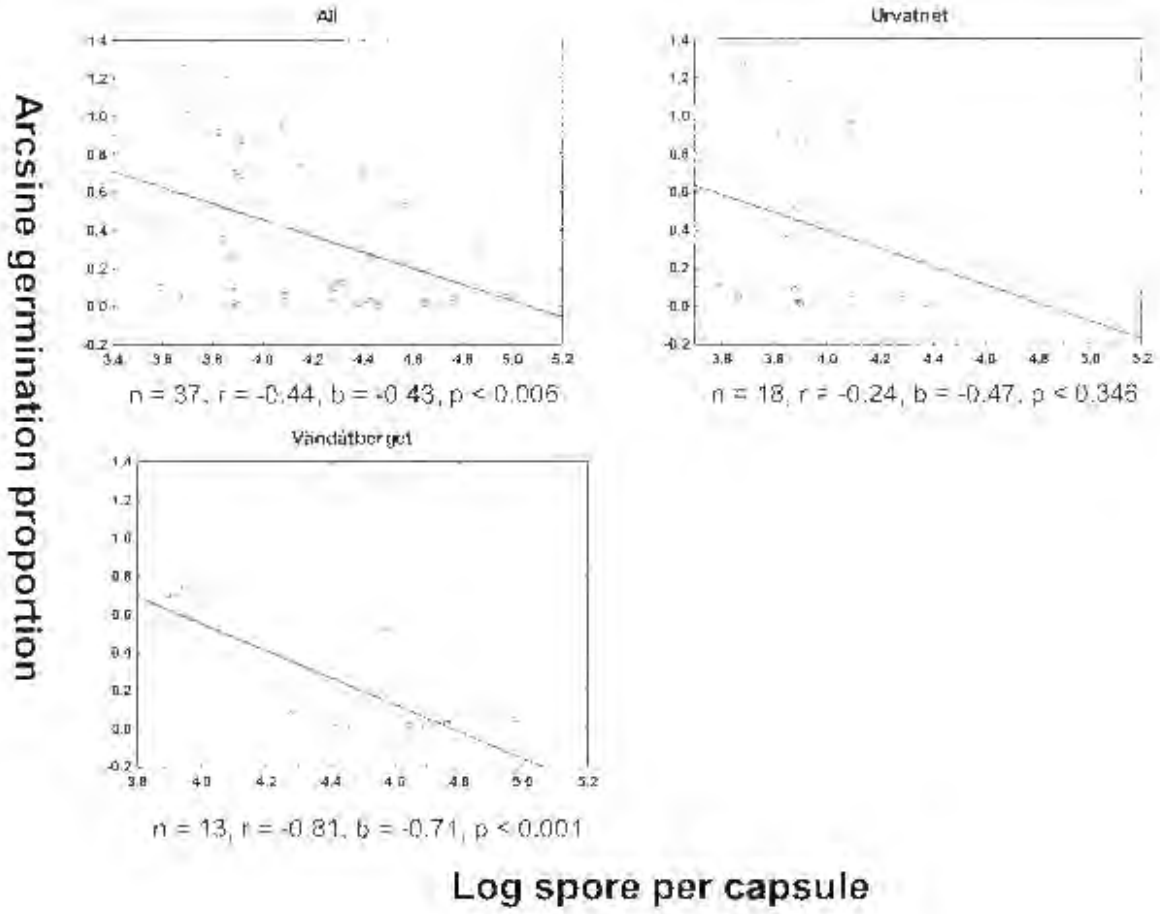

Figure 5.4. Scatter plots of spore numbers per capsule versus proportion of germination in *Lophozia ciliata* for all the localities combined, and then for separate localities. Included below each plot are sample size (n), correlation coeffient (r), regression coefficient (b) and p-values from the regression analysis.

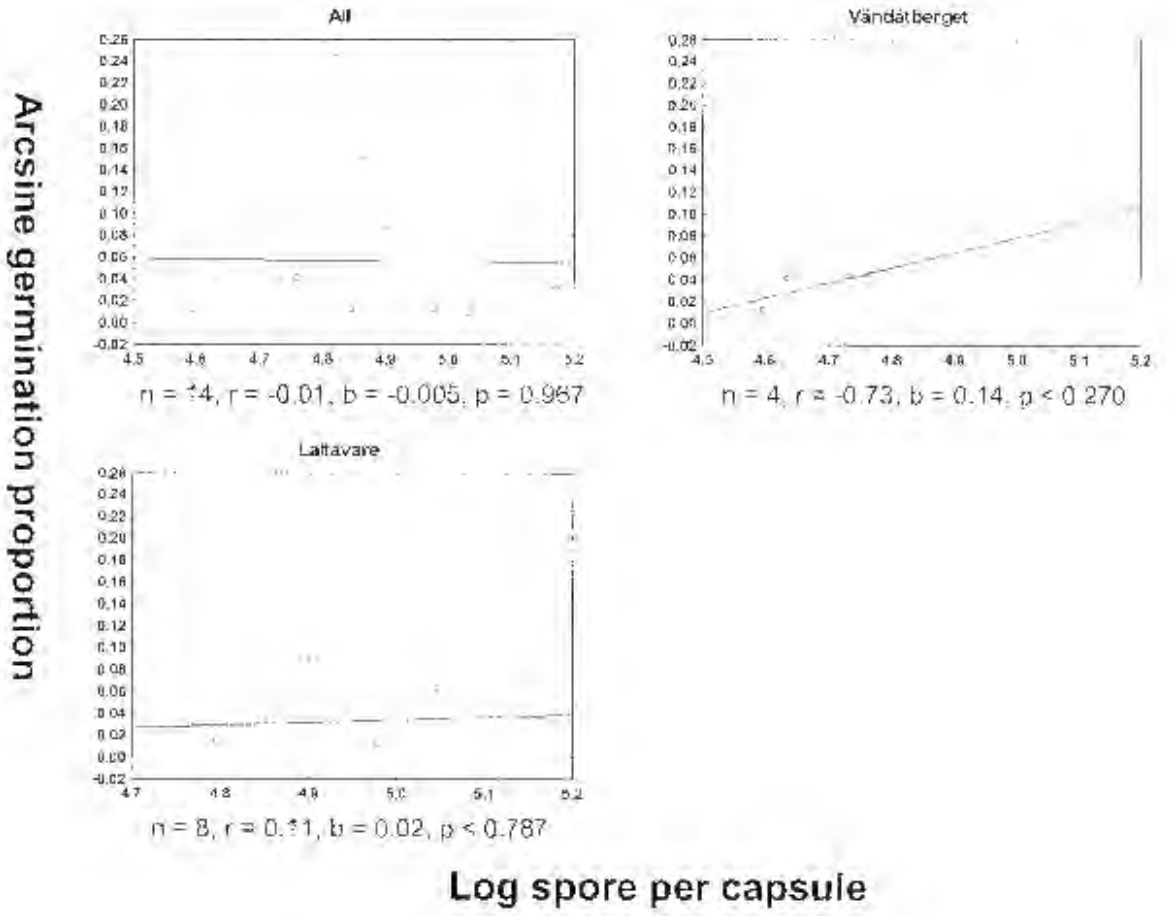

Figure 5.5. Scatter plots of spore numbers per capsule versus proportion of germination in *Lophozia longiflora* for all the localities combined, and then for separate localities. Included below each plot are sample size (n), correlation coeffient (r), regression coefficient (b) and p-values from the regression analysis.

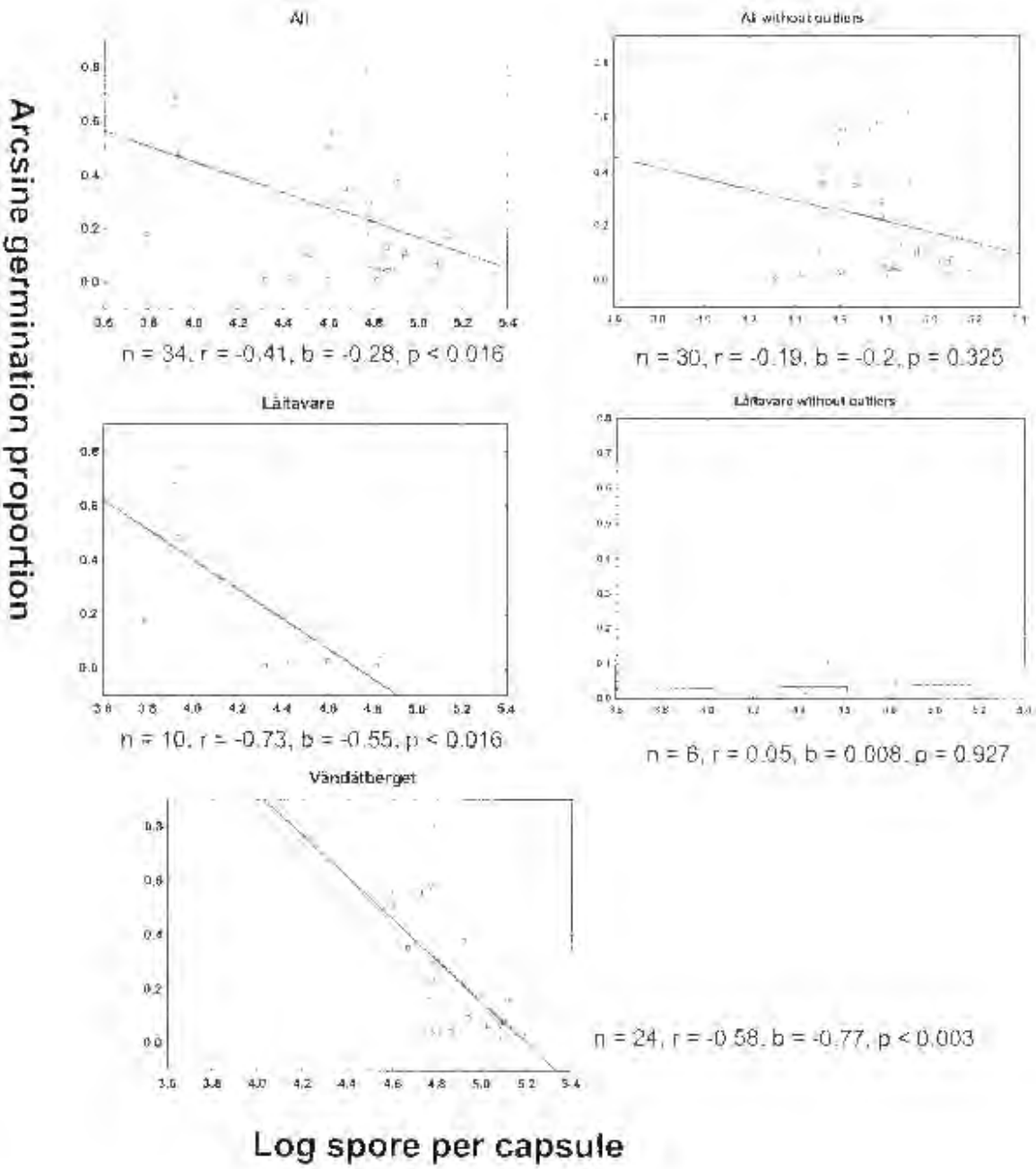

Figure 5.6. Scatter plots of spore numbers per capsule versus proportion of germination in *Lophozia ventricosa* for all the localities combined, and then for separate localities. Included below each plot are sample size (n), correlation coeffient (r), regression coefficient (b) and p-values from the regression analysis.

gemma germination significantly differs between *L. longidens* and *L. ventricosa* but not between *L. longidens* and *B. hatcheri*.

5.3.5.2 Spatial variation

Patterns of variation in germination proportions among the localities of each of the species are summarised in Table 5.5. *B. hatcheri* and *L. ventricosa* show no significant difference in the average proportion of gemmae that germinated among localities, among colonies within localities, or among shoots within colonies. *L longidens* shows significant differences in the average proportion of gemmae that germinated among localities and among colonies within localities but not among shoots within colonies, for all the shoots tested. For the positives only, it shows significant differences among localities but not among colonies and shoots. The only significant difference is between Urvatnet and Lidarende. Table 5.6 summarises the contributions made by each of the levels of variation to the total variation in mean germination proportions.

5.3.6 Correlation between gemma size and gemma germination

L. longidens shows a statically significant positive correlation between mean gemma size and the mean rate of gemma germination (Figure 5.8). In *B. hatcheri* (Figure 5.7) and *L. ventricosa* (Figure 5.9) these two are not significantly correlated for either all the capsules (positive) or only the positives (negative).

5.3.7 Correlation between gemma production and gemma germination

None of the species show a significant correlation between the mean numbers of gemmae produced per shoot and the mean rate of gemma germination (Figures 5.10 to 5.12), for all shoots tested as well as for the positives only. However, the correlation and regression coefficients are in the expected (negative) direction in all the species examined

Table 5.4 Summary table showing the mean and standard deviation of gemma germination proportions for each of the species tested. Positives are germination results based only on sporangia where at least some germination occurred.

Species	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
L. ventricosa	0.044	0.035	0.070	0.055	45	62.2
L. longidens	0.135	0.105	0.150	0.100	45	86.6
B. hatcheri	0.061	0.064	0.096	0.054	30	63.3

Table 5.5 Summary tables showing the mean and standard deviation of the proportions of gemma germination for the different localities of each of the species.

a. *Lophozia ventricosa*

Locality	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
Urvatnet	0.036	0.035	0.054	0.029	15	66.7
Gråkallen	0.039	0.015	0.064	0.034	18	61.1
Huesta	0.060	0.015	0.103	0.093	12	58.3

b. *Lophozia longidens*

Locality	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
Urvatnet	0.197	0.020	0.215	0.084	12	91.7
Gråkallen	0.131	0.039	0.138	0.102	9	77.8
Lidarende	0.066	0.039	0.084	0.049	24	91.7

Table 5.5.c. *Barbilophozia hatcheri*

Locality	Total		Positives		Total	Percentage
	Mean	SD	Mean	SD	Tested	Positive
Lérida	0.061	0.029	0.084	0.057	18	72.2
Lézidan	0.061	0.027	0.123	0.038	12	50

Table 5.6. Summary of Nested ANOVA in mean rate of gemma germination per shoot for three species. Numbers indicate the percentage of total variance attributable to each source. Residual tern represents variation among shoots within collection. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$.

Species	Total			Positives		
	Localities	Colonies	residual	Localities	Colonies	residual
L. ventricosa	19.61	46.5	9.86	38.38	20.18	10.57
L. longidens	59.81**	24.23*	8.72	61.35*	24.08	5.32
B. hatcheri	0.007	52.93	14.06	41.06	33.44	8.33

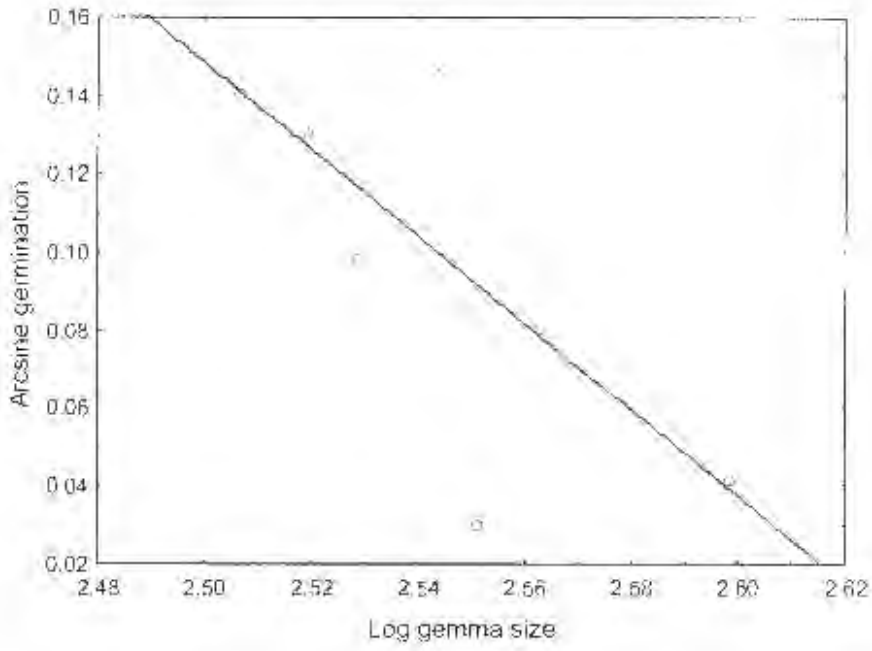

Figure 5.7. Scatter plots of gemma size versus proportion germinating for *Barbilophozia hatcheri*, n = 8, r = -0.70, b = -0.45, p < 0.052.

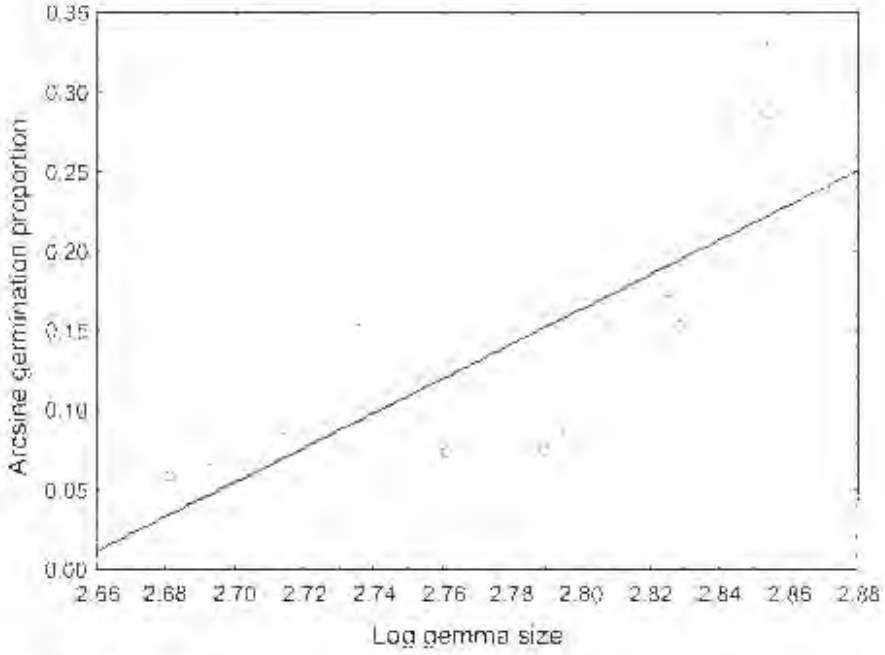

Figure 5.8. Scatter plots of gemma size versus proportion germinating for *Lophozia longidens*, n = 15, r = 0.71, b = 1.09, p < 0.003.

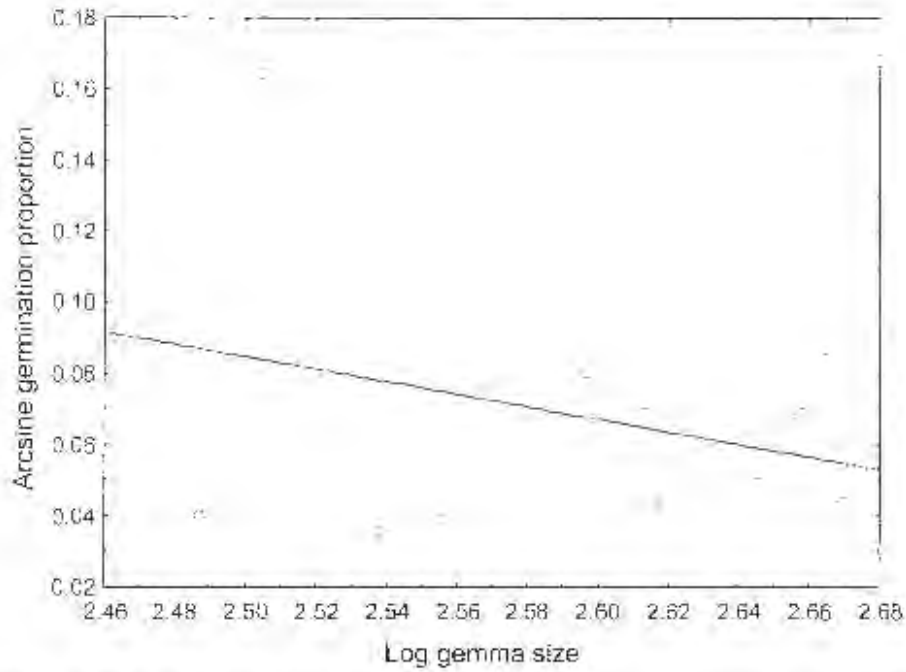

Figure 5.9. Scatter plots of gemma size versus proportion germinating for *Lophozia ventricosa*, n = 13, r = -0.30, b = -0.18, p < 0.328.

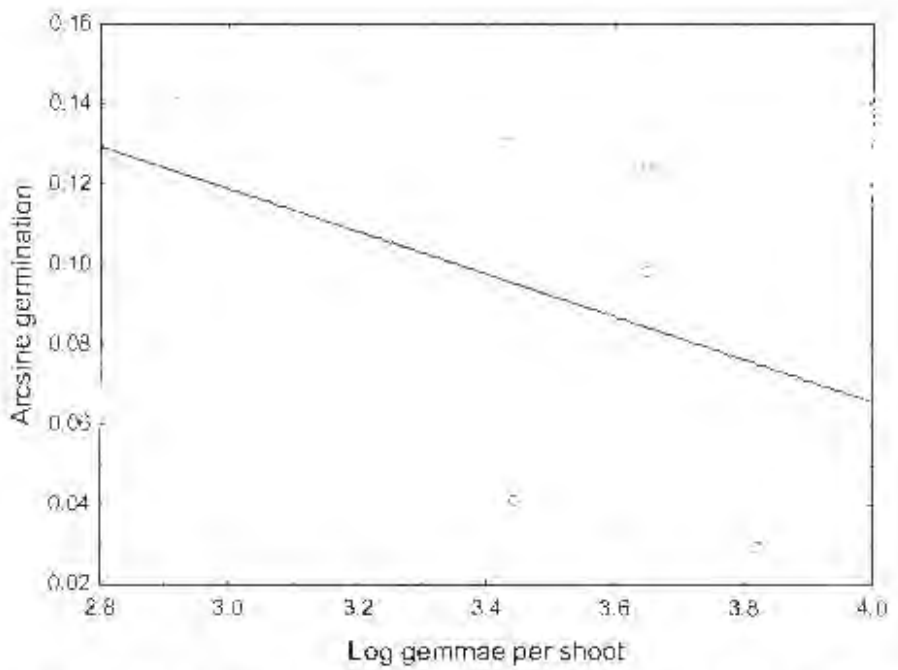

Figure 5.10. Scatter plots of number of gemma per shoot versus proportion germinating for *Barbilophozia hatcheri*, n = 8, r = -0.41, b = -0.05, p < 0.317.

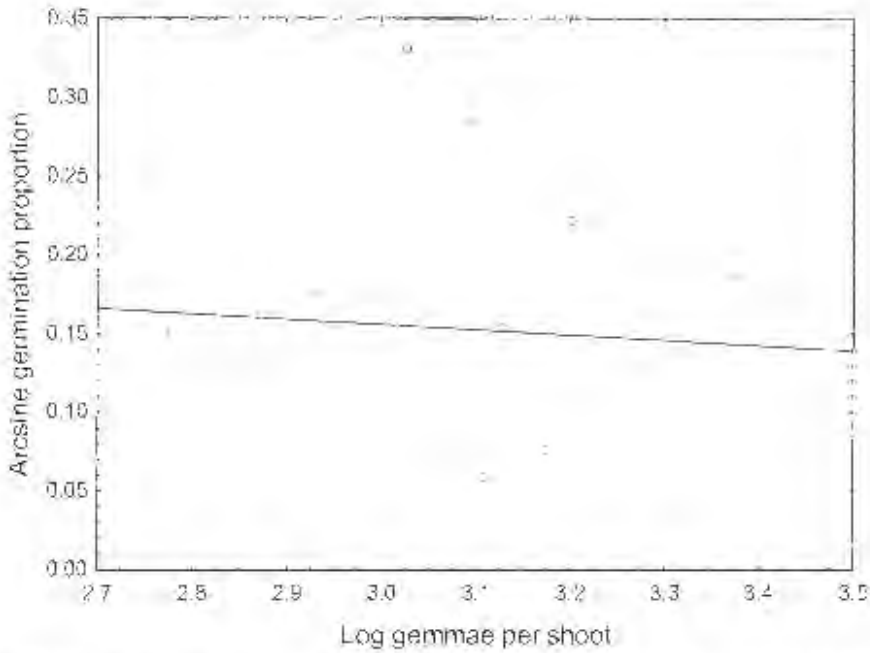

Figure 5.11. Scatter plots of number of gemmae per shoot versus proportion germinating for *Lophozia longidens*, n = 15 r = -0.07, b = -0.03, p < 0.809.

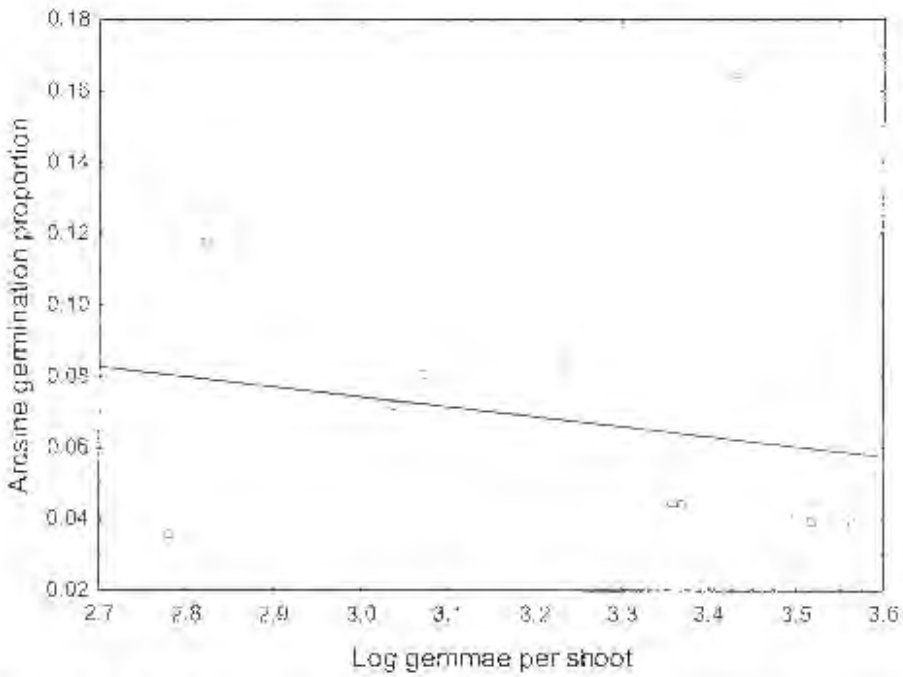

Figure 5.12. Scatter plots of number of gemmae per shoot versus proportion germinating for *Lophozia ventricosa*, n = 13, r = -0.19, b = -0.03, p < 0.538.

5.4 Discussion

5.4.1 Spore germinability

The study here shows marked differences in spore germination percentages among the species. Species with higher percentages of "positive" capsules also had greater proportions of spore germination in those capsules. Spore germination in liverworts is said to be influenced by both evolutionary lineage and the environment (Nehira, 1987). The results here are likely to be showing the germination patterns that are influenced more by the species. However differences among species may also be due to selection of the individual species for better survival in the different habitats that the different species occupy. Of the three species studied, *L. ciliata* and *L. ventricosa* have higher germination percentages and *L. longiflora* has the least. *L. longiflora* produces sporophytes more frequently and gemmae rarely while *L. ciliata* and *L. ventricosa* produce sporophytes rarely and occasionally respectively and gemmae more frequently (Damsholt, 2002). *L. longiflora* therefore is dependent on spores for both local populations and colonisation of new localities. If it is assumed that the low germination percentages are due to dormancy, as suggested for gemmae (Laaka-Lindberg and Heino, 2001) and not death or other form of permanent non-viability, it would benefit more from dormancy should there be a season of unfavourable conditions.

The differences in germination between localities for the same species supports the idea that germination of spores is affected by the environmental conditions under which they are produced. Germination patterns in bryophytes are thought to be modified by habitat. It can be hypothesised that *L. ventricosa* which occupies a variety of different micro-habitats is likely to have different germination percentages depending on the micro-habitat on which each of the colonies is growing within the same locality. In such a case differences among micro-habitats would have more effect than differences among localities. *L. longiflora* would have significant variation among localities because it is more restricted to micro-habitats with similar conditions that do not vary much within localities. The difference would be greater between

localities thus making locality differences more important in determining the germination proportions. This study unfortunately did not consider differences in the microhabitats or substrates for species like *L ventricosa*.

A positive relationship between diaspore size and germination proportion as is displayed by *L. ciliata* and *L. longiflora* (Figures 5.1 and 5.2) has been shown for some species in higher plants (Gómez, 2003). The explanation is that big diaspores can provide adequate food for the growing seedling while small seeds do not guarantee that, and hence might need more favourable conditions. However, in contrast *L. ventricosa* generally shows a negative relationship between spore size and proportion of spore germination (Figure 5.3). *L. ventricosa* produces sporophytes occasionally and gemmae frequently (Appendix 1). In such species sexual spores are likely to form diaspore banks that become active after periods of unfavourable condition. Low germination percentages may therefore be due to spore dormancy with the gemmae germination functioning to maintain current populations.

The generally negative relationship between number of spores per capsule and the proportion of spore germination (Figures 5.4 to 5.6) could indicate a trade-off between viability and number of spores produced. If germinability can be taken as a measure of fitness, then when limited resources are used to produce few offspring, they are likely to be fitter than when the same resources produce many offspring. However it could just be due to trade off between spore size and spore numbers; capsules with few spores are likely to have large ones that germinate better and vice versa.

5.4.2 Gemma germinability

Gemma germination rates are generally lower than reported in a previous study on *Lophozia silvicola* (Laaka-Lindberg and Heino, 2001). Only the germination proportions of the positives for *L. longidens* are comparable to the germination percentages of *L. silvicola* at the end of growing season. The proportion of gemma germination also differs significantly across species. The species with high percentage of positives also have high germination proportions (Table 5.1). *L. longidens,* which always produces gemmae, shows

the highest proportion of germination. *B. hatcheri* and *L. ventricosa* which produce gemmae frequently have lower germination proportions. It could be that since both these species are capable of reproducing both sexually and asexually, the lower germination proportions in gemmae would mean more gemmae go dormant and contribute to diaspore banks. These gemmae would help in the maintenance of local populations after periods of unfavourable conditions.

L. longidens also shows significant difference among localities with localities contributing the greatest to total variation. *B. hatcheri* and *L. ventricosa* show no significant variation in germination proportion among localities. For both colonies contribute the greatest to variation in the percentage of shoots where some germination occurs while localities contribute the most difference in the proportion of gemmae that germinate.

A positive correlation between gemma size and proportion of germination, as is shown by *L. longidens,* can be explainable if the size is related to maturity of the gemmae and mature gemmae germinate better than immature gemmae. However *B. hatcheri* and *L. ventricosa* show negative relationships which are not significant, and this makes it difficult to maintain the above explanation. However, it could also be that gemmae are designed to last longer in the diaspore banks, because they mainly function in maintenance of local population. In such a situation bigger gemmae are likely to have thicker walls, enabling dormancy.

Germinability of gemmae in *Lophozia silvicola* has been shown to decrease with the growing season in Finland (Laaka-Lindberg and Heino, 2001). It is thought that the decline is due to increasing frequency of dormant gemmae being produced but there is no evidence for this thought. Most of the *L. ciliata, L. longiflora* and *L. ventricosa* specimens used for this study were collected between mid-July and the end of August. For this period Laaka-Lindberg and Heino (2001) recorded germination proportions (0.308 and 0.307 for July and August, respectively) that were less than half of those recorded for May (0.690). The proportions recorded in this research are comparable for *L.*

ciliata but lower *L. longiflora* and *L. ventricosa* (Tables 5.1 and 5.2).However, the significant differences among the different localities of each species do suggest influence of other environmental factors.

CHAPTER 6

GENERAL DISCUSSION

Under classical life history theory (Roff, 1992, Stearns, 1992), the general features of a plausible explanation of evolution of life history traits are that; (1) life histories are shaped by the interaction of extrinsic and intrinsic factors, (2) the extrinsic factors are ecological impacts on survival and reproduction, and (3) the intrinsic factors are trade offs among life history traits and lineage-specific constraints on the expression of genetic variation (Stearns 2000). The main idea behind the study of life history evolution is to be able to explain the patterns of variation in characteristics observed among organisms. It is predicted that environmental conditions select for trait combinations that enhance the fitness of organisms living in that particular habitat (Stuefer, *et. al.*, 2002).

This study provides support for the idea of occurrence of recurrent suites of co-variation in life history traits within the family Lophoziaceae. The patterns of co-variation show close association with variation in other aspects of species' biology such as habitat characteristics like wetness and duration of conditions permitting metabolic activity. Besides supporting previous views (During 1979, 1992; Longton & Schuster 1983; Hedderson and Longton, 1995) that it is possible to classify bryophyte life histories into ecologically meaningful groups, it also suggests that within a group of closely-related species such classification might not be closely related to phylogeny. This therefore supports the idea that life histories are free to adjust to the environment independently of phylogeny and thus cannot be used to infer evolutionary processes.

Also supported is the idea of occurrence of constraints leading to trade-offs between life history traits. Evident from this study is a trade-off between spore size and number of spores produced per capsule (chapter 4) with species or individuals either producing many small spores or producing few large spores.

The positive correlations between germination proportion and spore size (chapter 5) may mediate variation along this axis. Gemmae did not show this trade-off between size and number of propagules.

6.1 Sexual and asexual reproduction

The study supports the expectation that monoiecisim is more conducive to sporophyte development than dioiecism (Crum (2001). The results in Chapter 2 show a relationship between sexuality and sporophyte production. However, production of gemmae was shown to be independent of sexuality. The same was shown for British hepatics (Longton, 1997; Laaka-Lindberg, *et al.* 2000). This, as put by Laaka-Lindberg *et al.* (2000), contradicts suggestions that asexual reproduction serves to compensate for uncertain fertilisation due to separation of sexes in dioicous species (Longton and Greene, 1969; Riemann, 1972; Une , 1986; Longton, 1992)

Also shown here is the dominance of dioicous species in the family Lophoziaceae, making up about 90% of the species studied (Chapter 2). The same has been shown in Jungermanniales generally, where monoicous species make 15 – 20% of the species (Schuster, 1983) and in British liverworts (Laaka-Lindberg *et al.*, (2000). The majority of dioicous species (ca 80%) either produce sporophytes rarely or are sterile, which in most cases could be explained by absence of plants of one sex. Most of these plants are therefore forced to reproduce vegetatively (Glime, 2006). Asexual reproduction has been thought to be associated with very little if any genetic variability, and in bryophytes where the dominant state is the haploid (*n*) gametophyte it has been assumed this would be detrimental to their ability to survive rapidly changing habitats. Recent studies (Mishler, 1988; Wyatt, 1994; Newton and Mishler 1994; Shaw and Beer 1999) have, however, shown that levels of genetic variation in bryophytes are at least comparable with those in angiosperms, and that sexual variation might thus be a source of genetic variation in bryophytes. Observations have shown that male and female gametophytes are dimorphic in size, maturation rates, and reproductive output (Shaw and Beer, 1999).

It has been thought that even when sexual reproduction does occur, cross fertilisation may be difficult because of the short distances that sperms are able to travel (Crum 2001). Self fertilisation is, therefore, thought to be common among the bryophytes. However, the identification of a relatively large proportion of recombinant sequences (ca. 9%) in *Homalothecium sericeum* (Hedw.) Br. Eur. (Hedderson and Nowell, 2006) is evidence that sexual reproduction and the production of spores play a significant role in the population biology of some bryophytes species. Although this study does not show the role of sexual reproduction, molecular studies would help explain the exact role and significance of spores in bryophyte reproduction. Being monoicous and gametophyte means that all the gametes of the same sex (either male or female) produced are identical, except only when possible mutations occur. Any self fertilization therefore results in a sporophyte that is homozygous for every trait and thus limits the ability of bryophytes to store recessive alleles. This has also been though to reduce fitness and thus reduce the variation upon which natural selection is expected to act during the process of evolution. However, mechanisms that prevent self-fertilisation have been demonstrated in some bryophytes (Ashton & Cove 1976; Crum 2001). More recent evidence seems to suggest that cross fertilisation might be more common than has always been thought. Cross fertilisation distances may be greater than previously thought due to, for example, microarthropod mediation in sperm transfer (Cronberg *et al.*, 2006).

Asexual propagules are important for maintenance of local populations, because they are larger and generally lack specialised dispersal mechanisms while sexually produced spores mainly help in dispersal to new localities or, since in some species they are known to survive for long periods of time, they provide spore banks that help re-establishment on the same locality following a disturbance. Pohjamo *et al.* (2006) found that in *Anastrophyllum hellerianum*, where spores and gemmae are of the same size, both diaspore types contribute to long distance dispersal. Of importance to the current research is the idea that asexual reproduction is said to require less energy (Longton & Schuster 1983; Newton & Mishler 1994; Pohjamo and Laaka-

Lindberg, 2004), and therefore can still take place under stressful conditions. This would also explain why gemmae did not show a significant trade-off between sizes and numbers.

6.2 Tradeoffs and life history variation

In terms of reproductive trade-offs bryophytes have a choice between producing many small spores, few large spores and producing no spores at all. For those species that never produce spores, it can be assumed that asexual reproduction is the means by which they maintain the population and even spread to new localities. However, it is possible, in those plants that are capable of reproducing both ways, to include production of specialised asexual propagules which may add an extra force to the trade-off patterns. Specialized reproductive structures like gemmae require energy and thus compete with production of sexual structures.

Further interesting trade-offs in bryophytes may include for example 1) expenditure of parental energy *versus* providing offspring energy, and 2) between having many offspring *versus* few. This study shows evidence of a trade-off between spore size and number of spores per capsule at the inter-species level as well as within species and among localities (Chapter 4). It appears to be more strong at the within species level than between species. This may be due to genetic effect but high plasticity of the traits within species may also limit the ability to detect the between species correletions. Trade-off between expansion to new areas and staying on the same locality is also evident (Chapter 2). Species in less stable or short duration habitats produce small spores as an adaptation for dispersal, however they risk a high juvenile mortality rate (During, 1992), which might be catered for by large numbers. Species in stable conditions in contrast would be expected to select more for competitive advantage on the same habitat. This would mean producing large asexual diaspores that establish easily and large spores that can stay long in the diaspore bank. Large diaspores would also give enhanced survival and juvenile growth rates. More of the species in cluster 2 (Chapter 2) do exhibit

these characters. Their big spores would be expected to disperse less hence limiting the species range expansion capability.

No evidence of trade-off between expenditure of parental energy versus providing for the offspring could be assessed from this study. It is known that spore production comes at a great energy cost to the parent plant (Rydin, 1997; Ehrlén *et al.*, 2000; Bisang and Ehrlén, 2002). A negative relationship between such traits as parental gametophyte size and number or size of spores, would be expected. Bisang and Ehrlén (2002) found that *Dicranum polysetum* females invest 16% of their productivity into reproduction when they produced sporophytes, as compared to only 1.3% when perichaetia remained unfertilised.

Still there are some species that do not seem to show some of the expected trade-offs. For example *Lophozia excisa* (chapter 2) produces many large diaspores of both types frequently. This species occupies very stable habits that remain suitable for metabolic activity for longer periods and are likely to have lots of energy for reproduction. Although not the biggest of the species studied, *L. excisa* plants are among the big plants in the family.

If inference can be made from studies on seed size (Westoby and Leishman, 1994; Westoby *et al.*, 2002; Moles and Westoby, 2004), spore size can be viewed as a measure of fitness, where bigger spores are expected to germinate and establish faster and give competitive advantage to the growing plant. This would mean that spore size and percentage of germination would be positively correlated. This study has shown such a relationship for *Lophozia ciliata* and *Lophozia longiflora.* However, if low germination percentages are a result of dormancy (Laaka-Lindberg and Heino, 2001) the percentage of germination might not always be positively related to spore size. For some species small spores might be adapted for dispersal while larger spores are adapted for long storage in the diaspore banks. *L. ventricosa* shows a negative relationship between spore size and proportion of germination; since it produces both spores and gemmae frequently, it might fit the above explanation. In this case local populations would be maintained

by gemmae while small spores function in dispersal. The bigger spores would therefore be functioning in diaspore banks and thus would display more dormancy properties than small spores.

Some issues for further research

The Lophoziaceae is a very large and not well defined family (Chapter 1). Trying to gather and generalise life history information on the family is therefore a very difficult task. However, it also makes it a good study target since it is also likely to show great variation in life strategies. This study, has shed light on some aspects of life histories in some members of the family. It however can be complemented in a number of ways. A number of questions on the reproductive life history aspects of the family which can form areas of further research on the family's life histories, do arise as a result of the outcome from this study.

A key observation emerging from this study is that the production of sporophytes is related to environmental conditions such as water availability i.e. in drought years very few sporophytes are produced while in wet years lots of sporophytes are produced. Future studies could follow specific species colonies over a number of years and observe how they respond to changes in the environment. The variations in the characters could be assessed in relation to real data measurements from the field. In future variations in characters may be assessed in relation to measurements of soil moisture content, mean rainfall, temperature, etc. Plasticity of life characters such as spore size and spore number could also be asses. Given a particular genetic composition, how much variation in the characters would be brought about by varying the environmental conditions? Reciprocal transplants might help answer this, where plants from the same population are grown under different conditions and their difference in the traits of interest is examined. Also of important to establish is the amount genetic variation among the populations that show significant variation in the studied life traits.

It would also be interesting to know if the size of spores or gemmae on a single colony varies depending on whether the colony has produced one or

both types of propagules. This could help understand the nature of trade-off between the different modes of reproduction? In situ studies observing populations or specific colonies of some species over a longer period may stretching a couple of years would also help get information This would give a better idea of how the responses to changes in the environment.

REFERENCES

Aarssen, L. W. and Jordan, C. Y. 2001. Between-species patterns of co variation in plant size, seed size and fecundity in monocarpic herbs. *Ecoscience 8: 471 - 477.*

Anderson, L. E. and Lemmon, B. E. 1974. Gene flow distances in the moss, *Weissia controversa. Journal of the Hattori Botanical Laboratory. 38: 67-90.*

Andersson, K. 2002. Dispersal of spermatozoids from splashcups of the moss *Plagiomnium affine. Lindbergia 27: 90-96.*

Ashton, N. W. and Cove, D. J. 1976. Auxotrophic and developmental mutants of *Physcomitrella patens. Bullitin of British Bryological Society. 27: 10.*

Austrheim, G., Hassel, K. and Mysterud, A. 2005. The role of life history traits for bryophyte community patterns in two contrasting alpine regions. *The Bryologist* 108: 259-271

Bakalin, V. A. 2003. Notes on *Lophozia* II. On *Lphozia rufescens* Schljakov and *Lophozia sudetica* (Huebener) var. *anomala* (Schiljakov) Schiljakov with notes on allied taxa. *Annales Botanici Fennici. 40:47–52*

Bapna, K. R. & Kachroo, P. 2000. Hepaticology in India II. Himanshu Publications, New Delhi.

Baraloto, C., Forget, P. and Goldberg, D. E. 2005. Seed mass, seedling size and neotropical tree seedling establishment. *Journal of Ecology 93: 1156 – 1166.*

Baskin J. M. C. C. Baskin 1989 Physiology of dormancy and germination in relation to seed bank ecology. *In* M. A. Leck, V. T. Parker, and R. L. Simpson [eds.], Ecology of soil seed banks, pp. 53–66. Academic Press, San Diego, California, USA

Bateman, A. J. 1947. Contamination of seed crops III. Relations with isolation distance. *Heredity 1. 303 – 336.*

Bates, J. W. 2000. Mineral nutrition, substratum ecology, and pollution. *In*: Shaw, A. J. and Goffinet, B. (eds): Bryophyte Biology, pp 248-312. Cambridge University Press.

Bednarek-Ochyra, H., Vana, J., Ochyra, R. and Lewis Smith, R. I. 2000.The liverwort flora of Antarctica. Polish Academy of Science, Cracow.

Bell, G. 1989. A comparative method. *American Naturalist 133: 553 -571.*

Benson-Evans, K. 1961. Environmental factors and Bryophytes. *Nature. 191: 255-260*

Bennett, P. M. and Owens, I. P. F. 2002. Evolutionary Ecology of Birds: Life History, Mating System and Extinction. Oxford University Press.

Begon, M., Harper, J. L., and Townsend, C. R. 1996. Ecology: Individuals, populations and communities. Blackwell Scientific Publications, London.

Bisang, I. and Ehrle´n, J. 2002. Reproductive effort and cost of reproduction in female *Dicranum polysetum. Bryologist: 105:384–397.*

Bowker, M. A,, Stark L. R., McLetchie, D. N. and Mishler, B. D. 2000. Sex expression, skewed sex ratios, and microhabitat distribution in the dioecious desert moss *Syntrichia caninervis* (Pottiaceae). *American Journal of Botany* 87: 517–526

Brown, C. A. 2003. Offspring size-number trade-offs in scorpions: an empirical test of the Van Noordwijk and De Jong model. *Evolution 57: 2184–2190*

Cao, T. and Vitt, D. H. 1986. Spore surface structure of *sphagnum. Nova Hedwigia 43: 191 – 220.*

Clarke, G. C. S. 1979. Spore morphology and bryophyte systematics. In Clarke, G. C. S. and Duckett, J. G. (Ed.) Bryophyte Systematics. Systematics Association Special Volume 14: 231-250. Academic Press Incl. London.

Cody, M. 1966. The consistency of intra and inter- continental grassland bird species counts. *American Naturalist 100: 371 – 376.*

Cronberg, N., Natcheva, R. and Hedlund K. 2006. Microarthropods mediate sperm transfer in mosses. *Science 313: 1255.*

Crum, H. A. 1972. The geographic origins of the mosses of North America's eastern deciduous forests. *Journal of the Hattori Botanical Laboratory* 35: 269 – 298.

Crum, H. 2001. Structural diversity of bryophytes. The University of Michigan Herbarium, Ann Arbor

Dalen, L, and Söderström, L. 1999. Survival of moss diaspores in water - an experimental study. *Lindbergia 24: 49–58.*

Damsholt, K. 2002. Illustrated Flora of Nordic Liverworts and hornworts. Nordic Bryological Society. Lund.

Davis, C. E. 2004. A molecular phylogeny of leafy liverworts (Jungermanniidae: Marchantiophyta). *Systematic Botany Missouri Botanical Garden. 98: 61-86.*

Degreef, J. Rocha, O. J. Vanderborght, T. and Baudoin, J. P. 2002. Soil seed bank and seed dormancy in wild populations of lima bean (Fabaceae): considerations for in situ and ex situ conservation. *American Journal of Botany* 89: 1644-1650.

De Roo, R.T., Hedderson, T. A. and Söderström, L. 2007. Molecular insights into the phylogeny of the leafy liverwort family Lophoziaceae Cavers. *Taxon 56: 301-314.*

Duckett, J. G. and Ligrone, R. 1992. A survey of spore liberation mechanisms and germination patterns in mosses. *Journal of Bryology 17: 335 – 354.*

Duckett, J. G. and Renzaglia, K. S. 1993. The reproductive biology of the liverwort *Blasia pusilla* L. *Journal of Bryology 17: 541–552.*

During, H. J. 1973. On the difference between *Physcomitrium pyriforme* and *Funaria fascicularis. Lindbergia 2: 94 – 98.*

During, H. J. 1979. Life History strategies of Bryophytes: a preliminary review. *Lindbergia 5: 2 – 17.*

During H. J. 1992. Ecological classification of Bryophytes and Lichens. In Bates J. W. and Farmer A. M. eds. Bryophytes and Lichens in changing environments pp 1-31. Oxford. Clarendon Press.

During, H. J. 1997. Bryophyte diaspore banks. *Advances in Bryology 6: 103 – 134.*

Dzwonko, Z. and Loster, S. 1992. Species richness and seed dispersal to secondary woods in southern Poland. *Journal of Biogeography. 19: 195 - 204.*

Egunyomi, A. 1978. Comparative culture studies on the spores and gemmae of *Octoblepharum albidum* Hedw. *Journal of the Hattori Botanical Laboratory 44: 25 – 30.*

Ehrlén, J., Bisang, I. and Hedenäs, L. 2000. Costs of sporophyte production in the moss, *Dicranum polysetum. Plant Ecology 249: 207-217.*

Eiken, G. E. and Springer, T. L. 1995. Seed size distribution, germination, and emergence of 6 switchgrass cultivars. *Journal of Range Management 48: 455 – 458.*

Elgar, M. A. 1990. Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos 59:283–287*

Engel, J.J., Braggins, J.E., 1998. The genus *Anastrophyllum* (Spruce) Steph. (Jungermanniales) in Australasia, with a synopsis of austral taxa. In *Austral Hepaticae. 27. pp 371*

Foster, A. S. and Janson C. H. 1985. The relationship between seed size and establishment conditions in Tropical woody plants. *Ecology 66: 773 – 780.*

Frisvoll, A. A. 1982. The status of *Lophozia kiaerii* Jørg. *The bryologist 85: 142-144.*

Frye T. C. & Clark L. 1937. Hepaticae of North America. University of Washington Publications in Biology Volume 6. No 3 pp 337-431

Fulford, M. 1951. Distribution pattern of the genera of leafy Hepaticae of South America. *Evolution 5: 243 – 264.*

Glime, J. M. 2006. Bryophyte Ecology. Volume 1. Physiological Ecology. Published online at http://www.bryoecol.mtu.edu/

Gómez, J M. 2003. Bigger is not always better: Conflicting selective pressures on seed size in *Quercus ilex. Evolution 58: 71 – 80.*

Grime, J. P. 1979. Plant strategies and vegetation process. Wiley, Chichester.

Grolle R. and Long D.G. 2000. An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia. *Journal of Bryology 22: 103–140.*

Hammond, D. S. and Brown, V. K. 1995. Seed size of woody plants in relation to distance, dispersal, soil type in wet neotropical forests. *Ecology 76: 2544 – 2561.*

Hanski, I. 2001. Spatially realistic theory of metapopulation ecology *Naturwissenschaften 88: 372–381*

Harper, J. L. 1957. The ecological significance of dormancy and its importance in weed control. *Proceedings of the 4th International Congress on Crop Protection,* Hamburg *415-420.*

Harper, J. L. 1967. A Darwinian approach to plant ecology. *Journal of Ecology. 55: 242 – 270.*

Hassel, K. 2003. Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum.* PhD thesis, Norwegian University of Science and Technology, Trondheim.

Hedderson T. A. 1992. Rarity at range limits; dispersal capacity and habitat relationships of extraneous moss species in a boreal Canadian National Park. *Biological Conservation 59: 113 – 120.*

Hedderson T. A. and Longton R.E. 1995. Patterns of life history variation in the Funariales, Polytrichales and Pottiales. Journal of Bryology 18: 639 – 675.

Hedderson T. A. and Longton R.E. 1996. Life history variation in mosses: water relations, size and phylogeny. OIKOS 77: 31 – 43.

Hedderson T. A. and Nowell, T. 2006. Phylogeography of *Homalothecium sericeum* (Hedw.) Br. Eur.; toward a reconstruction of glacial survival and postglacial migration. *Journal of Bryology 28: 283-292*

Hendrix, S. D. 1984. Variation in seed weight and its effects on germination in *Pastinaca sativa* L. (Umbelliferae). *American Journal of Bottany. 71*: 795-802

Hendrix. S. D. 1988. Herbivory and its impact on plant reproduction. In Lovett Doust, J. and Lovett Doust, L. (Ed.) Plant reproductive ecology: Patterns and strategies pp. 246-266. Oxford University Press. Oxford.

Hock, Z., Szövényi P. and Zoltán, T. 2004. Seasonal variation in the bryophyte diaspore bank of open grasslands on dolomite rock. *Journal of Bryology 26: 285–292*

Hoffman, G. R. 1970. Spore viability in Funaria hygrometrica. *Bryologist 73: 634 – 635.*

Hohe, A. and Reski, R. 2005. From axenic spore germination to molecular farming: One century of bryophyte in vitro culture. *Plant Cell Reports 8:513 - 21.*

Howe, M. A. and Underwood, L. M. 1903. The genus *Riella* with descriptions of new species from North America and the Canary Islands. *Bulletin of the Torrey Botanical Club 30: 214 – 244.*

Hutchings, J. A. and Morris, D. W. 1985. The influence of phylogeny, size and behaviour patterns of co variation in salmonid life histories. *Oikos 45: 118 – 124.*

Jakobsson, A. and Eriksson, O. 2000. A comparative study of seed number, seed size, seedling size and recruitment in grassland plants. *Oikos 88: 494 - 502.*

Janzen, D. H. 1977. Variation in seed size within a crop of a Costa-Rican *Mucuna andreana* (Leguminosae). *American Journal of Botany 64 : 347 – 349.*

Joenje, W. and During, H. J.1977. Colonisation of a desalinating Wadden-polder by bryophytes. *Vegetatio 35: 177 – 185.*

Johansson, M. E. 1994. Life history differences between central and marginal populations of the clonal aquatic plant *Ranunculus lingua*: A reciprocal transplant experiment. *Oikos 70: 65 – 72.*

Jonsson, B. G. 1993. The bryophyte diaspore bank and its role after small-scale disturbance in a boreal forest. *Journal of Vegetation Science 4: 819–826.*

Jordan, M. A. and Howard, L. S. 2002. Life history trade-off and phenotypic plasticity of the reproduction of Galapagos lizards (Microlophus delanonis)

Kimmerer, R. W. 1991a. Reproductive ecology of *Tetraphis pellucida* I. Population density and reproduction. *Bryologist 94 : 255 – 260.*

Kimmerer, R. W. 1991b. Reproductive ecology of *Tetraphis pellucida* II. Differential success of sexual and asexual propagules. *Bryologist 94 : 284 – 288.*

Krzanowski, W. J. and Marriott, F. H. C. (1994). Multivariate Analysis Part 2: Classification, Covariance Structures and Repeated Measurements. London: Edward Arnold.

Kürschner, H. 2004. Life strategies and adaptations in Bryophytes from the near and Middle east. *Turkish Journal of Botany. 28 : 73 - 84*

Kürschner, H. and Parolly, G. 1999. The *epiPterygio-Riccietum frostii* ass. nov.: Ecology and life strategies of an ephemeral bryophyte community in western Turkey. *Lindbergia 24: 84 – 92.*

Laaka-Lindberg, S. 2000. Substrate preference in *Lophozia Silvocola* (Hepaticopsida). In Southern Finland. *Annales Botanici Fennici 37: 85 – 93.*

Laaka-Lindberg, S. 2000b. Ecology of asexual reproduction in hepatics. PHD. Thesis. Helsinki.

Laaka-Lindberg, S. 2001. Biomass allocation to sexual and asexual reproduction in a leafy hepatic *Lophozia silvicola* Buch. *Journal of Bryology 23: 3 – 8.*

Laaka-Lindberg, S. 2005. Reproductive phenology in leafy hepatic *Lophozia silvicola* Buch in Southern Finland. *Journal of Bryology 27: 253 – 259.*

Laaka-Lindberg, S., Hedderson, T. A. And Longton, R. E. 2000. Rarity and reproductive charactors in the British hepatic flora. *Lindbergia 25: 78 – 84.*

Laaka-Lindberg, S. and Heino, M. 2001. Clonal dynamics and evolution of dormancy in the leafy hepatic Lophozia silvicola. *Oikos 94: 525 – 532.*

Lambrinos J. G. 2004. How interactions between ecology and evolution influence contemporary invasion dynamics. *Ecology 85: 2061-2070*

Lauder, G. V.1982. Historical biology and the problem of design. *Journal of Theoretical biology 97: 57 -67.*

Law, R. 1979. The ecological determinants in the evolution of life histories. In Anderson, R. M., Turner, B. D. and Taylor, L. R. (eds), Population dynamics pp 81-103. Blackwell, Oxford

Le Maitre, D. C. 1998. Life history reproductive ecology of selected Proteaceae in the mountain fynbos vegetation of the south west Cape. PhD. thesis from the Department of Botany University of Cape Town.

Longton, R. E. 1979. Studies on growth, reproduction and population ecology in relation to microclimate in the bipolar moss *Polytrichum alpestre*. *Bryologist 82: 325 – 67.*

Longton, R. E. 1988. Life history strategies among bryophytes of arid regions. *Journal of the Hattori Botanical Laboratory 64: 15 – 28.*

Longton, R. E. 1992. Reproduction and rarity in British mosses. *Biological Conservation 59: 89-95*

Longton, R. E. 1997. Reproductive biology and life history strategies. *Advances in Bryology 6: 65 – 101.*

Longton, R. E. 2006. Reproductive ecology of Bryophytes: what does it tell us about significance of sexual reproduction? *Lindbergia 31: 16 – 23.*

Longton, R. E. and Greene, S. W. 1969. Relationship between sex distribution and sporophyte production in *Pleurozium schreberi* (Brid.) Mitt.. – *Annals of botany, 33*: 107–126.

Longton, R. E. and Miles C. J. 1982. Studies on the reproductive biology mosses. *Journal of the Hattori Botanical Laboratory 52: 219 – 240.*

Longton, R. E. and Schuster, R. M. 1983. Reproductive biology. In Schuster, R. M. (ed.) *New manual of bryology, Volume 1;386 – 462.* Nichinan: Hattori Botanical Laboratory.

Mabry, C. M. 2004. The number and size in common vs restricted woodland herbaceous species in central Iowa, USA. *Oikos 107: 497 – 504.*

MacArthur, R. H. 1962. Some generalised theorems of natural selection. *Proceedings of National Academic Science USA 48: 1893 – 1897.*

Macvicar, S. M. 1926. The student's handbook of British Hepatics. 2nd. Edition. V. V. Sumfield. Eastbourne.

Matlack, G. R. 1994. Plant species migration in a mixed-history forest landscape in eastern North America. *Ecology 75: 1491 - 1502.*

McArthur, R. H. and Wilson, E. O. 1967. Theory of Island biogeography. Princeton University Press, Princeton.

McClymont, J. W. 1954. Spores of music: Their structure and significance in systematic research. Ph.D. thesis, University of Michigan, Ann Arbor.

McLetchie, D. N. 1999. Dormancy/non-dormancy cycles in spores of the liverwort *Sphaerocarpos texanus. Bryologist 102: 15–21.*

Miles, C. J. and Longton, R. E. 1992. Deposition of moss spores in relation to distance from parent gametophytes. *Journal of Bryology 17: 355-365.*

Mishler, B. D. 1988. Reproductive ecology of bryophytes. In Lovett Doust, J. and Lovett Doust, L. (Ed.) Plant reproductive ecology: Patterns and strategies, pp. 284-306. Oxford University Press. Oxford.

Mogensen, G. S. 1978. Spore development and germination in *Cinclidium* (Mniaceae, Bryophyta) with special reference to spore mortality and false anisospory. *Canadian Journal of Botany 56; 1032 – 1060.*

Mogensen, G. S. 1981. The biological significance of morphological characters in bryophytes: The spore. *The Bryologist 84: 187 – 207.*

Moles, A. T. and Westoby, M. 2004. Seedling survival and seed size: A synthesis of the Literature *Journal of Ecology 92:372–383*

Murphy, M. T. 1989. Life history variability in North American breeding flycatchers: phylogeny, size or ecology? *Oikos 54: 3-14.*

Murray, B. R., Thrall, P. H., Gill, A. M. and Nicotra, A. B. 2002. How plant life-history and ecological traits relate to species rarity and commonness at varying spatial scales. *Austral Ecology. 27: 291 - 310.*

Nakosteen, P. C. and Hughes, K. W. 1978. Sexual life cycles of three species of Funariaceae in culture. *The Bryologist 81: 307-314.*

Nehira, K. 1987. Some ecological correlations of spore germination patterns in liverworts. *The Bryologist* 90:405-408.

Nehira, K. 1988. Germination and protonema. *Methods in Bryology* (ed J. M. Glime), pp 113-117. Hattori Botanical Laboratory, Nichinan.

Newton, M. E. 1972. Sex ratio differences in *Mnium hornum* Hedw. Amd M. undulatum Sw. in relation to spore germination and vegetative regeneration. *Annals of Botany 36: 163 - 178.*

Newton, A. E. and Mishler, B. D. 1994: The evolutionary significance of asexual reproduction in mosses. *Journal of the Hattori Botanical Laboratory. 76: 127-145.*

Olesen, P. and Mogensen, G. S. 1978. Ultrastructure, histochemistry and notes on germination stages of spores in selected mosses. *The Bryologist 81: 493-516.*

Parihar, N. S. 1961. *An Introduction to Embryophyta, Volume I.* Allahbad: Central Book Depot.

Parker, G. A. and Begon, M. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. *American Naturalist 128; 573 – 592.*

Partridge, L. and Sibly, R. 1991. Constrains in the evolution of life histories. *Philosophical Transactions of the Royal Society London 332; 3 – 13.*

Paton, J. 1999. The liverwort flora of the British Isles. Harley Books. Essex.

Paton, J. A. & Birks, H. J. B. 1967. *Lophozia perssonii* Buch & S. Arnell - new in Britain

Pohjamo, M. and Laaka-Lindberg, S. 2003. Reproductive modes in the epixylic hepatic *Anastrophyllum hellerianum. Perspectives in Plant Ecology, Evolution and Systematics 6: 159 –168*

Pohjamo, M., Laaka-Lindberg, S., Ovaskainen, O. and Korpelainen, H. 2006. Dispersal potential of spores and asexual propagules in the epixylic hepatic *Anastrophyllum hellerianum. Evolutionary Ecology 20: 415–430.*

Rees, M. 1995. Community structure in sand dune annuals: is seed weight a key quantity? *Journal of Ecology. 83: 857 - 863.*

Renzaglia, K. S. 1978 A comparative morphology and developmental anatomy of the Anthocerotophyta. *Journal of Hattori Botanical Laboratory 44, 31-90.*

Riemann, B. 1972. On the sex-distribution and the occurrence of sporophytes in *Rhytidiadelphus triquetrus* (Hedw.) Warnst. in Scandinavia. *Lindbergia 1: 219–224.*

Rice K. J. 1989. Impacts of seed banks on grassland community structure and population dynamics. *In* M. A. Leck, V. T. Parker, and R. L. Simpson [eds.], Ecology of soil seed banks, pp. 211–230. Academic Press, San Diego, California, USA.

Roff, D. A. 1992. The evolution of life histories; Theory and analysis. Chapman and Hall. London.

Roff, D. A. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *Journal of Evolutionary Biology. 13: 434-445*

Rydin, H. 1997. Competition among bryophytes. *Advances in Bryology 6:135–168.*

Sakai, S. and Sakai, A. 2005. Nature of size-number trade-off: test of the terminal-stream-limitation model for seed production of *Cardiocrium cordatum. Oikos, 105: 105–114.*

Salisbury, S. E. 1974. Seed size in relation to environment. *Proceedings of the Royal Society of London Board on Biological Sciences 186: 83 – 88.*

Sans, F. X. Garcia-Serrano, H. and Afan, I. 2004. Life-history traits of alien and native senecio species in the Mediterranean region. *Acta oecologica 26: 167-178.*

Schaal, B. A. 1980. Reproductive capacity and seed size in *Lupinus texensis. American Journal of Botany, 67*: 703-709.

Schill, D. and Long, D. G. 2003. A revision of *Anastrophyllum* (Spruce) Steph. (Jungermanniales, Lophoziaceae) in the Himalayan region and Western China. *Journal of Hattori Botanical Laboratory 94: 115–157*

Schill, D. B., Long, D. G., Moeller M. and. Squirrell, J. 2004. Phylogenetic relationships between Lophoziaceae and Scapaniaceae based on chloroplast sequences. *Systematic Botany Missouri Botanical Garden. 98: 141-149.*

Schuster, R. M. 1969. The Hepaticae and Anthocerotae of North America. Volume II. Columbia University Press. New York.

Shaw, J. and Beer, S. C. 1999. Life history variation in gametophyte populations of the moss *Ceratodon purpureus* (Ditrichaceae). *American Journal of Botany 86: 512-521.*

Shiv Ram Kashyap. 1923. Liverworts of the Western Himalaysa and the Panjab plain.

Silvertown, J. and Charlesworth, D. 2001. Introduction to plant population biology. 4th Edition. Blackwell Science. Edinbrough.

Simpson, R. L., Leck, M. A. and Parker, V. T. 1989. Seed banks: general concepts and methodological issues. *In* Leck, M. A. Parker, V. T and V. T Simpson [eds.], Ecology of soil seed banks, pp. 3–8. Academic Press, San Diego, California, USA.

Smith, C. C. and Fretwell, S. D. 1974. The optimal Balance between size and number of offspring. *The American Naturalist 108: 499 – 506.*

Sokal, R. R. and Rohlf, F, J. 1995. Biometry. Freeman. New York.

Söderström, L. and Gunnarsson, U. 2003. Life History Strategies. A Catalogue of population Biology Parameters for Bryophytes occurring in North-Western Europe. Manual v. 1.0 — BryoPlanet, Trondheim.

Söderström, L. and Herben, T. 1997. Dynamics of bryophyte metapopulations. *Advances in Bryology 6: 205 – 240.*

Söderström, L and During H. J. 2005. Bryophyte rarity viewed from the perspectives of life history strategy and metapopulation dynamics. *Journal of Bryology 27: 261 – 268.*

Söderström, L., Séneca, 2006. A. World distribution patterns in the Lophoziaceae / Scapaniaceae complex (Hepaticae, Bryophyta). *Journal of Hattori Botanical Laboratory 100: 431-441*

Söderström, L., Séneca, A. and Santos, M. 2007. Rarity patterns in the northern hemisphere members of the Lophoziaceae/Scapaniaceae complex (Hepaticae, Bryophyta). *Biological. Conservation 135: 352-359.*

Sommer, S.and Hommen, U. 2000. Modelling the effects of life-history traits and changing ecological conditions on the population dynamics and persistence of the endangered Malagasy giant jumping rat (*Hypogeomys antimena*). *Animal Conservation 3: 333-343*

Stark, L. R., Mishler, B. D. and McLetchie, D. N. 2000. The cost of realized sexual reproduction: assessing patterns of reproductive allocation and sporophyte abortion in a desert moss. *American Journal of Botany* 87:1599–1608.

Statsoft 2003. Statistica electronic textbook. Statsoft. website www.statsoft.com/textbook/stfacan.html.

Stearns, S. C. 1976. Life history tactics: A review of the ideas. *The Quarterly Review of Biology. 51: 3-47.*

Stearns, S. C. 1977. The evolution of life history traits: A critique of the theory and a review of the data. *Annual Review of Ecology and Systematics 8: 145 - 171*

Stearns, S. C. 1982. On fitness. In: *Environmental Adaptation and Evolution* (Eds D. Mossakowski & G. Roth) pp. 3–17. Gustav Fischer, Stuttgart.

Stearns, S. C. 1989. Trade-offs in Life history evolution. *Functional Ecology 3: 259 – 268.*

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press. Oxford.

Stearns, S.C. 2000. Life history evolution: successes, limitations, and prospects. *Naturwissenschaften 87: 476-486*

Stearns, S. C. and Hoekstra, R.. 2005. Evolution, an introduction, 2nd Edition. Oxford University Press, Oxford.

Stuefer, J. F., Van Hulzen, J. B. and During, H. J. 2002. A genotype trade-off between number and size of clonal offspring in stoloniferous herb *Potentilla reptans. Journal of Evolutionary Biology 15: 880 – 884.*

Sundberg, S. 2000. The ecological significance of sexual reproduction in Peat mosses (*Sphagnum*). - PhD thesis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 581: 1-37.

Sundberg, S. 2002. Sporophyte production and spore dispersal phenology in sphagnum: the importance of summer moisture and patch characteristics. *Canadian Journal of Botany 80: 543 – 556.*

Sundberg, S. and Rydin, H. 1998. Spore number in *Sphagnum* and its dependency on spore and capsule size. *Journal of Bryology 20: 1 – 16.*

Tallis, J. H. 1962. The identification of *Sphagnum* spores. *Transactions of the British Bryological Society 4: 209 – 213.*

Thompson, R. H. 1941. The morphology of *Riella affins* 1. Germination of the spore and development of the thallus. *American Journal of Botany 28: 845 – 855.*

Thompson, K. and Robinowitz, D. 1989. Do big plants have big seeds? *The American Naturalist 133: 722 – 728.*

Une, K. 1986. Sexuality of the Japanese mosses. *Hikobia 9: 339-344.*

van Mölken T., Jorristima-Wieknk, L. D., van Hoek, P. H. W. and de Kroon, H. 2005. Only seed size matters for germination in different populations of the dimorphic *Tragopogon pratensis* subsp. *pratensis* (Asteraceae). *American Journal of Botany 92: 432 – 437.*

Weis, M. I. 1982. The effects of propagule size on germination and seedling growth in *Mirabilis hirsuta*. *Canadian Journal of Botany, 60: 1868-1874.*

Westoby, M., Falster, D. S., Moles, A. T., Vesk, P. A. and Wright, I. J. 2002. Plant ecological strategies: Some leading dimensions of variation between species. *Annual Review of Ecology and Systematics 33: 125 – 159.*

Westoby, M. and Leishman M. R. 1994. Hypotheses on seed size: Testing using semi-arid flora of western New South Wales, Australia. *The American Naturalist 143: 890 – 906.*

Wiklund, K. 2003. Phosphorus concentration and pH in decaying wood affect establishment of the red-listed moss *Buxbaumia viridis*. *Canadian Journal of Botany, 81: 541-549.*

Wiklund, K. and Rydin, H. 2004. Ecophysiological constrains on spore establishment in bryophytes. *Functional Ecology 18 : 907 – 913.*

Wilbur, H.M., Tinkle, D. W. and Collins, J. P. 1974. Environmental certainty, trophic levels and resource availability in life history evolution. *American Naturalist 108: 805 – 817.*

Wilson, M. F. 1983. Plant reproductive ecology. John Wiley & Sons. New York.

Wyatt, R. 1977. Spatial pattern and gamete dispersal distances in. *Atrichum angustatum*, a dioicous moss. *The Bryologist 60:. 284-291.*

Wyatt, R. 1994: Population genetics of bryophytes in relation to their reproductive biology. *Journal of the Hattori Botanical Laboratory.* 76: 147-157.

Yatsentyuk, S. P., N. A. Konstantinova, M. S. Ignatov, J. Hyvönen. & A. V. Troitsky. 2004. On phylogeny of Lophoziaceae and related families (Hepaticae, Jungermanniales), based on *trn*L-*trn*F intron-spacer sequences of chloroplast DNA. *Systematic Botany Missouri Botanical Garden. 98: 243-260.*

Zhang, J. and Maun, M. A. 1990. Seed size variation and its effects on seedling growth in *Agropyron psammophilum*. Botanical Gazette, *151*: 106-113.

APPENDICES

Appendix 1. Species that were used in the multivariate analysis and the values for the five variables.

Cluster	Species	Reproductive mode	Sexuality	Sporophyte Frequency	Spore size (µm)	Gemmae Frequency	Gemma Size(µm^3)	Sources
1	*Lophozia excisa*	gemmae/spores	paroicous	frequent	16	frequent	48581.7	**1;2;3;5;6;7;8**
2	*Lophozia longidens*	gemmae/spores	diocious	very rare	13	always present	27746.6	**1;2;7;8**
2	*Lophozia sudetica*	gemmae/spores	dioicous	very rare	12	frequent	12158.1	**1;2;5;8;17**
2	*Lophozia ciliata*	gemmae	dioicous	not seen	13	frequent	11837.5	**1**
2	*Barbilophozia hatcheri*	gemmae/spores	dioicous	very rare	13.75	frequent	11219.7	**1;2;3;5;6;7;8**
2	*Lophozia perssonii*	gemmae/spores	dioicous	very rare	16	always present	6953.4	**1;2;19**
2	*Barbilophozia attenuata*	gemmae/spores	dioicous	very rare	13	frequent	13307.8	**1;2;3;7;8**
3	*Lophozia ventricosa*	gemmae/spores	dioicous	occasional	14	frequent	19653.9	**1;2;3;7;8**
3	*Lophozia incisa*	gemmae/spores	dioicous	occasional	13	frequent	31466.3	**1;2;5;7;8;11**
3	*Lophozia bicrenata*	gemmae/spores	paroicous	frequent	13	frequent	22787.1	**1;2;3;7;8**
3	*Tritomaria scitula*	gemmae/spore	dioicous	rare	15	frequent	14719.4	**1;3**
3	*Lophozia capitata*	gemmae/spores	dioicous	rare	14.5	occasional	17473.6	**1;2;3;7;15**
3	*Lophozia ascendens*	gemmae/spores	dioicous	rare	13.5	frequent	5510.9	**1;7**
3	*Anastrophyllum assimile*	gemmae/spores	dioicous	rare	13	rare	19905.2	**1;3;7;9**
4	*Barbilophozia floerkei*	gemmae/spores	dioicous	very rare	12	very rare	3380.6	**1;2;3;7;8**
4	*Barbilophozia lycopodioides*	gemmae/spores	dioicous	very rare	13	rare	3380.6	**1;2;3;7;8**
4	*Barbilophozia kunzeana*	gemmae/spores	dioicous	very rare	12	rare	5259.1	**1;2;3;7;8**
4	*Barbilophozia binsteadii*	gemmae/spores	dioicous	very rare	12	rare	12751.5	**1;3;7**
4	*Lophozia obtusa*	gemmae/spores	dioicous	rare	12.5	very rare	9001.7	**1;2;3;7;8**
4	*Tritomaria polita*	gemmae/spores	dioicous	rare	15	rare	7588.1	**1;3;7;8**
4	*Leiocolea collaris*	gemmae/spores	dioicous	rare	12.5	rare	7242.4	**1;3;7;8;11**
4	*Tetralophozia setiformis*	spores	dioicous	rare	14	lacking	16411.7	**1;2;7**
4	*Anastrophyllum sphenoloboides*	gemmae/spores	heteroicous	rare	13.5	rare	6382.2	**1;3**
4	*Tritomaria quinquedentata*	gemmae/spores	dioicous	frequent	13.5	rare	4974.2	**1;3;8**
4	*Anastrophyllum joergensenii*	spores	dioicous	occasional	12	lacking	1494.1	**1;9**
5	*Lophozia longiflora*	gemmae/spores	dioicous	frequent	9	rare	9655.2	**1;2;3;7**
5	*Anastrophyllum hellerianum*	gemmae/spores	dioicous	frequent	10.5	always	2343.1	**1;3;7;8;9**
6	*Lophozia wenzelii*	gemmae/spores	dioicous	rare	10	always	12465.9	**1;3;7;8**
6	*Tritomaria exsectiformis*	gemmae/spores	dioicous	very rare	10.5	frequent	6861.3	**1;3;7;8**
6	*Leiocolea heterocolpos*	gemmae/spores	dioicous	rare	12	always	5929.8	**1;3;7;8**
6	*Tritomaria exsecta*	gemmae/spores	dioicous	never	10.5	common	2315.4	**1;3;7;8**
6	*Anastrepta orcadensis*	gemmae/spores	dioicous	never	10	common	13439.8	**1;2;7**

Sources; **1** = Damsholt, 2002; **2** = Paton, 1999; **3** = Schuster, 1969; **5** = Bapna, & Kachroo, 2000; **6** = Bednarek-Ochyra *et al.*, 2000; **7** = Frye & Clark, 1937; **8** = Macvicar, 1926; **9** = Schill & Long, 2003; **11** = Shiv Ram Kashyap, 1923; **15** = Frisvoll, 1982; **17** = Bakalin, 2003; **19** = Paton & Birks, 1967.

Appendix 2. Summary of spore production and spore sizes for different localities.

Species	Locality	Spores per capsule				Spore sizes			
		Min	Max	Mean	SD	Min	Max	Mean	SD
B. barbata	Tavastia australis, Kotinen	95746.4	152169.5	121706.6	28241.48	14.58	19.44	16.038	0.3712
B. quadriloba	TorneLappmark, Låktatjåkka	20632.6	108281.9	20632.6	30554.82	13	19.5	15.119	1.1425
L. bandriensis	Sør-Trøndelag, Oppdal, Unndalen	39145.6	99269.3	99269.3	28868.44	14.3	21.87	17.197	0.8771
L. collaris	TorneLappmark, Låktatjåkka	53040.4	53040.4	53040.4		11.7	14.3	13.52	
L. heterocolpos	Sør-Trøndelag, Trondheim Estenstadmarka	75441.7	203677	203677	76575.65	10.4	15.6	12.675	0.9007
L. bicranata	Tavastia australis, Kotinen	26950	123218.4	65162.8	33363.51	12.15	19.44	13.815	0.7291
L. ciliata	Ångermanland, Vändåtberget	7825.8	123399.1	57685	30264.92	10.4	16.9	13.196	0.6613
L. ciliata	Åsele lappmark, Låitavare	5066.8	26944.6	5066.8	12283.93	10.4	15.6	12.068	2.16
L. ciliata	Åsele lappmark, Rödberget	37827.2	94423.8	94423.8	28483.63	9.1	11.7	10.898	0.1876
L. ciliata	Sør-Trøndelag, Meldal, Urvatnet	4631.7	34758.9	6654.5	7851.919	11.7	15.6	13.536	0.4663
L. excisa	Sør-Trøndelag, Oppdal, Unndalen	10805.4	23929.4	23929.4	5977.585	13	19.5	15.616	0.7661
L. grandirietis	TorneLappmark, Låktatjåkka	24108	111564.8	70765.65	38591.35	10.4	14.3	12.188	0.2883
L. longiflora	Ångermanland, Vändåtberget	24893	261660.7	101090.3	48650.86	10.4	14.3	12.783	0.4275
L. longiflora	Åsele lappmark, Låitavare	52191.4	189862.5	86924.22	29620.31	10.4	18.2	13.075	0.846
L. longiflora	Åsele lappmark, Rödberget	52820	114750.5	75290.53	22177.26	11.7	15.6	13.759	0.4024
L. sudetica	Sør-Trøndelag, Oppdal, Unndalen	45053.3	145843	83097.56	32972.81	10.935	17.01	14.131	0.936
L. ventricosa	Ångermanland, Vändåtberget	26736.9	152023	74300.65	30827.39	10.4	19.5	15.85	1.1356
L. ventricosa	Åsele lappmark, Låitavare	6133.3	68955.3	31734.61	20162.33	11.7	19.5	16.123	1.8967
L. ventricosa	Sør-Trøndelag, Trondheim Lidarende	32376.3	135439	61913.88	42650.86	11.7	15.6	13.834	0.2658
L. ventricosa	Sør-Trøndelag, Oppdal, Unndalen	49156.9	124611.3	81907.13	28241.48	13	16.9	14.576	0.5651

Appendix 3. Summary of gemma production and gemma sizes for different localities.

Species	Locality	No. gemmae per shoot				Gemmae size (area) μ			
		Mean	Min	Max	SD	Mean	Min	Max	SD
B. hatcheri	Lérida, Bonaigua Pass Spain	3369.62	587	9542	2466.31	364.1	220	558	22.47
B. hatcheri	Lézidan, Àren, Vall Femera, Spain	2536.63	481	5441	1471.04	319.9	143	473	44.42
L. longidens	Sør-Trøndelag, Meldal, Urvatnet	1522.88	679	3981	881.799	666.6	373	1217	66.16
L. longidens	Sør-Trøndelag, Trondheim, Gråkallen	1585.39	612	2830	602.614	634.6	380	1014	55.97
L. longidens	Sør-Trøndelag, Trondheim, Lidarende	1145.27	289	2347	641.282	513.7	379	958	33.24
L. ventricosa	Huesta, benasque, valley of Vallirierna, Spain	1788.54	261	3847	1269.66	332.7	203	521	26.1
L. ventricosa	Sør-Trøndelag, Meldal, Urvatnet	2399.03	353	5140	1500.09	416.9	242	634	45.58
L. ventricosa	Sør-Trøndelag, Trondheim, Gråkallen	1457.73	359	5421	1190.56	421.1	284	811	42.97