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MAINTENANCE OF SEXUALLY DIMORPHIC PATTERNS OF GROWTH AND REPRODUCTION IN MARCHANTIA INFLEXA

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ABSTRACT OF DISSERTATION

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A dissertation submitted in partial fulfillment
of the requirements for the degree of Doctor of Philosophy in the
College of Arts and Sciences
at the University of Kentucky

By
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Lexington, Kentucky

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MAINTENANCE OF SEXUALLY DIMORPHIC PATTERNS OF GROWTH AND REPRODUCTION IN *MARCHANTIA INFLEXA*

Sexual dimorphism in life history traits may influence the distribution of the sexes, population sex ratios, the maintenance of sex in populations, and the evolutionary potential of a species. In bryophytes, sexual dimorphism in traits related to growth and reproduction may be responsible for female-biased population sex ratios and a lack of sexual reproduction. I examined the roles of natural selection in maintaining sexual dimorphism in the context of impacts on bryophyte population sex ratios, using *Marchantia inflexa* as a model system. My studies included an assessment of among-population variation in habitat use by the sexes, comparison of phenotypes between single-sex and both-sex populations, a field study of natural selection, and a comparison of the influence of selection on asexual and sexual fitness components.

The sexes of *M. inflexa* were sexually dimorphic in investment in growth, asexual and sexual reproduction. The sexes were spatially separated in populations, but the sexes overlapped in habitat use. Populations differed in growth, asexual reproduction rates, degrees of sexual dimorphism, and strength of among-trait correlations. Plants from single-sex and both-sex populations differed in investment in growth and asexual reproduction, but the two population types showed the same degree of sexual dimorphism. Thus, local environment may be more influential than the presence of the opposite sex in maintaining sexual dimorphism.

Selection on sexually dimorphic traits was both sex-specific and environmentally dependent. Between-sex correlations were not significant in the greenhouse but were

significant in the field thus, evolution and expression of sexual dimorphism in nature may be constrained by among-trait and between-sex correlations. Additionally, females incurred a cost of plasticity that males did not. Because there was a negative trade-off between sexual and asexual fitness, overall lifetime selection may result in a different picture of how the sexes experience selection. The combination of sex-specific and environment-dependent selection, and sex-specific costs to plasticity may not only maintain sexually dimorphic traits but also ensure the persistence of both sexes in a population.

KEYWORDS: Bryophyte, sexual dimorphism, selection, plasticity, liverwort

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March 6, 2004

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Chapter One: Sexual dimorphism in liverworts

Introduction

Sexual dimorphism in life history traits may influence the distribution of the sexes, population sex ratios, the maintenance of sex in populations, and the evolutionary potential of a species. An understanding of the maintenance of sexual dimorphism in a species provides insight into the biology, population structure and evolution of the species. The work I present in this dissertation focuses on the influence of sex-specific and environment-dependent selection in maintaining sexual dimorphism in a dioicous bryophyte. In bryophytes, the maintenance of sexually dimorphic traits may be particularly important because sexual dimorphism in growth and reproductive traits may influence sex ratios (Crowley and McLetchie, 2002; McLetchie, Garcia-Ramos, and Crowley, 2002), and biased sex ratios can lead to a lack of sexual reproduction. Thus, I examine factors that influence the maintenance of sexual dimorphism in the context of impacts on bryophyte population sex ratios and the persistence of both sexes in populations.

Sexual dimorphism may be defined in a variety of ways, but here sexual dimorphism refers to differences between two classes of plant (the sexes of a species) in secondary sexual characteristics. Lloyd and Webb (1977) define secondary sexual differences in plants as differences in any characters other than the "primary sex organs" the gynoecia and androecia (gamete producing organs of females and males). Dioecious seed plants can be sexually dimorphic in secondary sex characteristics including: life history traits, vegetative morphology, physiology, longevity of vegetative and reproductive organs, resource acquisition and allocation, competitive ability, and susceptibility to herbivores and pathogens (Delph and Meagher, 1995; Dawson and Geber, 1999; Delph, 1999; Eckert, Dorken, and Mitchell, 1999; Geber, Dawson, and Delph, 1999; Delph, Knapczyk, and Taylor, 2002).

Most studies of sexual dimorphism in plants focus on non-clonal angiosperms, whereas, bryophytes are nearly absent from the literature on sexual dimorphism. In this introductory chapter, I provide a brief overview of background information pertinent to the examination of the maintenance of sexual dimorphism in bryophytes and results of a review of sexually dimorphic characters in liverworts. I do not review literature on the theories of evolution and maintenance of sexual dimorphism because there is a current and in-depth review for angiosperms available

(Geber, 1995; Geber, Dawson, and Delph, 1999). I do relate these theories to the maintenance of sexual dimorphism in bryophytes (Chapters 4,5 & 6).

Maintenance of sexual dimorphism

Principal processes leading to the evolution and maintenance of sexual dimorphism are natural and sexual selection (Geber, 1995). Natural selection arises when character variation is related to variation in survival and/or fertility whereas, sexual selection arises when trait variation is related to mating success (reviewed in Geber, 1999). The maintenance of sexual dimorphism through natural selection has gained the most empirical support (Geber, 1999), but neither process has been applied to understanding the maintenance of sexual dimorphism in bryophytes.

Sexually dimorphic characters may evolve through natural selection when selection favors different phenotypic optima for the sexes, i.e., when selection is sex-specific. Sex-specific natural selection may occur because the sexes inhabit different microhabitats and are exposed to different selection pressures, but may also occur when the sexes share habitats. In both cases, sexual dimorphism is hypothesized to occur because of differences between the sexes in the costs of sexual reproduction (Geber, 1995).

In cases where the sexes do not overlap in habitat use, typically, the sex that incurs the higher cost of sexual reproduction, usually females, experiences higher mortality in more stressful habitats (Lloyd and Webb, 1977; Charnov, 1982a; Bierzychudek and Eckhart, 1988). Thus, the sexes "specialize" on different habitats, ones that confer the greatest fitness advantages (Charnov, 1982a; Charnov, 1982b; Bierzychudek and Eckhart, 1988), as a result of sex-differential mortality (for examples see Lloyd and Webb, 1977). Sex-specific microhabitat specialization may result in the spatial segregation of the sexes, correlation of sex ratios with an environmental gradient (Bierzychudek and Eckhart, 1988), and maintenance of sexually dimorphic vegetative and reproductive characters in a population (Lloyd and Webb, 1977; Kohorn, 1994; Dawson and Geber, 1999; Delph, 1999). For example, in wind-pollinated dioecious seed plants, males may exhibit higher survival rates in open areas that are beneficial for pollen dispersal whereas, females have lower mortality in protected areas that optimize fruit development and maturation (Dawson and Bliss, 1989; Bertiller et al., 2002). In plants that rely on abiotic factors such as wind for pollination, spatial segregation of the sexes does not impede

fertilization success, but for plants that require close proximity of males and females for successful fertilization, (e.g., bryophytes), spatial segregation may result in low levels or absence of sexual reproduction in a population. Few studies present empirical evidence of microhabitat specialization in the sexes resulting in spatial segregation along an environmental gradient (Charnov, 1982a; Charnov, 1982b; Bierzychudek and Eckhart, 1988). I investigated spatial segregation of the sexes and sex-specific habitat use by a thallose liverwort to determine if habitat use, and selection in different habitats, was important to the maintenance of sexual dimorphism (Chapter 2).

When the sexes share habitats, the maintenance of sexual dimorphism is closely tied to the sex-specific costs of sexual reproduction. However, in clonal plants that can persist through asexual reproduction, life history trade-offs, especially between asexual and sexual fitness, will influence the outcome of selection. Sexual dimorphism in growth, asexual and sexual reproduction in clonal plants may result from the combination of sex-specific selection on these traits and on relationships or trade-offs among the traits. I examined sex-specific selection on both sexual and asexual components of fitness to assess the importance of natural selection to the maintenance of sexual dimorphism in bryophytes.

Sexual selection as a mechanism of the evolution of sexual dimorphism was originally proposed by Darwin (Darwin, 1871) and included both intrasexual and intersexual components. The study of sexual selection in plants has been rife with controversy over how to define sexual selection as it applies to plants, but still in keeping with principles described by Darwin (1871). Arnold (1994) reviews definitions and views of sexual selection and proposes that sexual selection be defined very generally as selection "that arises from differences in mating success (among individuals) measured as the number of mates that bear or sire progeny over some specified period of time." The definition is neither gender specific nor does it invoke competition, which is difficult to demonstrate empirically. Mating success is not equivalent to fecundity or fecundity selection (Arnold, 1994) and requires the identification of both progeny and parents. However, it may be difficult to distinguish natural and sexual selection based on this definition because mating success may be determined by many traits not involved in interaction with mates or among plants of the same sex (Andersson, 1994). An alternative view of sexual selection in plants argues that competition over mates is the cornerstone of the definition

(Andersson, 1994). Trait variation must lead to variation in pollination and fertilization success as a result of competition among rivals.

Application sexual selection theory to maintenance of sexually dimorphic traits in bryophytes has not been investigated, but has the potential to explain sex-specific life history strategies observed in a number of liverwort taxa. In particular, in liverworts, sexual dimorphism in asexual propagule production may be a mechanism by which males compete among themselves for access to mates. To test this empirically would require demonstrating a positive relationship between variation in asexual propagule production and mating success, and intrasexual competition. In a review of literature presented in this section, I explored the potential for sexual selection to explain patterns of dimorphism in liverworts.

Costs of sexual reproduction

As stated above, hypotheses invoking the maintenance of sexually dimorphic traits via natural selection depend on sex-specific differences in reproductive ecology. In most organisms, females are expected to incur a higher cost of reproduction because they must support an embryo and bear the costs of producing and dispersing propagules (Bateman, 1948; Charnov, 1982a). In dioecious angiosperms, males incur a lower cost of reproduction than females and inhabit more stressful environments whereas, females have a greater cost of sexual reproduction and live in more nutrient rich or comparatively benign habitats (Freeman, Klikoff, and Harper, 1976; Cox, 1981; Bierzychudek and Eckhart, 1988; Meagher, 1988; Dawson and Ehleringer, 1993; Ramadan et al., 1994). Allocation of resources to sexual reproduction differ for the sexes and throughout the sexual season (reproductive bout) of adult plants. For example, in angiosperms, male reproductive effort peaks early in the reproductive season and female reproductive effort peaks later, when females must allocate energy to seeds and fruits (Wolfe and Schmida, 1997).

In dioicous bryophytes, the occurrence of sporophytes (produced only by successful sexual reproduction) is low (Rohrer, 1982; Stark and Castetter, 1987), and in some species, sporophyte abortions early in development are common (Stark, Mishler, and McLetchie, 2000). Therefore, most female plants in a population do not expend resources on sexual reproduction beyond the production of gametes. Consequently, the realized cost of sexual reproduction may be higher for male than female bryophytes (Stark, Mishler, and McLetchie, 2000), i.e., antheridiophores may be more costly to produce than archegoniophores. Antheridia take longer

to mature (Lackner, 1939) than archegonia, and males produce fewer gametophores but more gametes, than females (Fuselier and McLetchie, 2002; Voth and Hamner, 1940), indicating a higher cost of gamete production (and realized cost of sexual reproduction) for males (Stark et al., 2000). Costs of sexual reproduction are tied to physiological trade-offs, and if these trade-offs differ between the sexes, trade-offs between reproductive modes may be the key to understanding the maintenance of sexual dimorphism in bryophytes.

Trade-offs

The sex that exhibits the greater sexual reproductive investment should also incur higher somatic and demographic costs (Obeso, 2002). In other words, investment in sexual reproduction is hypothesized to trade-off with investment in other processes (Stearns, 1992). Trade-offs play a central role in life history evolution because they may constrain the simultaneous evolution of the traits involved (Stearns, 1992), and, because trade-offs alter the balance between fecundity and survival, they affect the fitness of the individual (Sutherland and Vickery, 1988). Trade-offs between sexual reproduction and vegetative propagation are well documented in plant literature (Sohn and Policansky, 1977; Sutherland and Vickery, 1988; Snow and Whigham, 1989; Méndez and Obeso, 1993; Worley and Harder, 1996; Prati and Schmid, 2000; Ronsheim and Bever., 2000). In bryophytes, trade-offs between asexual reproduction and growth (McLetchie and Puterbaugh, 2000), and sexual and asexual reproduction (Laaka-Lindberg, 2001; Fuselier and McLetchie, 2002) have been detected. Trade-offs are important to the maintenance of sexual reproduction in populations because increased investment in clonal growth results in reduced sexual reproduction, and the longer the clonal growth period, the less likely that sexual reproduction will occur (Klekowski, 1997). I document a trade-off between sexual and asexual fitness in a liverwort, and discuss the consequences of this trade-off, combined with sex-specific life history strategies, in relation to the impacts of sexual dimorphism on population sex ratios (Chapter 6).

Constraints to the expression of sexual dimorphism

The degree to which sexual dimorphism is expressed will reflect a balance among constraining factors (Lande, 1980). Expression of dimorphic characters is constrained by: 1) the amount of genetic variation in dimorphic traits present in a population, 2) correlations among

traits within a sex, and 3) between-sex genetic correlations that can prevent or slow the evolution of dimorphism, (Lande, 1980; Lande and Arnold, 1983; Meagher, 1994; Delph, Knapczyk, and Taylor, 2002). Because between-sex and among-trait correlations constrain the evolution of sexual dimorphism, Lande (1980) predicted that trait means will evolve faster than sexual dimorphism across populations. However, because these correlations may change among environments (Lyons, Miller, and Meagher, 1994; Galen, 2000; Pigliucci and Kolodynska, 2002), sexual dimorphism can vary among populations. Thus, examination of among-population genetic architecture is also important in understanding the evolution and maintenance of sexually dimorphic traits (Delph, Knapczyk, and Taylor, 2002). I estimated constraints to the expression of sexual dimorphism among populations of a liverwort to identify factors important to the evolution of sexual dimorphism in bryophytes (Chapter 3).

Bryophytes

Bryophytes are non-vascular, spore producing plants that pre-date polysporangiophytes in evolutionary history and have a haploid-dominant life cycle rather than a diploid-dominant life cycle ((Schuster, 1969; Schofield, 1985). This difference in life cycles (Figure 1.1) is important evolutionarily and ecologically. The literature reviewed above on the maintenance of sexual dimorphism is entirely based on plants with diploid-dominant life cycles, primarily angiosperms. In bryophytes, the gametophyte is the "green plant" that is seen in the field and, in my experiments, examined in terms of exposure to selection. Whereas, in angiosperms (for example), hypotheses of the maintenance of sexual dimorphism via selection come from models based the diploid "green plant" exposed to selection. In bryophytes, mutations are not masked by dominant alleles as in a diploid organism, thus, population genetic structure of bryophytes may differ dramatically from angiosperms. In particular, mutations will be immediately exposed to selection and detrimental mutations will not persist in bryophyte populations through dominance masking. Whether these differences between haploid and diploid-dominant life cycles influences the maintenance of sexual dimorphism via selection has not been thoroughly examined.

Bryophytes comprise a non-monophyletic group that includes mosses, liverworts and hornworts. The placement of hornworts and the relationship of the bryophyte groups to polysporangiophytes are equivocal though, recent analyses converge on a phylogenetic

hypothesis where liverworts and mosses are sister groups (Goffinet, 2000). Bryophytes reproduce both clonally and sexually, and provide an excellent study system in which to examine the forces that maintain sexually dimorphic characters. Female-biased population sex ratios are common in bryophyte populations and spatial segregation of the sexes has been implicated as the cause of the low rates of sexual reproduction observed in bryophyte populations (Gemmel, 1950; Longton and Schuster, 1983; Fuselier and McLetchie, 2002). Many bryophytes exist in single-sex populations that are maintained solely via asexual reproduction. In the case of the study organism I use, numerous single-sex and 2-sex populations provide a natural experiment in which to examine how selection maintains sexually dimorphic traits.

Mating systems

Two basic mating systems in bryophytes are dioicy and monicy; dioicous taxa have sexes on separate plants whereas, monicious taxa have sexes on the same plant. Within dioecy and monicy there is a wide array of mating system variation. For example, dioicous systems include phyllodioicy, where male plants are dwarfed and epiphytic on females, and monicious taxa range from having both sexes on the same inflorescence to males and females on different stems connected by rhizoids (Shaw, 2000). I will limit my delineation of mating systems to dioecy and monecy (usually autoicous). Note that dioicy in bryophytes and dioecy in angiosperms are not homologous traits, hence the use of dioicous in reference to unisexual gametophytes of bryophytes versus dioecious in reference to unisexual sporophytes of seed plants (Shaw, 2000 and references therein). Thus, comparisons of bryophytes to polysporangiophytes will be kept to a minimum. Within my dissertation, I use the term “dioecious” for both bryophytes and angiosperms for consistency, and because I am not explicitly examining the evolution of dioicy.

Sex ratios

My research on the maintenance of sexual dimorphism in liverworts is based on the fact that bryophytes often have biased sex ratios, and differences between the sexes maintain sex ratio bias. Female-biased sex ratios occur so often in bryophytes that they are considered a general characteristic of dioicous species (Longton, 1990; Wyatt, 1994; Shaw, 2000; Stark, Mishler, and McLetchie, 2000; Bowker et al., 2000). Female-biased adult sex ratios in bryophytes may result from habitat specializations of the sexes (Cameron and Wyatt, 1990), greater clonal expansion ability of one sex compared to the other (Wyatt, 1977; McLetchie and Puterbaugh, 2000; Crowley and McLetchie, 2002), differential costs of reproduction between the sexes (Stark,

Mishler, and McLetchie, 2000), and/or lack of sex expression on the part of males (Newton, 1971). Sexually dimorphic traits related to growth and reproduction have been proposed as an explanation for female-biased adult population sex ratios observed in many bryophytes. Thus, sex-specific and environment-dependent selection may be important in determining population sex ratios as well as the frequency of sexual reproduction in populations of clonal plants.

Liverworts

Because my research focuses on the maintenance of sexual dimorphism in a thallose liverwort, I will focus my discussions on liverworts (Marchantiophyta), and leave reviews of sexual dimorphism in the remainder of the Bryophyta for further studies. Liverworts comprise a monophyletic group of two classes, Marchantiales and Jungermanniales, the thallose and leafy liverworts, respectively. Relationships among the Marchantiales are better known than among the Jungermanniales primarily because of the wealth of tropical Jungermanniales that remain undescribed (Shaw, 2000; Crum, 2001). Monecy and dioecy are found in all groups in all major lineages within the order Marchantiales (Bischler, 1998).

Approximately 68% of the 3000-5000 species of liverworts are dioicous (Crum, 2001) whereas, only about 6% of the world's angiosperms are dioecious (reviewed in Geber, Dawson and Delph, 1999). In Marchantiales 56.4 % of taxa are monicious whereas in Jungermanniales 28-36% are monicious. Sex determination in liverworts occurs via "sex chromosomes"; sex-specific chromosomes that differ in size or heterochromatin content (Allen, 1919; Ramsay and Berrie, 1982). Sometimes the sexes have different numbers of chromosomes, for example, in *Frullania raddi*, the female has 9 and the male, 8 chromosomes (Schuster, 1969; Smith, 1990). Recent research indicates sex chromosome-specific genetic sequence (Fujisawa et al., 2003) and genes on the male sex chromosome specific to male sexual reproductive function (Ishizaki et al., 2002).

Evolution of sexual dimorphism among liverworts

There is no exhaustive phylogenetic study of the evolution of sexual dimorphism among liverworts. The earliest ancestor of the liverwort clade is hypothesized to have been dioicous and monomorphic (Schuster, 1969; Crandall-Stotler, 1981; Crandall-Stotler and Stotler, 2000). Character state changes on a proposed phylogeny of liverworts proceed from dioicy without sexual dimorphism (hypothetical ancestor to liverworts) to dioicous and sexually dimorphic (Crandall-Stotler and Stotler, 2000). Numerous groups of liverworts are monicious and some

that are dioicous are not sexually dimorphic. Thus, it appears that sexual dimorphism in liverworts may have evolved numerous times (Crandall-Stotler and Stotler, 2000). The best way to quantify differences between sexes is to grow plants in a common garden and measure traits. Unfortunately, few such common garden studies have been performed, and information on sexual dimorphism in most species is lacking. More data on sexual dimorphism and additional phylogenetic studies are needed to examine hypotheses of the evolution of sexual dimorphism among the Marchantiophyta.

Sexually dimorphic traits in liverworts

I conducted a review of literature on sexual dimorphism in dioicous liverworts in search of taxa with secondary sex characters described as sexually dimorphic. My view of sexually dimorphic traits differs from that typically presented for angiosperms in that I did not include the bryophyte equivalents of features of flowers and inflorescences. Androecial and gynoecial branches may be considered secondary sex structures, but I did not include differences between the sexes in these structures in my review. Androecial branches are specialized branches bearing antheridia (male sex organs) and gynoecial branches are specialized branches bearing gynoecia (female sex organs), and associated paraphyses and modified leaves. Schuster (1969) provides details on differences in ontogeny of female and male sex structures. Archegonia, unlike antheridia, are commonly surrounded by modified leaves that may form a sheath (perianth) around the archegonia, and hairs that may prolong water retention (Schuster, 1969). Only in extreme cases do archegonia occur naked (Schuster, 1969), and although shape, size and composition (hairs, paraphyses, etc.) of the structures associated with archegonia vary, the occurrence of "protective" structures surrounding the archegonia is a common feature among liverwort groups. Thus, the difference between "naked" antheridia and "protected" archegonia were not considered among the secondary sex characters reviewed here.

I reviewed primary and secondary sources for accounts of sexual dimorphism in dioicous liverwort taxa only. Primary literature sources on topics including, species descriptions, sex ratios and bryophyte ecology often did not include information on sexual dimorphism. Secondary sources, such as books of regional taxonomic accounts provided more descriptions of sexually dimorphic characters. One of the most useful sources was a taxonomic treatment (So, 2001) that documented morphology of the sexes of 80 species of *Plagiochila* in China, and

included sexually dimorphic characters as well as aspects of species ecology seldom included in primary species descriptions. Unfortunately, most accounts of sexual dimorphism were anecdotal and rarely come from controlled experiments where dimorphic characters are quantified. In some cases, the mating system of the species was not known or unmentioned. I included these taxa in my summaries since there were few that fit this category (Table 1.2). Additionally, there may be differences in investment in female and male structures on monecious liverworts. Thus, any conclusions from this compilation of data are examined with this consideration in mind. I do not list sources that I examined but that yielded no accounts of sexual dimorphism. So, while my review is non-exhaustive, it does encompass more literature than listed herein.

I categorized sexually dimorphic characters into three categories: morphological, physiological and life history (Table 1.1). I included an "unknown" category for taxa where the accounts did not describe the sexually dimorphic characters. Within the three main categories I distinguished various traits and discussed their possible ecological or evolutionary significance. The "morphological" category included leaf morphology, subfloral innovations, the presence or absence of structures such as hairs or teeth on leaves, branching patterns, stature shape, and size. The "physiology" category included differences between the sexes in oil body characters and color or pigmentation. The "life history" category included differences in asexual reproduction, sexual reproduction, longevity, survival and spore germination. The "unknown" category included those species that were considered sexually dimorphic, but the characters that differed between the sexes were not given. These taxa were not included in any other trait category. The categories overlapped and were not mutually exclusive (taxa may appear in more than one category), but provided a framework from which to begin examination of the types of characters important to the evolution of sexual dimorphism in liverworts.

Sexually dimorphic characters

There were 34 genera and 99 species described as sexually dimorphic in some trait. Patterns that emerged from this compilation are indicative of sexual size dimorphism where females are the larger sex, males are small and often more slender than females, and males are more likely to have reddish pigmentation, more branches and higher investment in asexual reproduction than females (Table 1.1). Only 6 of the accounts of sexually dimorphic traits come

from primary literature detailing experiments with taxa grown in culture or under observation (Table 1.2). Most accounts are from taxonomic descriptions given in secondary literature. I will review results for each of the three main categories and address possible ecological and/or evolutionary implications of sexual dimorphism associated with the traits. It is tempting to formulate adaptive stories to explain these sexually dimorphic traits but, there is little evidence to support such explanations, and not all sexually dimorphic traits are adaptive (Geber, 1995). Sexual dimorphism in size, asexual/sexual reproduction and pigmentation, have emerged in literature or are related to hypotheses regarding patterns of bryophyte sexual dimorphism and sex ratios, and will be considered here in more detail.

Morphological differences

Morphological differences included structural, branching, stature, shape, size, and. Structural differences were primarily the presence of subfloral innovations on females and the presence of hairs on female thalli. Arguably, these structures are likely involved in protection of female gametangia (Schuster, 1969) or in facilitation of fertilization. Five of six taxa with differences in branching patterns had male plants more branched than females. Increased branching may be related to asexual propagation in that a highly branched individual can survive destruction of the main generative center (Crandall-Stotler, 1981), and each branch likely has the potential to grow into a physiologically independent ramet. I found one anecdotal account of difference in stature; males of *Ptilium ciliare* were less erect than females. In my discussion of morphological sex differences, I will concentrate primarily on sexual size dimorphism.

Of the dioicous taxa that were documented as sexually dimorphic, 56% exhibited sexual size dimorphism, and of those, only two taxa had males larger (*Cheilolejeunea rigidul;* Schuster, 1969), or possibly larger (*Marchantia berteronana;* Bischler, 1984) than females. Differences in size between the sexes ranges from obvious, e.g., in *Sphaerocarpos* males may be half the size of the female (Allen, 1919), to very subtle, and not noticable without detailed measurement, e.g., most *Marchantia inflexa* I've grown in the greenhouse. In one case, males were described as less "robust" than females, e.g., *Solenostoma pyriflorum,* (Schuster, 1969) and this was taken to mean that males were smaller than females. While I considered "robustness" as a size character, "slender" was considered a shape character. Twenty five species exhibited sexual dimorphism in shape and of these, males were more "slender" than females (which were described as

"wider") in 22 species. Given that females were often larger than males, the description of female plants as "wider" than males may be an allometric component to sexual size dimorphism. Note that when most authors discuss size of plants, because of the modular nature of clonal plants, they are not identifying individual plants but possibly ramets (genetically identical parts of the parent plant) of a genet (the parent plant), though this is not made explicit in most descriptions.

Sexual size dimorphism may be an important determinant of patch sex ratios in bryophyte populations. Sexual size dimorphism may be related to the costs of sexual reproduction and associated or correlated with many additional sexually dimorphic traits. For example, larger plants may overgrow and outcompete smaller, slower growing plants, and larger plants survive better and produce more asexual and sexual reproductive structures.

In bryophytes, growth rate is also a measure of clonal expansion. If size can be considered a surrogate measure of growth rate, sexual dimorphism in plant size may have implications for patch sex ratios and the occurrence of sex in populations. In a model using parameters taken from liverwort biology, patch sex ratio was dependent primarily on growth and asexual reproduction in combination with disturbance frequency (McLetchie, Garcia-Ramos, and Crowley, 2002). Sex differences in clonal expansion may lead to overgrowth and dominance of fast-growers (females) in a patch (Crowley, Stieha, and McLetchie, submitted) with low disturbance but in areas of high disturbance, plants with higher asexual reproduction (males) are expected to dominate. So, under the right conditions (i.e., low disturbance), females could outgrow and out compete males for space within a patch, forcing the patch sex ratio toward 100% female. In some seed plants, higher rates of clonal expansion by males are associated with male-biased sex ratios. In mosses, this pattern varies (see review in (Stark et al., 2004).

Females tend to be larger than males in smaller species of plants. In animals this is known as Rensch's rule (Rensch, 1960; Obeso, 2002) and is hypothesized to be related to sex-specific costs of sexual reproduction, but in plants it is inconclusive whether this pattern occurs because of sex-specific costs of reproduction, physiological differentiation or sexual selection (Obeso, 2002). Lack of sexual reproduction is common in dioicous bryophytes and overall, sexual reproduction is lower in dioicous than in monicious taxa (During, 1979). In a desert moss, females were larger and had a lower realized cost of sexual reproduction than males in populations where sexual reproduction was rare (Stark et al., 2000). Realized cost of sexual

reproduction refers to the fact that successful sexual reproduction in bryophyte populations is typically low, and females often do not realize their full cost of sexual reproduction, but males that reach gamete production do realize their cost of sexual reproduction. If production of gametangia is more costly for males than females, males will have a higher realized cost of sex.

If females incur a lower cost of sexual reproduction, because sexual reproduction typically trades-off with other investments, females could invest more energy in growth, asexual reproduction or other somatic processes than males. If , in males, sexual reproduction occurs at a cost to somatic investments, than the somatic costs may reduce probability of survival and future reproduction (Obeso, 2002). In my dissertation research, I found that larger plants, i.e., faster growers, survive better, produce asexual propagules earlier, and are more likely to produce sex structures than slow growers. The general pattern that females are the larger and that sexual reproduction and somatic processes trade-off, supports the hypothesis that males incur a higher cost to realized sexual reproduction in most bryophyte populations.

Physiology

Presence and, presumably, contents of oil bodies differs between the sexes of some taxa. In *Solenostoma*, female plants contain more oil bodies than male plants and, in one case, *Conocephalum conicum*, the sexes differed in odor, indicating possible presence of different chemical composition in the thalli (Table 1.2). Oil bodies are membrane-bound organelles filled with a wide variety of terpenoid oils (reviewed in Mues, 2000). The function of the contents of oil bodies has been a subject of speculation with hypotheses ranging from herbivory repellants to cold protection (reviewed in Schuster, 1969). Indeed, if sexes of a species consistently differ in oil body content, further investigation of oil body function and how this plays out as a sexually dimorphic character needs to be considered.

Six of seven species with a color dimorphism had males that were purplish to red, and females without a specific color or green. The color may occur on leaves or on bracts surrounding sex structures. The presence of red to purple pigmentation in liverworts may be associated with costs of sexual reproduction or sex-specific habitat use. Flavonoids, compounds that give plants their red coloration, occur almost universally in land plants, and are the most widespread phenolic compounds in mosses and liverworts (Mues, 2000; Crum, 2001). Approximately 238 flavonoids have been isolated from liverworts including red cell wall

pigments from both thalloid and leafy liverworts (Mues, 2000). Red to purple pigmentation in liverwort cell walls was considered a response to a combination of strong light and high temperatures (Garjeanne, 1932; cited in Voth and Hamner, 1940). In seed plants, an increased synthesis and an accumulation of flavonoids occurs in response to high light exposure (Kolb et al., 2001; Merzlyak and Solvchenko, 2002; Newsham, 2003), and may act as a sun screen to protect plants from damaging radiation (Flint, Jordan, and Caldwell, 1985; Mazza et al., 2000). UV-B irradiation, a natural component of solar radiation to which all plants are exposed in varying amounts, can cause severe damage to plants via direct and indirect damage to photosystem II (Solovchenko and Schmitz-Eiberger, 2003). Consequently, the production of red pigments in liverworts may be associated with protection from UV radiation. Additionally, UV-tolerance is positively associated with desiccation tolerance in bryophytes (Csintalan et al., 2001). In mosses, desiccation tolerance and tolerance of extreme temperatures is more common in females than males (Newton, 1972; Une, 1985a). Thus, sexually dimorphic pigmentation may have significant ecological consequences.

In *Marchantia inflexa*, scales with red pigmentation surround antheridia embedded in the surface of antheridiophores whereas, females do not show red pigmentation in the scales surrounding archegoniophores. In this species, the antheridia are exposed to sunlight on the splash-surface of the antheridiophore whereas, the archegonia are under the relatively thick plant tissue of the archegoniophore with layers of somatic tissue (the thickness of the thallus) between the light and archegonia. The plant tissue, even without the accumulation of pigments, probably attenuates UV radiation (Krauss, Markstadter, and Riederer, 1997). The degree of red pigmentation on the thallus of *M. inflexa* differs depending on the degree of exposure to sunlight (personal observation), indicating that the presence of pigmentation may be ephemeral.

Production and maintenance of pigments to protect gametes or for other purposes may be another cost to males and their investment in sexual reproduction that is not incurred by females. Or, more often pigmented males may indicate that males either use habitats with more sunlight exposure or have a greater propensity to process pigments than do females. It would be interesting to examine degrees of pigmentation between females and males of the same species that are equally exposed to sunlight to determine if there is a difference between the sexes in the propensity to produce protective pigments. If the pigments indeed protect against solar radiation, they may reflect an adaptation by males to optimize habitat use via light environment plasticity

and may indicate sex-specific mortality in relation to the light environment. Further studies on the production and significance of UV attenuating pigments in liverworts will provide insights into the adaptive significance of flavonoids.

Life History

In two taxa, female plants were reported to live longer than male plants and in *Sphaerocarpos*, males had lower germination success than females. Differences in survival/germination success, especially where males have lower survival, may be important in explaining commonly observed female-biased sex ratios reported for bryophytes (McLetchie, 1992). However, differences in longevity and germination success are reported for so few taxa, and longevity may be related to many different physiological processes. Also, in my own research, (Chapter 4), I found no differences in survival between sexes in *Marchantia inflexa*.

In 6 out of 7 of the species that showed a dimorphism in asexual reproduction, males produced more asexual propagules than females. Males produced more cupules, had more flagelliform leaves or produced caducous (readily deciduous) leaves when females did not. In one taxon, *Tritomaria exsectiformis*, Shuster's (1969) text implied that males produced gemmae and females did not, but this was unclear. In addition to more asexual reproduction via specialized propagules in males, males were also described as more frequently branched than females (branching is partly covered in the "morphological" category). For a review of asexual processes found in liverworts, see (Laaka-Lindberg, 2000).

In clonal plants, there is likely a tripartite trade-off among growth, asexual reproduction and sexual reproduction. Thus, if males experience a high cost of sexual reproduction, they may shunt resources away from growth and invest in asexual reproduction or vice versa. In liverworts, females are larger than males, but males invest in asexual reproduction more than females. In general, males produce more asexual propagules or exhibit a greater degree of branching than females. In species that produce caducous leaves, more branches may yield more leaf fragments for asexual propagation. If, within a patch, males are likely to be overgrown and outcompeted by females for space(Crowley, Stieha, and McLetchie, submitted), production of asexual propagules may be the most important aspect of sex-specific life history strategies that allow males to persist in populations. Male propagule production may be a mechanism for males to escape female overgrowth.

Investment in asexual propagules in males may also be related to intrasexual competition. Sexual selection, as defined by Arnold (1994), may explain the relatively higher investment by males in asexual propagule production. In bryophytes, successful fertilization is dependent on close proximity of the sexes. Sperm dispersal distances are short (Wyatt, 1977; Longton, 1990; McLetchie, 1996), often less than 10 cm. Because asexual propagules provide mechanisms for both short range and long range dispersal (Laaka-Lindberg, Korpelainen, and Pohjamo, 2003), males that produce more asexual propagules may be more likely to encounter females in a population consisting of separated patches of plants (McLetchie and Puterbaugh, 2000). In the dioicous moss *Polytrichum formosum* which produces underground rhizomes for clonal spread, there was no significant among-patch dispersal of asexual propagules, but males closest to females sired more offspring than males further away (Van der Velde et al., 2001). Much empirical evidence is needed to examine further the hypothesis of intrasexual competition among males, and further paternity experiments are needed to apply sexual selection theory to the maintenance of sexual dimorphism and influence on sex ratios in liverworts.

Summary

Dioicy is common in liverworts and many dioicous liverwort taxa are sexually dimorphic. However, few studies quantify sexual dimorphism thus, it's true prevalence in Marchantiophyta remains speculative. Given the wealth of dioicous liverwort taxa, sexual dimorphism is likely more common than is reflected in the literature. The presence of sexual dimorphism is hypothetically related to sex-specific costs of sexual reproduction, but this hypothesis has not been fully tested in liverworts. Patterns of sexual dimorphism among liverwort taxa support this hypothesis, but hypotheses of sexual selection and physiological differentiation deserve further consideration. Liverworts may be sexually dimorphic in a number of morphological, physiological and life history characters. In general, liverworts show trends toward sexual size dimorphism with larger females than males, and greater male investment in asexual reproduction. Additionally, males are more likely than females to carry red pigmentation that may be associated with increased UV light tolerance. These sexually dimorphic characters may have considerable ecological and evolutionary implications for liverworts and, in particular, may be influential in skewing sex ratios of liverwort populations and influencing probability of successful sexual reproduction. Consequently, it is instructive to

investigate the mechanisms responsible for the maintenance of sexual dimorphism in dioicous liverworts.

My dissertation research provides an in-depth investigation of sexual dimorphism of one liverwort taxon with the aim of understanding the population demography of sexual dimorphism, and the role of natural selection in maintaining sexual dimorphism. Sex-specific and environment-dependent selection potentially maintain sexual dimorphism and may promote the coexistence of sexes in populations (despite the tendency for females to overgrow male under certain conditions) and genetic variation within populations. My research reveals a trade-off between sexual and asexual reproductive fitness for females, and a surprising importance of sexual reproduction to the expressed phenotype of females. I also examine the significance of morphological plasticity and sex-specific selection on plasticity to determine if there is sexual dimorphism in plasticity and whether natural selection is the driving force behind the maintenance of sexually dimorphic characters.

Table 1.1 Characters sexually dimorphic in liverworts.
+ indicates that entire genera were referenced but not single taxa within the genera. M = male, F = female; Taxa and citations are given in Table1.2.

Category	Genera	No. species	Category description	Trends
Morphology				
Structural	10	11+	Presence/absence (P/A) of subfloral innovations, hairs, scales, setae, location of pores & air chambers, arch of segments, leaf morphology	Females with subfloral innovations and hairs
Branching	4	5	Pattern and degree of branching	M > F
Stature	1	1	Plant more or less erect	F > M
Shape	10	25+	Plant shape, cell shape	Females wide, males slender
Size	25	51+	Larger vs. smaller	F > M
Physiology				
Color	6	8+	Plant colors	Males reddish
Oil bodies	2	3	Numbers,	F > M
			contents	Male lacks fragrance
Chemistry	1	3	Ion absorption, amino acids present,	Sex-specific amino acids, enzymes,
Life History				
Survival, germination			longevity,	F > M
			germination, survival related to environment	F > M
Asexual reproduction	4	8	P/A caducous leaves, # asexual propagula structures	M > F
Sexual reproduction	1	2	# sex structures	F > M
Unknown	7	8	Taxa described as dimorphic but no characters given	

Table 1.2. Sexually dimorphic traits in liverworts from accounts in literature.
Taxa are in alphabetical order by genus. Summary of these data are given in Table 1. Orders are as given in Cradall-Stotler and Stotler (2000) A = autoicious, D= dioicous. * indicates experimental study.

Category	Trait	Genera	Species	Reprod	order	citation	Description
Morphology	**Structural**	*Ceratolejeunea*	*Ceratolejeunea laetefusca*	D	Porellales	Schuster, 1989	females with subfloral innovations
		Drepanolejeunea	*Drepanolejeunea*	A OR D	Porellales	Schuster, 1989	females with subfloral innovations
		Eremonotus	*Eremonotus myriocarpus*	D	Jungermanniales	Smith, 1990	females produce innovations below inflorescences
		Lejeunea	*Lejeunea ulicina*	D	Porellales	Schuster, 1989	females with subfloral innovations
			Lejeunea (Microlejeunea) ruthii	D	Porellales	Schuster,1989	females with subfloral innovations
		Lophozia	*Lophozia capitata*	D	Jungermanniales	Schuster, 1969	male shoots 2-lobed, female robust and 3-4 lobed
		Metzgeria	*Metzgeria furcata*	D	Metzgeriales	Smith, 1990	male hairless midrib
			Metzgeria crassipilis	D	Metzgeriales	Schuster, 1992	female branches setose or pilose male, smooth
			Metzgeria pubescens	D	Metzgeriales	Kasyap, 1972	males with hairs on postical surface, females hairs on both surfaces
			Metzgeria	A OR D	Metzgeriales	Schuster, 1992	males lack hairs
			Metzgeria leptoneura	D	Metzgeriales	Schuster, 1992	females with hairs
		Moerckia	*Moerckia hibernica*	D	Metzgeriales	Smith, 1990	females lack dorsal scales
		Riccia	*Riccia howei*	D	Ricciales	Schuster, 1992	females spongy with air chambers
		Targionia	*Targionia hypophylla*	A OR D	Marchantiales	Schuster, 1992	female segments arch upward
	Branching	*Aneura*	*Aneura indica*	D	Metzgeriales	Kasyap, 1972	males irregularly branched
		Frullania	*Frullania ericoides*	D	Porellales	Schuster, 1992	males less ramified
		Lophozia	*Lophozia longidens*	D	Jungermanniales	Schuster, 1969	male more freely branched
		Marchantia	*Marchantia papillata*	D	Marchantiales	Bischler, 1984	males palmate, females dichotomous
		Plagiochila	*Plagiochila defolians*	D	Jungermanniales	So, 2001	males more branched
			Plagiochila poeltii	D	Jungermanniales	So, 2001	males more branched
	Stature	*Ptilidium*	*Ptilidium ciliare*	D	Lepicoleales	Schuster, 1969	males less erect
	Shape	*Aneura*	*Aneura maxima*	D	Metzgeriales	Schuster, 1992	males slender
		Aneura		D	Metzgeriales	Schuster, 1992	male sublinear, simple
		Diplophyllum	*Diplophyllum taxifolium*	D	Jungermanniales	Schuster, 1974	females longer leaves, males stout
		Frullania	*Frullania tamarisci*	D	Porellales	Schuster, 1992	male slender
			Frullania taxodiocola	D	Porellales	Schuster, 1992	male shoots slender
		Lophozia	*Lophozia capitata*	D	Jungermanniales	Schuster, 1969	male more slender
			Lophozia latifolia	D	Jungermanniales	Schuster, 1969	male more slender
			Lophozia alpestris polaris (L. sudetica)	D	Jungermanniales	Schuster, 1969	male slender

Table 1.2 continued.

		Lophozia marchica	D	Jungermanniales	Schuster, 1969	male more slender
		Lophozia collaris	D	Jungermanniales	Schuster, 1969	male more slender
		Lophozia attenuata	D	Jungermanniales	Schuster, 1969	male more slender
		Lophozia binsteadii	D	Jungermanniales	Schuster, 1969	male more slender
		Lophozia obtusa	D	Jungermanniales	Schuster, 1969	male more slender
		Lophozia longidens	D	Jungermanniales	Schuster, 1969	male more slender
	Metzgeria	*Metzgeria acuta*	D	Metzgeriales	Schuster, 1992	males tapered toward apex, females acute
		Metzgeria furcata	D	Metzgeriales	Schuster, 1992	male branches button shaped
	Moerckia	*Moerckia hibernica*	D	Metzgeriales	Schuster, 1992	male thalli slender
	Nowellia	*Nowellia curvifolia*	A OR D	Jungermanniales	Schuster, 1974	males more slender
	Pellia	*Pellia megaspora*	D	Fossombroniales	Schuster, 1992	males slender, linear
		Pellia endiviifolia	D	Fossombroniales	Schuster, 1992	males slender, linear
	Porella	*Porella swartziana*	D	Porellales	Schuster, 1989	males slender
	Solenostoma (Jungermmania)	*Solenostoma hyalinum*	D	Jungermanniales	Schuster, 1969	male more ovate
		Solenostoma crenuliformis	D	Jungermanniales	Schuster, 1969	male slender
		Solenostoma caespiticum	D	Jungermanniales	Schuster, 1969	male slender
		Solenostoma cordifolium (J. exsertifolia)	D	Jungermanniales	Schuster, 1969	male slender
		Solenostoma atrovirens (Jungermannia atrovirens)	D	Jungermanniales	Schuster, 1969	male slender
		Solenostoma gracillimum	D	Jungermanniales	Schuster, 1969	male slender
Size	*Aneura*	*Aneura pinguis*	D	Metzgeriales	Hicks, 1992	males smaller
		Aneura indica	D	Metzgeriales	Kasyap, 1972	males smaller
	Blasia	*Blasia pusilla*	D	Blasiales	Kasyap, 1972	males smaller
		Blasia pusilla	D	Blasiales	Smith, 1990	males smaller
	Cephalozia	*Cephalozia macrostachya*	A OR D	Jungermanniales	Smith, 1990	male smaller
	Cheilolejeunea	*Cheilolejeunea rigidula*	D	Porellales	Schuster, 1989	females smaller
	Cryptocolea	*Cryptocolea imbricata*	D	Jungermanniales	Schuster, 1969	male smaller
	Cryptothallus	*Cryptothallus mirabilis*	D	Metzgeriales	Schuster, 1992	males smaller
	Diplophyllum	*Diplophyllum taxifolium*	D	Jungermanniales	Hicks, 1992; Schuster, 1974	males smaller
	Frullania	*Frullania ericoides*	D	Porellales	Schuster, 1992	males smaller
	Geothallus	*Geothallus*	D	Sphaerocarpales	Crum, 2001	males smaller
	Haplomitriales	*Haplomitriales*	D	Haplomitriales	Crum, 2001	males smaller
	Lophozia	*Lophozia capitata*	D	Jungermanniales	Hicks, 1992	males smaller

Table 1.2 continued.

	Marchantia	*Marchantia inflexa*	D	Marchantiales	McLetchie and Puterbaugh, 2000	males smaller	*
		Marchantia foliacea	D	Marchantiales	Bischler, 1984	males smaller	
		Marchantia berteroana	D	Marchantiales	Bischler, 1984	males larger?	
	Marsupella	*Marsupella sphacelata*	D	Jungermanniales	Hicks, 1992	males smaller	
	Metzgeria	*Metzgeria macrocellulosa*	D	Metzgeriales	Schuster, 1992	males smaller	
		Metzgeria macrospora	D	Metzgeriales	Schuster, 1992	males smaller	
		Metzgeria furcata	D	Metzgeriales	Schuster, 1992	males smaller	
		Metzgeria myriopoda	D	Metzgeriales	Schuster, 1992	males smaller	
	Mylia	*Mylia taylori*	D	Jungermanniales	Schuster, 1969	males smaller	
	Pallavicinia	*Pallaviciniaceae*	D	Metzgeriales	Schuster, 1992	males smaller	
	Pellia	*Pellia neesiana*	D	Fossombroniales	Schuster, 1992	males smaller	
	Plagiochila	*Plagiochila salacensis*	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila aspleniodies porelloides	D	Jungermanniales	Schuster, 1989	males smaller	
		Plagiochila emeiensis	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila ghatiensis	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila secretifolia	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila gymnoclada	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila elegans	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila delavayi	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila bishleriana	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila shangaica	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila pseudofirma	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila gracilis	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila chenii	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila retusa	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila fordiana	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila pecularis	D	Jungermanniales	So, 2001	males smaller	
		Plagiochila sichuanensis	D	Jungermanniales	So, 2001	males smaller	
	Ptilidium	*Ptilidium pulcherrimum*	D	Lepicoleales	Smith, 1990	males smaller	
		Ptilidium ciliare	D	Lepicoleales	Schuster, 1969	male smaller	
		Ptilidium ciliare	D	Lepicoleales	Smith, 1990	males smaller	
	Riccia	*Riccia sanguinea*	D	Ricciales	Kasyap, 1972	males smaller	
		Riccia curtisii	D	Ricciales	Schuster, 1992	males smaller	
		Riccia howei	D	Ricciales	Schuster, 1992	males smaller	

Table 1.2 continued.

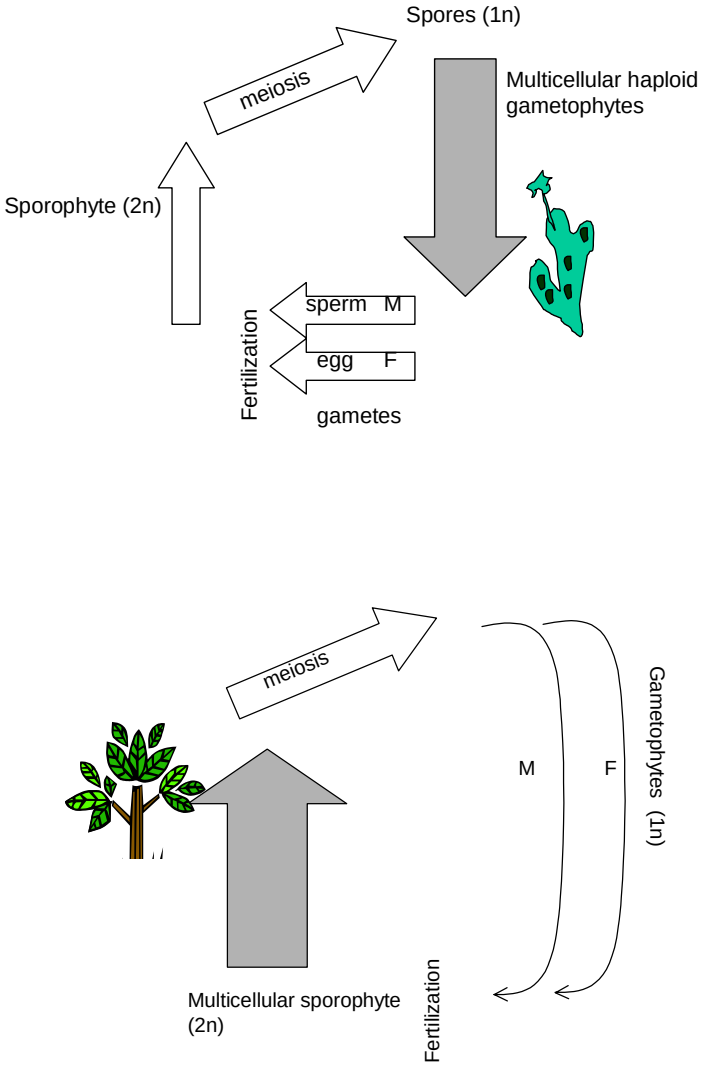
		Scapania	*Scapania gracilis*	D	Jungermanniales	Smith, 1990	male bracts smaller	
			Scapania aequiloba	D	Jungermanniales	Smith, 1990	male bracts smaller	
		Solenostoma	*Solenostoma cordifolium (Jungermannia exsertifolia)*	D	Jungermanniales	Schuster, 1969	male smaller	
			Solenostoma pyriflorum	D	Jungermanniales	Schuster, 1969	male less robust	
			Solenostoma hyalinum	D	Jungermanniales	Schuster, 1969	male smaller	
		Sphaerocarpos	*Sphaerocarpos*	D	Sphaerocarpales	Crum, 2001; Schuster, 1992	males smaller	
			Sphaerocarpos texanus	D	Sphaerocarpales	Hicks, 1992	males smaller	
			Sphaerocarpos donnellii	D	Sphaerocarpales	Schofield, 1985; Schuster, 1992; Allen, 1919	males smaller, slower growth	
			Sphaerocarpos michelii	D	Sphaerocarpales	Schuster, 1992	males smaller	
		Tritomaria	*Tritomaria exsecta*	D	Jungermanniales	Schuster, 1969	male smaller	
Physiology	**Color**	*Conocephalum*	*Conocephalum conicum*	D	Marchantiales	Smith, 1990	male receptacle violet, female green	
		Lophozia	*Lophozia longidens*	D	Jungermanniales	Schuster, 1969	male reddish	
			Lophozia ventricosa confusa	D	Jungermanniales	Schuster, 1969	male	
		Pellia	*Pellia neesiana*	D	Fossombroniales	Schuster, 1992	purplish to reddish	
		Riccia	*Riccia sanguinea*	D	Ricciales	Kasyap, 1972	male red, female green	
		Solenostoma	*Solenostoma pyriflorum*	D	Jungermanniales	Schuster, 1969	female more purple	
		Sphaerocarpos	*Sphaerocarpos michelii*	D	Sphaerocarpales	Smith, 1990	males reddish	
		Sphaerocarpos		D	Sphaerocarpales	Schuster, 1992	males red	
		Sphaerocarpos		D	Sphaerocarpales	Crum, 2001	males red	
	Oil bodies	*Conocephalum*	*Conocephalum conicum*	D	Marchantiales	Crum, 2001	females with spicy odor; males lack fragrance	
		Solenostoma	*Solenostoma hyalinum*	D	Jungermanniales	Schuster, 1969	female more	
			Solenostoma crenuliformis	D	Jungermanniales	Schuster, 1969	female more than sterile (male usually sterile)	
	Chemical	*Sphaerocarpos*	*Sphaerocarpos donnellii*	D	Sphaerocarpales	Schofield, 1985	males absorb ammonium ions	*
Life History	**Survival, Germination**	*Cryptothallus*	*Cryptothallus mirabilis*	D	Metzgeriales	Lewis and Benson-Evans, 1960	females live longer	*?
			Sphaerocarpos donnellii	D	Sphaerocarpales	Allen, 1919	Females may have better germination rate	*
			Sphaerocarpos texanus	D	Sphaerocarpales	McLetchie, 1992	females live longer, males susceptible to environment, males lower germination success	*
	Asexual reproduction	*Marchantia*	*Marchantia inflexa*	D	Marchantiales	McLetchie and Puterbaugh, 2000	males more cups	*
			Marchantia polymorpha	D	Marchantiales	Voth and Hamner, 1940	males produce more cupules	*
		Plagiochila	*Plagiochila tagawae*	D	Jungermanniales	So, 2001	males with caducous leaves	
			Plagiochila trabeculata	D	Jungermanniales	So, 2001	males with caducous leaves	

Table 1.2 continued.

			Plagiochila poeltii	D	Jungermanniales	So, 2001	male abundant flagelliform branches	
			Plagiochila sciophila	D	Jungermanniales	So, 2001	males with caducous leaves	
		Tritomaria	*Tritomaria exsectiformis*	D	Jungermanniales	Schuster, 1969	male produce gemmae?	
	Sexual reproduc-tion	*Marchantia*	*Marchantia polymorpha*	D	Marchantiales	Voth and Hamner, 1940	males produce more sex structures	*
			Marchantia inflexa	D	Marchantiales	Fuselier and McLetchie, 2002	females more sex structures	*
Unknown		*Haplomitrium*	*Haplomitrium hookeri*	D	Haplomitriales	Crandall-Stotler, 2000		
			Haplomitrium mnioides	D	Haplomitriales	Crandall-Stotler, 2000		
		Makinoa	*Makinoa crispata*	D	Metzgariales	Crandall-Stotler, 2000		
		Monoclea	*Monoclea gottschei*	D	Monocleales	Crandall-Stotler, 2000		
		Pallavicinia	*Pallavicinia lyellii*	D	Metzgeriales	Crandall-Stotler, 2000		
		Petalophyllum	*Petalophyllum ralfsii*	D	Fossombroniales	Crandall-Stotler, 2000		
		Porella	*Porella pinnata*	D	Porellales	Crandall-Stotler, 2000		
		Symphyogyna	*Symphyogyna brasiliensis*	D	Metzgeriales	Crandall-Stotler, 2000		

Figure 1.1. Life cycles

Sexual reproductive cycle of a dioicous bryophyte and a flowering plant. Dominant stages are indicated by gray arrows and illustrations. Bryophytes undergo haploid-dominant alternation of generations and angiosperms, diploid-dominant alternation of generations.



Study organism

The study organism that I used throughout my research, *Marchantia inflexa* (Nees et Mont.) is a dioicous, thallose liverwort that ranges from the state of Tennessee in the southern USA to northern Venezuela (Bischler, 1984; Schuster, 1992). Most populations throughout the species' range include both sexes but some populations on the range periphery in the USA are unisexual, and maintained solely through asexual reproduction (Schuster, 1992). Plants typically grow in distinct patches along stream banks and on rocks in streams, and these patches are often separated in space by unsuitable substrate. The species is sexually dimorphic in growth and reproductive traits. Female *M. inflexa* tend to be larger than males, and males tend to produce more cupules than females (McLetchie and Puterbaugh, 2000). In the Marchantiaceae, sex determination is under genetic control of sex chromosomes (Bischler, 1986) and, in the genus *Marchantia*, sex-specific life history strategies have been documented (Voth and Hamner, 1940; McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002).

Bryophytes exhibit an alternation of generations where, unlike angiosperms, the gametophyte (haploid) is the dominant, independent, longer-lived generation, and the sporophyte (diploid) generation is short-lived and dependent upon the gametophyte. Reproduction occurs both asexually and sexually. In *M. inflexa*, clonal expansion occurs in both sexes by two mechanisms: (1) new thallus branches that are produced by a mericell (analogous to the meristem of seed plants) dividing into two mericell regions (splitting) and (2) specialized asexual propagules called gemmae that are produced inside cupules (cups) on the thallus surface. Gemmae are water-dispersed, disc-shaped propagules that grow into a gametophytes identical to the parent plant.

Sexual reproduction occurs when motile sperm from a male's antheridiophore fertilize eggs within a female's archegoniophore. Throughout this dissertation, I refer to antheridiophores and archegoniophores as "sex structures". Successful fertilization results in a diploid sporophyte that develops on the female plant and produces spores, which germinate to form female and male, haploid, gametophytes. Sexual reproduction in *M. inflexa* occurs seasonally in USA populations (LCF, personal observation), but in Trinidad populations lower levels of sexual reproduction may occur year round (McLetchie and Puterbaugh, 2000).

New ramets of *M. inflexa*, may establish themselves in unoccupied space through dispersal and growth of asexual propagules and fragments of parent plants, or via growth of a parent plant with subsequent severing of connections between ramet and genet. New genets are established by dispersal and growth of sexual propagules (spores). Because a growing region can invest in only one strategy at a time, ultimately, growth, and cupule production are constrained by the number of mericells produced (McLetchie and Puterbaugh, 2000). *Marchantia inflexa* displays trade-offs between mericell (growing tip) production and cupule formation (McLetchie and Puterbaugh, 2000).

Chapter Two: Microhabitat and sex distribution in *Marchantia inflexa*

Summary

Spatial segregation of the sexes in bryophytes has been implicated as the primary reason for the lack of sporophyte production in many dioecious bryophyte populations. I examined habitat use of female and male *Marchantia inflexa* in two Trinidad populations and 10 populations in the USA to determine whether the sexes were spatially segregated within populations and if the sexes specialized on different microhabitats, with an emphasis on the light environment. Sex ratios varied among populations, and populations consisted mainly of single-sex or non-sex-expressing patches of plants. Despite the fact that *M. inflexa* is sexually dimorphic in life history strategies, female and male plants used areas of similar substrate, humidity, wind speed and exposure within and among populations. In USA populations, males were found in areas with more open canopy than females and, overall, there was a trend for males to live in areas that receive more light than females and use a wider range of light environments than females. The sexes of *M. inflexa* overlapped in habitat use and, although the sexes were spatially separated within populations, segregation did not occur along a light-environment gradient.

Introduction

Spatial segregation of the sexes (SSS) of dioecious plants may have important evolutionary consequences. Spatial segregation of the sexes may decrease chances of sexual reproduction, and, if segregation is associated with microhabitat specializations by the sexes, may result in biased population sex ratios and the maintenance of sexually dimorphic traits. Spatial segregation of the sexes may result from random processes, as in the case of post-Pleistocene colonization of habitats by a single-sex of a dioecious species (Longton and Schuster, 1983), or random dispersal of propagules within populations. More interestingly, spatial segregation of the sexes may occur when the sexes specialize on different microhabitats.

Sex-specific microhabitat specializations in dioecious plants can result in SSS along moisture, nutrient, light, temperature or salinity gradients (Freeman, Klikoff, and Harper, 1976; Cox, 1981; Bierzychudek and Eckhart, 1988; Lovett-Doust and Lovett-Doust, 1988; Dawson and Bliss, 1989; Korpelainen, 1993; Ramadan et al., 1994). In a plant that does not undergo sex choice, SSS along an environmental gradient may result from (reviewed for seed plants in

Bierzychudek and Eckhart, 1988): (1) differential mortality of the sexes, related to different costs of reproduction between the sexes, or differences in germination requirements for female and male sexual propagules (McLetchie, 2001), (2) competition between the sexes where females and males have different clonal expansion traits that can result in overgrowth and death of one sex (Wyatt, 1977; McLetchie, Garcia-Ramos, and Crowley, 2002; Crowley, Stieha, and McLetchie, submitted), (3) habitat selection where females and males actively choose microsites, or female and male propagules have different dispersal characteristics, and (4) sex-specific habitat requirements for gametangial formation (Longton, 1990).

In their review of the proximate mechanisms of SSS in seed plants, Bierzychudek and Eckhart (1988) found that differential mortality of the sexes in habitat patches was the most common cause for SSS. In plants that rely on abiotic pollination, SSS does not necessarily interfere with gamete transfer, but for species that depend on close proximity of the sexes for successful fertilizations, SSS may limit sexual reproduction. Dioecious bryophytes, unlike seed plants, rely on water to transport motile sperm to the eggs of a female plant. Sperm dispersal distances are short (Wyatt, 1977; Longton, 1990; McLetchie, 1996), and sexual reproduction is often seasonal (Gemmel, 1950; During, 1979; Longton, 1990). Gemmel (1950) noted that the major cause of the rarity of sporophytes in dioecious bryophytes may be the distance between the sexes. Despite the importance of spatial proximity, sexes of dioecious bryophytes often occupy separate patches in populations (Longton and Schuster, 1983; Longton, 1990), and population sex ratios are typically female-biased (Longton, 1990; Wyatt, 1994; Stark, Mishler, and McLetchie, 2000; Bowker et al., 2000).

Female-biased adult sex ratios in bryophytes may result from habitat specializations of the sexes (Cameron and Wyatt, 1990), greater clonal expansion ability of one sex compared to the other (Wyatt, 1977; McLetchie and Puterbaugh, 2000; Crowley and McLetchie, 2002; Stark et al., submitted), differential costs of reproduction between the sexes (Stark, Mishler, and McLetchie, 2000), and/or lack of sex expression on the part of males (Newton, 1971). Habitat specializations of the sexes of dioecious bryophytes may be related to sex-specific survival, germination, growth, dormancy and reproductive requirements. For example, male plants may be more susceptible to desiccation than female plants (Newton, 1972). Sex-specific dormancy and germination requirements were found in *Sphaerocarpos texanus* (McLetchie, 2001). Day length and temperature conditions that induce antheridia formation in *Plagiomnium undulatum*

are narrower than conditions that stimulate archegonia formation (Newton, 1972; Selkirk, 1979), and in species of *Riccia*, females and males differ in the optimal pH and nutrient concentrations for gametangial initiation (Selkirk, 1979).

Marchantia inflexa, a dioecious liverwort, is a prime suspect for SSS on an environmental gradient because the plants are sexually dimorphic in life history traits (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002), the sexes often occur in separate patches within populations, and single-sex populations of females and males are known (Schuster, 1992; McLetchie and Puterbaugh, 2000). The goals of this study were to describe general habitat characteristics of *M. inflexa*, and to examine whether the sexes were spatially segregated along an environmental gradient. It is important to note that any differences found in habitat occupation by the sexes may be indicative of conditions necessary for sex-structure production rather than differential mortality of the sexes in relation to the microhabitat, because sex determination in the field relies upon the presence of sex structures (antheridiophores or archegoniophores).

Materials and Methods

Populations

I measured environmental variables for female and male *M. inflexa* in 12 populations (Table 2.1) ranging from the southern to northern ends of the species' range. In 2001, I measured habitat characteristics at two populations in Trinidad and three in Oklahoma. Oklahoma populations were single-sex (SS) populations of either females (n = 2) or males (n = 1) whereas, the Trinidad populations contained both sexes. In April and May, 2002, habitat data were collected in 3 additional SS male populations in Oklahoma, and both-sex (BS) populations in Alabama (n = 2), Tennessee (n = 1), and Mississippi (n = 1), USA. I considered all plants in separate river or creek drainages a "population". For most populations in the USA, the beginning and end of the population was evident whereas, in Trinidad, the populations were large, and I sampled only a segment of the entire population.

A patch was defined as a group of plants separated by neighboring groups by at least one meter, typically with an obstruction (such as unsuitable substrate) between patches. A clump was a group of thalli within a patch. I described patch and clump occupancy as male (M), female (F), or both sexes (B) for patches along a linear transect through the sites. The populations differed in size so, transects ranged from approximately 1 to 1.5 km of stream. One population,

CC (Table 2.1), was smaller than all others, and the transect there was less than 0.5 km. Within each patch, I randomly selected a clump of plants (by tossing a marker onto the patch), placed a quadrat (157.5 cm^2) on a clump, and measured habitat of only those plants within the quadrat. Some male clumps were in BS patches because all-male patches were rare in some BS populations.

In all populations, antheridiophore production peaks before archegoniophore production (LCF unpublished data) thus, my estimates of female and male presence in populations were influenced by the phenology of sex expression. Plants in Trinidad begin sex expression in February, with antheridiophores appearing about 2 weeks before archegoniophores (McLetchie, Garcia-Ramos, and Crowley, 2002), and females have sporophytes in May. In USA populations, males begin sex expression in late April, females begin in May, and spores are dispersed in August (LCF, personal observations). I sampled Trinidad populations after some females had sporophytes but before all antheridiophores had been lost. Many young and older, dried antheridiophores were still present at the time of the surveys. Single-sex populations were sampled in late April and the both-sex USA populations in early May. All of the male populations, and one female population, contained numerous mature sex structures though, one of the female populations had delayed onset of sex expression relative to the other single-sex populations.

The phenology of sex expression in *M. inflexa* is such that samples may be biased against male patches in Trinidad and against female patches in USA populations because, if sex structures are not present, sex of the patch cannot be determined. Throughout this report, I use the numbers of antheridiophores and archegoniophores to represent proportions of males and females respectively, because the sexes are indistinguishable in the field unless they are sex-expressing. Thus, the range of habitats presented excludes those habitats in which non-expressing plants were found.

Sex ratios

I estimated sex ratios of gametangia-bearing adults in two Trinidad populations and 4 USA populations. In the Trinidad populations, I used counts of archegoniophores and antheridiophores within quadrats of both-sex patches to calculate the proportion of males present in a quadrat. Because my previous study showed that females produced approximately three times as many sex structures as males (Fuselier and McLetchie, 2002), I adjusted numbers of sex

structures by dividing the total number of archegoniophores by 3 and then calculating the proportion of males out of the adjusted total. Quadrats that had fewer than 3 archegoniophores were not included in calculations of sex ratios. To estimate sex ratios for Trinidad populations, I used the sum of the sex structures counted in both-sex patches and calculated the proportion of males present from these sums.

In the USA populations, sex ratios were estimated from counts of archegoniophores and antheridiophores in 50 randomly placed quadrats throughout the population. One person walked the center of the stream, stopped every 5 paces, threw a marker, placed a quadrat into a patch of plants and counted sex structures. There were no data available on sex structure production for plants in the USA populations so, I examined sex ratios on adjusted (for 3 times higher archegoniophore production) and unadjusted numbers of archegoniophores.

General habitat.— To describe the general habitat occupied by plants in each population, I measured water pH in populations sampled in 2001, and soil characteristics in populations sampled in 2002. Analyses of 2001 data indicated that water pH, did not differ among populations thus, in 2002, this measure was omitted, and soil samples were taken instead. Three soil samples, one from each end of the stream reach and one in the middle of the reach were taken from within a plant patch and analyzed at the University of Kentucky College of Agriculture Regulatory Services Department. Total soil nitrogen was determined from approximately 0.5 g of oven-dried and sieved soil using a LECO combustion instrument. Phosphorus, K, Ca, Mg, and Zn were determined using a Mehlich III extract and inductively coupled plasma spectroscopy. Percentage of sand, silt and clay in soil was estimated with dried and sieved soil using the pipette method, and reported as percent composition of oven-dried soil. Soil pH was measured with a pH meter and a slurry of soil.

Patch and plant characteristics

To describe patch characteristics within each population, I measured patch size, as the longest and widest axes of the patch in meters, and patch depth, measured with a ruler penetrated through plant thalli to the substrate. Patch disturbance may influence local adult sex ratios in populations (Crowley and McLetchie, 2002; McLetchie, Garcia-Ramos, and Crowley, 2002) thus, patch depth was included a surrogate measure for disturbance, assuming that the deepest patches are the oldest. This assumption may be invalid and may depend on colonization and growth rates, and disturbance frequencies specific to each population.

Sporophyte counts — To verify my classification of patches as both-sex or single-sex, and to quantify sperm travel distances, I conducted a survey of sporophyte-bearing archegoniophores in two Trinidad populations in 2001. A petri dish cover (9 cm diameter) was haphazardly placed into a patch with sex-expressing plants, distance to the nearest male was measured, and archegoniophores under the petri plate were harvested and dissected to quantify number of sporophytes. Patches were classified as either both sex or female depending on the sex structures present. If I were underestimating the presence of males by classifying patches as female rather than both-sex, I should have detected sporophytes in "female" patches, indicating that males were present in these patches but not detected because of termination of sex expression. The average distance from sporophyte-bearing archegoniophores to the nearest male was calculated to determine the average distance necessary for successful fertilization.
Microhabitat - To discern any differences in microhabitat features among patches of plants, the following variables were measured within each quadrat within a patch: substrate type, humidity, wind speed, direction of exposure ,and light environment. Substrate type was a categorical variable that included soil, logs, and rocks. Percent relative humidity and wind speed were measured with a Kestrel 3000 handheld weather meter, and were descriptive only of the exact time the measure was taken. Direction of exposure of a clump to the sky was recorded as a compass direction. I did not measure patch size, depth, substrate, humidity, wind or direction of exposure at field sites in 2002.

The light environment above each clump was quantified using hemispherical photographs, taken at low sun angles (to avoid scattering of light), through a 180^{o} fish-eye lens (Nikon FC-E8) on a tripod-mounted digital camera (Nikon 950, Nikon Corporation, Tokyo, Japan). Canopy photos were taken in May at Trinidad populations and during the early summer at USA populations when there was full canopy . Images were analyzed using Scanopy software (Reagent Instruments Inc, Quebec, Canada) to estimate canopy openness and the total amount of photosynthetically active photon flux density (PPFD, $mol/m^2/day$, both direct and indirect) reaching a clump over a specified time period. The software uses latitude and longitude to estimate the solar path over the site, and calculate the amount of light that a clump of plants receives throughout a specified period of time. I used a 12 month period for Trinidad populations, and May through October for all USA populations. May through October encompasses most of the time when the canopy is full (and similar to the conditions under which

the photo was taken). Canopy openness is assumed to stay constant throughout the time period specified whereas, PPFD changes throughout the day and season.

Microhabitat features that I chose to measure are known to be influential in distribution (Longton and Schuster, 1983; Longton, 1990) and sex expression (Hughes and Wiggin, 1969) of bryophytes. Light quantity and quality influence thallus morphology, gemmae cup production and sex expression in *M. polymorpha*, (Price, 1977), and *M. inflexa* females and males respond differently to different light environments in the greenhouse (Fuselier and McLetchie, 2002).

Analyses. — I examined differences between sexes in substrate use (soil and rock) and direction of sunlight exposure (N, S, W, E, skyward) using 2 by 2, and 2 by 4 contingency table analyses, and likelihood ratio tests for goodness of fit. In preliminary analyses (ANOVA with population and clump as main effects) relative humidity and wind speed, showed no significant differences within or among populations, and were dropped from analyses of habitat use (*F* statistics ranged from 2.41 to 0.04 and *p* values ranged from 0.14 to 0.83) . The ranges of relative humidity and wind speed values were small, primarily due to the lack of resolution provide by the instrument used (range of relative humidity = 62% - 100% and wind speed = 0 to 1 knot). I reported mean values of these habitat variables in a general description of *M. inflexa* habitat.

To explore differences between populations in soil composition and pH, I used an ANOVA with population type nested within population, and contrast statements for specific comparisons among populations. To examine differences in the range of light environments inhabited by the sexes, I used a contingency table analysis of the frequency distribution of the occurrence of patches under ranges of irradiance (1 umol/m^2/day) and openness. This provided a Chi-squared estimate to determine whether the sexes differed in the distribution of light environments that they used. I created a "patch-clump" designation to identify clumps within patches (e.g. a female clump within a both-sex patch was designated "BF" and there were five categories: FF, MM, BF, BM, BB). To examine differences in depth and area of patches, I used these patch-clump designations in an ANOVA with patch-clump and population as main effects, and probability values from t-distribution comparisons to determine which groups differed. This analysis included only those populations sampled in 2001 (Trinidad and Oklahoma populations).

I used unbalanced nested ANOVAs to examine differences between the sexes from both-sex populations, with population and clump nested within population as main effects. Differences between the sexes from single-sex populations in total light (ppfd) under the canopy

during the growing season, and canopy openness, were analyzed using general linear model (unbalanced) analyses of variance and contrast statements. To examine how the sexes were using light environments throughout the growing season, I used a repeated measures ANOVA. I examined results from univariate ANOVA by month for those months when sex structures develop and sporophytes appear.

To account for temporal differences in data collection, differences in phenology and differences in canopy closure dependent upon time of year, I assigned populations to three groups according to when light measurements were taken, and ran analyses of light habitat separately for the groups. I measured light environment in Trinidad in during two weeks in May 2001, in BS populations in the USA during two weeks in mid-May 2002, and in SS populations in Oklahoma during the first week of May 2002.

Sex ratios were calculated as the number of antheridiophores/ total number of sex structures. Log-likelihood ratio (G^2) tests were used to compare observed sex ratio estimates to the expected 1:1 ratio. I used linear regression of sex ratios onto amount of light received under the canopy to examine the relationship between clump sex ratio and light environment in Trinidad populations. I used correlations to quantify the relationships between both-sex USA population sex ratios and means of habitat variables measured in each population.

In all analyses, normality of data was improved by using arcsine-square root transformations of percentage data and log transformations of integer data. Type III sums of squares were used to generate probability values, and all analyses were conducted using SAS software (SAS, 1990).

Results

Population habitat characteristics

The general habitat in which plants were found across populations provided description of *Marchantia inflexa* habitat similar to that described by Bischler (1984; Table 2.2). Plants were found along streams with neutral to alkaline water and soil pH, and high relative humidity. Plants grew primarily on soil banks, exposed limestone and/or travertine rock and infrequently on logs. Although logs were present in the populations, only 2 patches of plants in all populations occurred on logs. In CC, plants occurred solely on rock substrate. Patch size was larger in the lower latitudes (n = 5 populations, r = -0.89, p = 0.04).

Patterns among populations in soil composition indicated a geographic effect rather than a population type or sex effect. Soil pH and P, K, and Zn did not differ among populations (p values ranged from 0.10 to 0.40). Total soil nitrogen ($df = 7$, $F = 9.69$, $p = 0.0004$) and Mg ($df = 7$, $F = 25.06$, $p < 0.0001$) varied among populations and were significantly higher in Oklahoma populations (male and female combined) than Tennessee (BD) and Mississippi (MS) populations ($df = 1$, $F = 16.95$ and 19.07 respectively, $p = 0.001$; Figure 2.1a). Soil composition followed the same pattern, with higher percentages of sand in Oklahoma populations ($df = 1$, $F = 13.61$ $p = 0.003$) and higher percentages of silt ($F = 9.08$, $p = 0.01$) and clay ($F = 18.88$ $p = 0.001$) in BD and MS (Figure 2.1b). Soil minerals, soil composition, mean light received, mean canopy openness, and range of light and openness in populations were not significantly correlated with either population sex ratio (adjusted or unadjusted) or latitude.

Sex ratios

Among the both-sex USA populations in which I conducted sex ratio estimates, 20% of the clumps were female, 21% were male, 10% contained both sexes, and the remaining clumps were non-expressing (Figure 2.2). Sex ratios varied among populations. Before adjusting for the difference between the sexes in sex structure production, BD had a significantly male-biased sex ratio, and LS and both Trinidad populations had female-biased sex ratios (Table 2.3). After adjusting for differences in sex structure production, assuming the difference between the sexes in USA populations was the same as in the Trinidad plants, 5 of the 6 populations had male-biased sex ratios (Table 2.3). If I had used an adjustment of only 2 times as many archegoniophores per antheridiophore, the results would have been similar with the exception of LS, which would remain female-biased.

Population sex ratios (unadjusted for any differences between the sexes in production of sex structures) for 4, both-sex populations in the USA, were significantly positively correlated with mean canopy openness ($n = 4$, $r = 0.99$ $p = 0.001$; Figure 2.3). Sex ratios of these 4 populations were not significantly correlated with latitude, range of light received at a site, mean light received at a site, total number of sex structures, or number of cups present in a clump (p values ranged from 0.38 to 0.83). The average number of archegoniophores and antheridiophores per quadrat were significantly positively correlated with the range of light received at a site through the growing season ($n = 4$ populations, $r = 0.97$ and 0.99, $p = 0.03$ and 0.001).

Proportion of males in both-sex clumps in Trinidad was not correlated with amount of light received for either adjusted or unadjusted values (Figure 2.4). Unadjusted sex ratios ranged from 0.03 to 0.84. Numbers of sex structures in the 14 both-sex clumps used in this analysis ranged from 7 to 86 with a mean = 32.6 sex structures/clump.

Patch and plant characteristics

A total of 223 archegoniophores from 43 patches on the Quare and 26 patches on the Turure River, Trinidad, was examined for presence of sporophytes. Only one patch that was classified as "female" had an archegoniophore with sporophytes, representing a 0.45% patch misclassification rate for the two populations. Thus, I had confidence in my ability to detect sex expression, and correctly classify patches that have sex expressing plants. In both-sex patches in Trinidad populations, mean distance from a fertilized archegoniophore to the nearest male was 12 cm (n = 20, max = 43 cm).

With Trinidad and Oklahoma populations grouped together, there was no significant relationship between substrate type and clump-sex in contingency table analysis (df = 6, G^2 = 6.32, p = 0.39). Likewise, there was no relationship between direction of clump exposure and clump-sex (df = 18, G^2 = 15.94, p = 0.60). Populations (n = 5, QR, R, TF, TC, BM) differed in clump depth (p = 0.0025), and patch-clumps (n = 5; BB, BF, BM, FF, MM) differed in depth (p = 0.04) and area (p = 0.02). All-female patches were significantly shallower than any both-sex patch, and both-sex clumps within both-sex patches were deepest of the patch-clump types (Figure 2.5a). Both-sex patches were larger than single-sex patches in the Trinidad populations (Figure 2.5b). Male patches (n = 33) exhibited a wider range of patch areas (range = 8.79 m^2) and patch depths (29 mm) than female patches (5.1 m^2, 18 mm, respectively, n = 53).

Light environment

Female and male clumps differed in their frequency distribution among available light environments (irradiance: n = 264, *Chisq* = 26.29, p = 0.05; openness: *Chisq* = 25.64, p = 0.05; Figure 2.6). Male clumps (n = 176) were found under a wider range of canopy openness (range = 38%) and total light received under the canopy (range = 35.1 umol/m^2/day) compared to females (n = 88, openness range = 26.7% and light range = 32.1 umol/m^2/day). The Quare and Turure River sites in Trinidad did not differ in canopy openness, and the sexes within these populations used areas of similar canopy openness. The Trinidad populations differed in amount

of light received under the canopy, but light received in areas where females and males were found did not differ within populations (Table 2.4).

There was a wider range of light available to plants in both-sex populations within the USA (range = 34.5 umol/m^2/day) compared to populations in Trinidad (range = 17.18 umol/m^2/day). In both-sex populations in the USA, males tended to be in patches with higher openness than females, but this difference was not significant (Table 2.4). Populations and sexes within populations did not differ in amount of light received under the canopy (Table 2.4). Because there were no significant differences among USA populations in openness and irradiance, I combined the three populations and examined sex differences in an ANOVA. Males were in patches with higher canopy openness than either female or both-sex patches (df = 2, F = 3.38, p = 0.04). There was a similar pattern for total light received though it was not significantly different among patch-sex types (p = 0.54; Figure 2.7 a & b). The Cook's Creek population (CC) was not included in these analyses because of small sample size (Table 2.1).

Single-sex populations differed in canopy openness but were not significantly different in total light received under the canopy through the growing season. Female (n = 2) and male (n = 4) populations did not differ in canopy openness or amount of light received throughout the growing season (Table 2.4). The range of canopy openness (33.58%) and light received under the canopy (33.62 umol/m^2/day) was higher for single-sex populations than either Trinidad or both-sex USA populations.

I combined all both-sex USA populations and compared how light varied across the growing season using a repeated measures ANOVA. Although, there was a pattern of male patches receiving more light than females, there was no significant sex effect (p = 0.85). Similarly, although the two Trinidad populations show the same pattern of males occurring in areas of higher light than females, there were no significant differences among patch types (Figure 2.8). In the Trinidad populations, population differences explained most of the variance in radiation received over time (p < 0.0001); the Quare River site received more light than the Turure.

Differences among single-sex populations in light received throughout the growing season were attributable to differences among populations (p < 0.0001), and results from contrast statements showed no differences between the sexes except in May when males were receiving more light than females (p = 0.01 in a univariate ANOVA). Overall, the three groups of

populations (USA both-sex, Trinidad both-sex and single-sex), showed similar patterns of females in lower light than males at some part of the growing season, but there were no significant differences for any populations (Figure 2.8).

Discussion

Populations of *M. inflexa* inhabit a well defined range of microhabitats, and female and male *M. inflexa* occur in separate patches within populations, and in single-sex populations, but based on the variables measured in this study, the sexes overlap in habitat use. Although the sexes are dimorphic in life history traits (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002), they did not use different substrates, or areas with different wind speed, humidity, or direction of exposure. There was considerable overlap between the sexes in their use of the light environment however, males inhabited a wider range of light environments than females, and tended to be found in microhabitats under more open canopy than females. Although there was an indication that males may use areas of higher light exposure than females, this pattern is difficult to tease apart from phenological effects involved in gametangial initiation and production.

General habitat

Populations occurred along banks of permanent streams, and conformed well to the general habitat description provided by Bischler-Causse (1989). Bischler-Causse (1989) described species of *Marchantia* as able to use all kinds of substrate, but limited in distribution primarily by availability of water and space. I detected wide variation in the amount of light received throughout the growing season among the populations. Additionally, there are populations along roads in Trinidad that may add to this variation and increase the range of habitat used by the species (DNM personal observations). This wide variation in microhabitat use is important because it is indicative of extreme phenotypic plasticity in habitat use. Specifically, genetic variation is hypothesized to be greater among isolated populations of bryophytes (that rarely, if ever, undergo gene flow) as compared to within populations. Thus, it is predicted that populations will diverge via drift or non-random processes, and variability within populations will be lower than among populations (Bischler and Boisselier-Dubayle, 1997). Because *M. inflexa* is plastic in habitat use, it is important to examine more populations rather than more individuals within a population to detect species-wide patterns of habitat use.

Distribution of the sexes

The sexes occurred primarily in single-sex patches within populations, but the sexes used the same light environments. The sexes differed in light received in particular months (in single-sex populations), but the pattern of monthly change did not differ across the growing season for the sexes. Comparisons of light received under the canopy throughout the growing season revealed a similar trend for males to occur in higher light environments, but this was not statistically significant for the three groups of populations examined.

The sexes showed different distributions within available light environments across populations, but this difference may be related to phenology of sex expression for the sexes. Our field sex structure counts may be biased against those males that live in low light and do not express sex. Data from a common garden experiment in which female and male *M. inflexa* were grown in a greenhouse under two shade treatments (Fuselier & McLetchie 2002) indicated that males under high light (55% shade) started sex structure production earlier than males under low light (73% shade) and very few males in low light produced sex structures after 180 days. We sampled populations when both sexes had sex structures, but before the peak production of archegoniophores (Fuselier, unpublished data). Thus, females may show a wider range or a different distribution among light environments later in the season when more plants have archegoniophores.

Sex ratio variation

Population sex ratios varied but tended toward female-bias or even ratios overall (for non-adjusted values) and, in temperate, both-sex populations, sex ratios were correlated with canopy openness. Because the four populations upon which this result is based were sampled within 2 weeks of one another but at different latitudes, this relationship may simply reflect a correlation of sex phenology with light environment, i.e., a long-day response to gametangial induction (Voth and Hamner, 1940; Longton, 1990). Further investigation into population sex ratios may reveal a geographic pattern to the sex ratio variation, or a relationship between sex ratios and other environmental factors. Bischler-Causse (1993) noted that the population sex ratio of *M. paleacea* varied geographically from the New World where males are common to the Mediterranean where only 5% of plants are male. More male (5) than female (2) populations of *M. inflexa* have been found in the USA (LCF unpublished data; Schuster, 1992) whereas, thus far, no single-sex populations have been documented from the tropic region of the species range.

A single patch model parametized for *M. inflexa* life-history traits implicates disturbance frequency as an important factor that influences patch sex ratios (McLetchie et al. 2002). The model predicts that high disturbance frequency results in all male patches whereas an intermediate disturbance frequency permits temporary coexistence of the sexes in a patch. (McLetchie et al. 2002). Population sex ratios in both-sex populations in the U.S.A. varied from male-biased to female-biased, and this pattern may be related to disturbance frequency and metapopulation structure.

McLetchie and Puterbaugh (2000) reported a sex ratio not significantly different from 1:1 for the Quare River population in March and no significant correlations between patch sex ratios and canopy openness (quantified with a densiometer; McLetchie & Puterbaugh 2000). Our sex ratio estimate in the Trinidad populations was based on 14 both-sex clumps within both-sex patches, whereas McLetchie and Puterbaugh (2000) based their estimate on total sex structure counts in 60 randomly chosen patches. In late May, we estimated female-biased population sex ratios, which would be expected if antheridiophores appear first in a population and gradually decrease as archegoniophores increase in number.

In Trinidad populations, there was a trend for the proportion of males in both-sex clumps to increase with decreasing light. After adjusting for differences in sex structure production between the sexes, this difference was not significant. One explanation for the contradictory nature of this result compared to analyses of the light environment that revealed males in areas of higher light, has to do with the time of year that the populations were censused, and plant phenology. Trinidad populations were censused when females had sporophytes in dehiscence whereas, in all other populations, archegoniophores were small and sporophytes were not yet visible. Personal observations of field populations and results from greenhouse studies indicate that in high light both females and males produce sex structures earlier than in low light (Fuselier and McLetchie, 2002), and patches in low light tend to have fewer sex-expressing plants (McLetchie and Puterbaugh, 2000). Thus, late in the season (after most females have sporophytes), males in low light will be expressing sex whereas those in higher light will have already expressed, and their antheridiophores may no longer be obvious. This phenology results in a an apparent pattern where males are in high light early in the season and low light late in the season.

Population sex ratios based on counts of sex structures do not necessarily reflect actual ratios of genetically distinct or physiologically independent individuals. However, McLetchie (unpublished data) found that, when non-expressing plants are grown to maturity in a greenhouse, field counts of sex structures are reliable indicators of greenhouse-grown plant sex ratios.

Patch and plant characteristics

Mean distance from a fertilized archegoniophore for *M. inflexa* was similar to mean sperm travel distances for other species of *Marchantia*.. Mean sperm flow distances for *M. chenopoda* were estimated at 4-10 cm (Moya 1993) and for *M. polymorpha*, 34 cm (Equihua 1987). This indicates that it is unlikely that archegoniophores in female patches will be fertilized.

I measured patch depth as a surrogate for patch age or stability. Both-sex patches were the largest, deepest and presumably, the most stable of the patch types. If scour of substrate during high water events is the primary form of disturbance in the Trinidad populations, large both-sex patches may experience less disturbance and thus, have time to grow, expansion and colonization of both sexes. Perhaps also, only portions of the BS patches are frequently disturbed, providing a mosaic of microhabitat within a patch such that males may continue patch residence in the face of competition from females. In Trinidad populations, male patches were significantly deeper than female patches. If patch depth is an adequate surrogate for disturbance, this indicates that male patches are the better established of the patches. This is contradictory to expectations because females have higher growth rates and were expected to create the deeper patches. On the other hand, patch depth may be a surrogate for patch persistence, but plants growing on the exposed surface of the patch may be new colonizers to the patch rather than the original inhabitants of the patch. As plants grow and cover those beneath them, plants on the bottom die (or at least do not appear to be photosynthetically active).

Despite the documentation of SSS in many bryophytes (Stark and Castetter, 1987; Stark, Mishler, and McLetchie, 1998; Bowker et al., 2000), intuitively, it should be maladaptive for bryophytes to exhibit SSS. Unlike dioecious angiosperms with abiotic pollination, *M. inflexa* relies on close proximity of females and males for successful fertilization. The proximity to females was the most influential trait in determining whether a male would sire offspring in a dioecious moss (Van der Velde et al., 2001). Thus, spatially segregated sexes must rely entirely

on asexual reproduction for continued existence. Asexual reproduction alone is often assumed to be detrimental for a species over the long term (Futumya, 1998). Thus, it is not surprising that there are no distinctly identifiable habitat use differences for the sexes but rather a continuum of overlapping habitat use characteristics for the sexes. The sexes are nevertheless, spatially segregated in that they occur in isolated patches outside of the range of average sperm dispersal or in geographically separated populations. This spatial separation may have ecological significance, and may result from sex-specific patterns of growth, reproduction, colonization and death related to disturbance frequencies, or in the case of single-sex populations, from colonization events in post-Pleistocene refugia (Longton and Schuster, 1983). Additionally, the fact that the species maintains sexual dimorphism (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002) when the sexes are not using different microhabitats within a population (at least to the extent that I detect here) points to other forces involved in the maintenance of sexual dimorphism rather than maintenance via differences in habitat use along an environmental gradient (Geber, Dawson, and Delph, 1999).

Table 2.1. Populations of *Marchantia inflexa* in which habitat parameters were measured. Location, population type, B = both-sex, F = female, M = male, and year sampled.

Site and abbreviation	Location	Latitude/longitude	Population type	No. Patches B, F, M	Year
Quare River (Q)	Trinidad	10.41/ 61.11	BS	25, 10, 8	2001
Turure River (R)	Trinidad	10.40/61.10	BS	21,7, 5	2001
Clark's Creek (MS)	Woodville, MS	31.017/91.506	BS	11, 7, 11	2002
Little Schultz (LS)	West Blocton, AL	33/87	BS	15, 10, 6	2002
Falls Creek (FC)	Dougherty, OK	34/97	M	-, -, 38	2002
Blue River (BR)	Connerville, OK	34/96	M	-, -, 35	2002
Turner Falls (TF)	Turner Falls, OK	34.425/97.148	M	-, 20 -	2001
Honey Creek Tributary (HCT)	Turner Falls, OK	34.425/97.148	M	-, -, 42	2002
Travertine Creek (TC)	Sulphur, OK	34.508/96.968	F	-, 10, -	2001
Byrd's Mill Creek (BM)	Fittstown, OK	34.615/96.634	F	-, 40, -	2001
Cooks Creek (CC)	Russellville, AL	34.62/87.719	BS	4, 3 , 3	2002
Fourth Creek (BD)	Bearden, TN	35.5/84	BS	11, 4, 15	2002

Table 2.2. General habitat description of *Marchantia inflexa* in the USA and Trinidad.

Habitat characteristic	Means	No. of populations
Water pH	7.9	4
Soil pH	7.87	9
Substrate	Rock and soil (>99% of plant locations)	4
Relative humidity	92%	4
Wind speed	0 – 1 knots	4
Soil particle content	48% sand, 36% silt, 15% clay	9
Soil P, K, Mg, Zn (lb/ac)	13, 222, 1250, 14	9
Total light received under canopy (ppfd)	13.5 umol/m^2/day	12

Table 2.3. Results from log-liklihood (G^2) tests for population sex ratios for *M. inflexa* from both-sex populations in the USA and Trinidad. Estimates are based on total numbers of sex structures counted. Estimates from USA populations are from randomly thrown quadrats whereas, estimates from Trinindad populations are sums of sex structures from both-sex patches only. Total area sampled is the sum of the number of 157-cm^2 quadrats. Adjusted estimates take into account 3 times higher production of sex structures by females.

		Unadjusted proportion male			Adjusted proportion male		
Population	Total m^2 sampled		G^2	*p*		G^2	*p*
BD	0.72	0.65	153.42	<0.0001	0.85	625.87	<0.0001
CC	0.33	0.49	0.01	ns	0.75	15.82	<0.0001
LS	0.79	0.25	60.19	<0.0001	0.51	0.10	ns
MS	0.79	0.35	2.28	ns	0.69	10.53	<0.0001
Q	0.19	0.44	4.00	0.05	0.70	26.33	<0.0001
R	0.17	0.41	10.42	0.001	0.67	23.70	<0.0001

Table 2.4. ANOVA results.
Results from analyses of variance for three groups of populations of *Marchantia inflexa*. Openness is percent of canopy open and ppfd is photosynthetically active photon flux density (umol/m^2/day) representing total irradiance throughout a growing season (12 mo for Trinidad and 7 mo for other populations). Patchsex was female, male or both-sex. Two female (F) and four male (M) populations were included in contrasts between single-sex populations.

Populations	Character	Effect	*df*	*F*	*p*
Q and R	Openness	Population	1	1.09	0.30
		Patchsex (Pop)	4	0.14	0.96
	PPFD	Population	1	7.55	0.01
		Patchsex (Pop)	4	0.46	0.76
USA both-sex	Openness	Population	2	1.62	0.20
		Patchsex (Pop)	6	1.89	0.09
	PPFD	Population	2	0.03	0.97
		Patchsex (Pop)	6	1.55	0.17
Single Sex	Openness	Population	5	9.59	<0.0001
	PPFD	Population	5	2.17	0.06
	Openness	F vs M contrast	1	0.11	0.73
	PPFD	F vs M contrast	1	0.32	0.57

Figure 2.1a. Soil N and Mg content.
Soil composition for 3 soil samples taken from 7 USA populations in 2002. X-axis indicates population name, type (B= both sex, M=male, F=female) and location (TN=Tennessee, MS=Mississippi, OK=Oklahoma). Population name abbreviations are given in Table 2.1

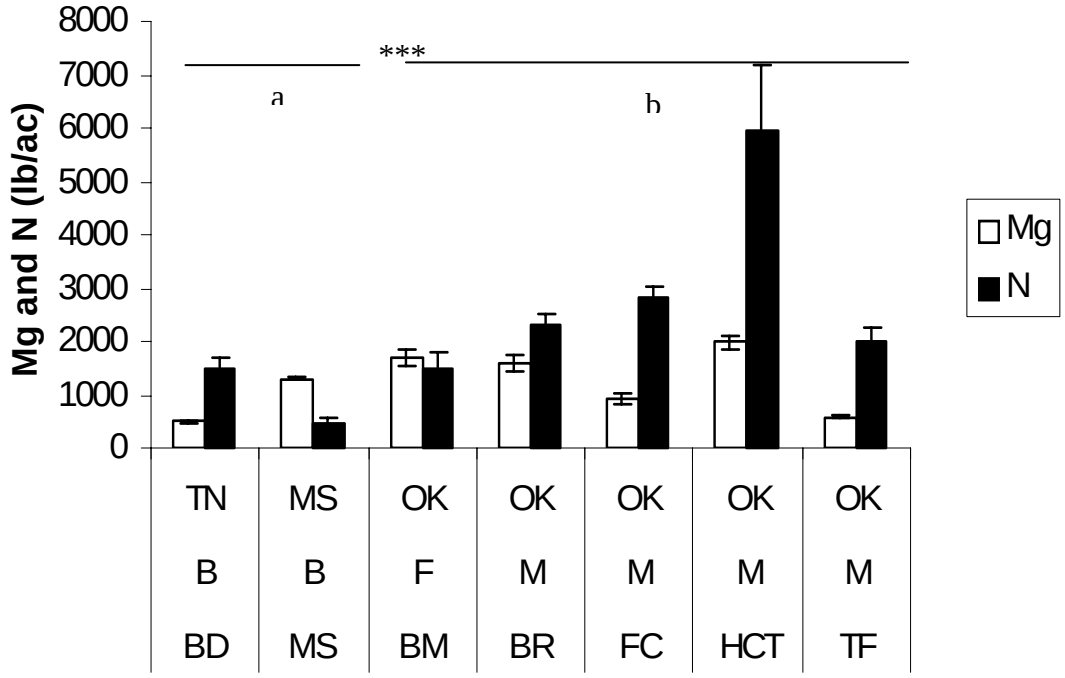


Figure 2.1b. Soil composition in percent sand, silt and clay in oven dried soil sample.

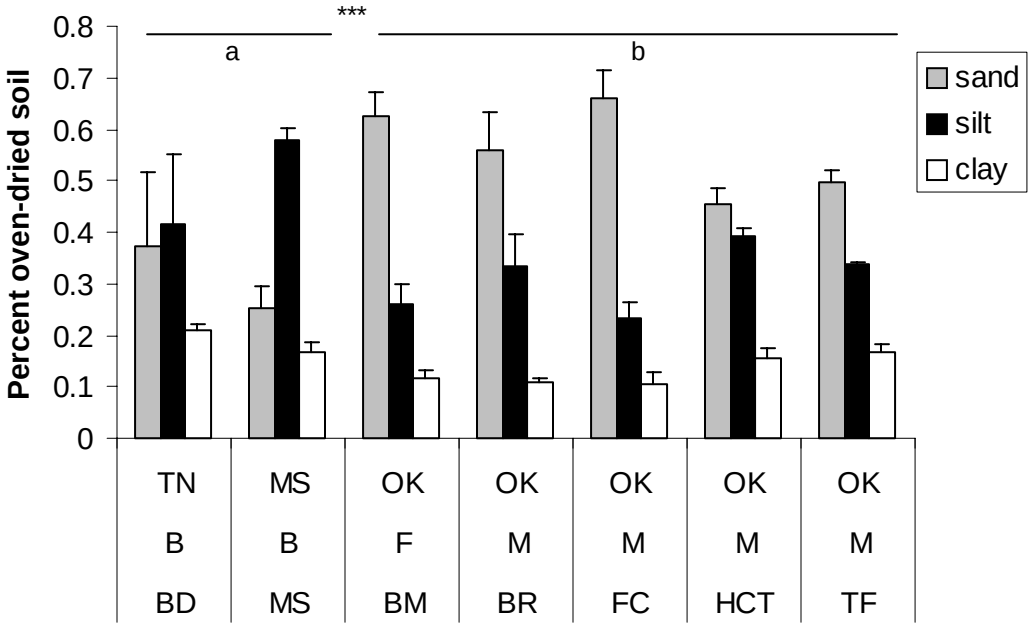


Figure 2.2. Patch type frequency in both-sex populations.
Frequency of occurrence of patch types in four both-sex populations in the USA.

B=both-sex, F=female, M=male, N=non-expressing, BD=Bearden, LS=Little Schultz,

MS=Clark's Creek, CC=Cooks Creek.

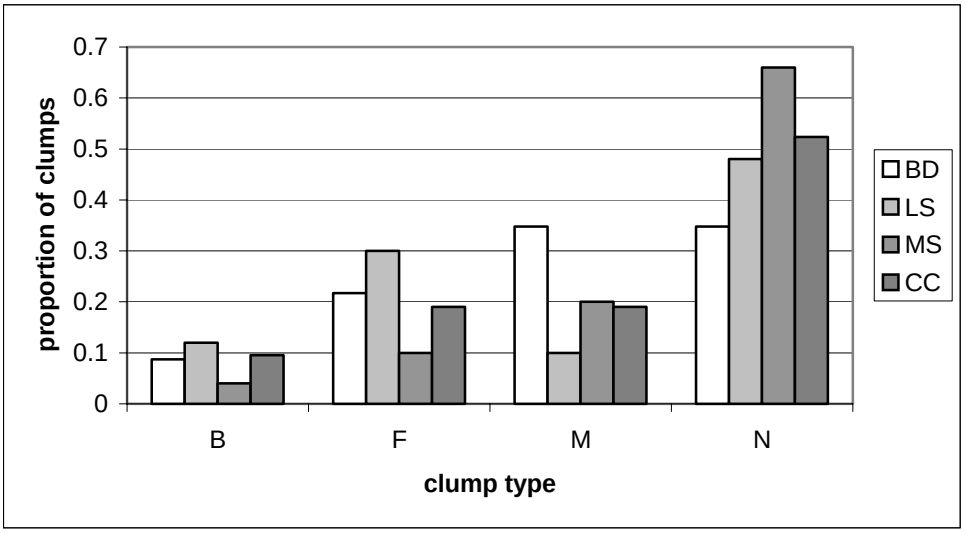


Figure 2.3. Population sex ratios.
Expressed as proportion of antheridiophores/total gametophores was directly positively related to mean canopy openness for four both-sex populations of *Marchantia inflexa* in the USA.

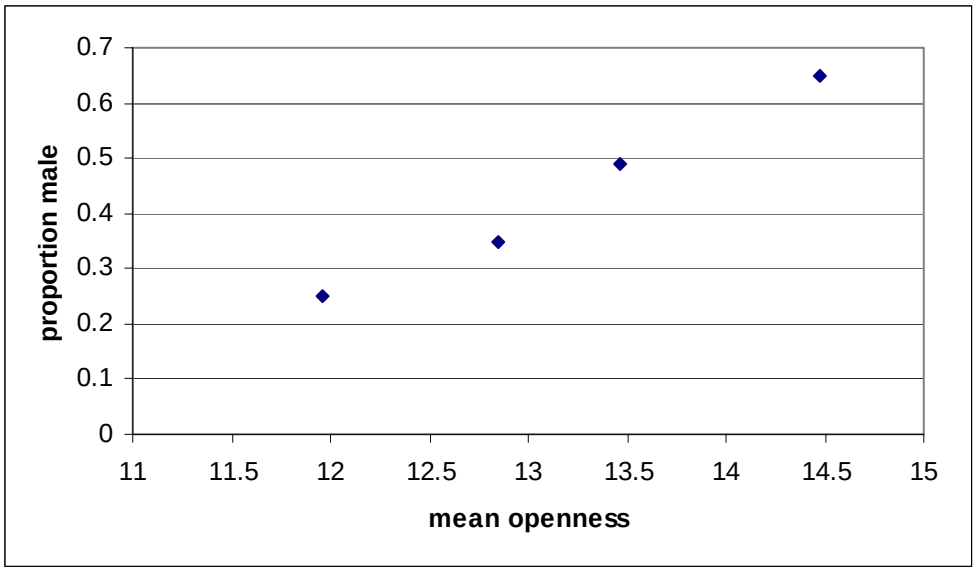


Figure 2.4. Patch sex ratios in Trinidad.
Sex ratio expressed as proportion of males in patches from two Trinidad populations combined, in relation to light received under the canopy. Ratios are not adjusted for differences in gametangiophore production between the sexes. Dashes indicate the upper (u95) and lower (l95) 95% confidence intervals.

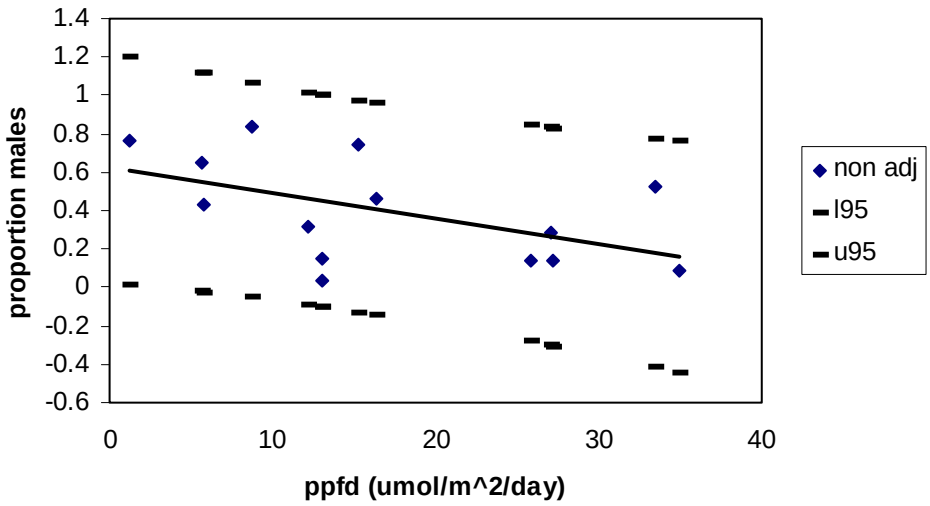
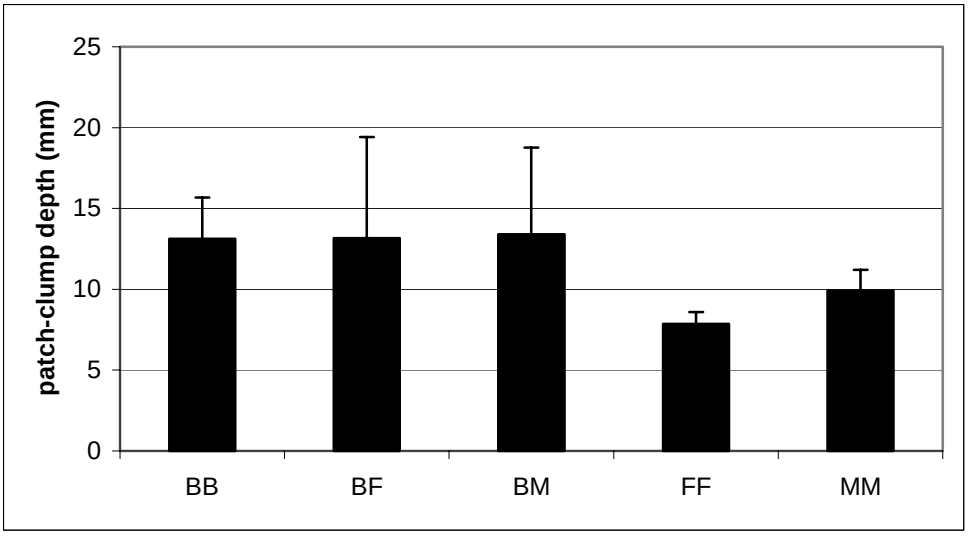


Figure 2.5 a. and b. Clump depth and patch area.
Depth of clumps measured from surface to substrate, and b) patch areas for both sex clumps in both sex patches (BB), female clumps in both sex patches (BF), male clumps in both sex patches (BM), and female (FF) and male (MM) patches in 2 Trinidad populations.



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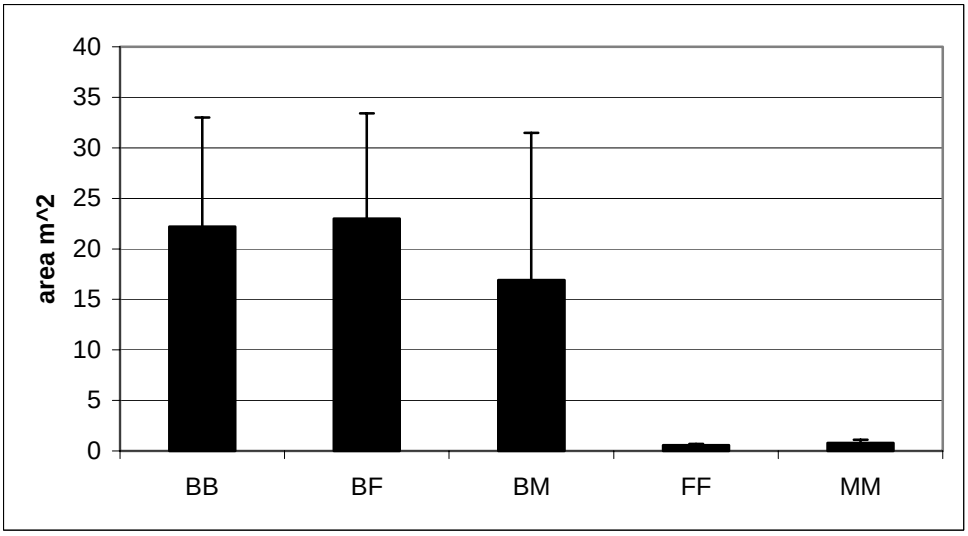
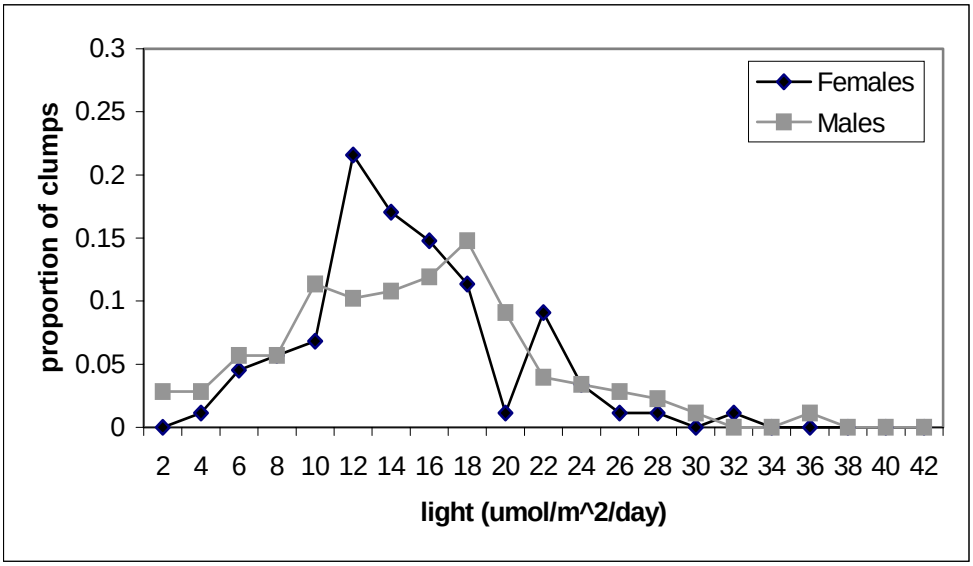


Figure 2.6. Distribution of sexes among light environments.
Frequency distribution based on proportion of patches out of the total number of patches of female (n=88) and male (n=176) *Marchantia inflexa* across light environments in 12 populations.



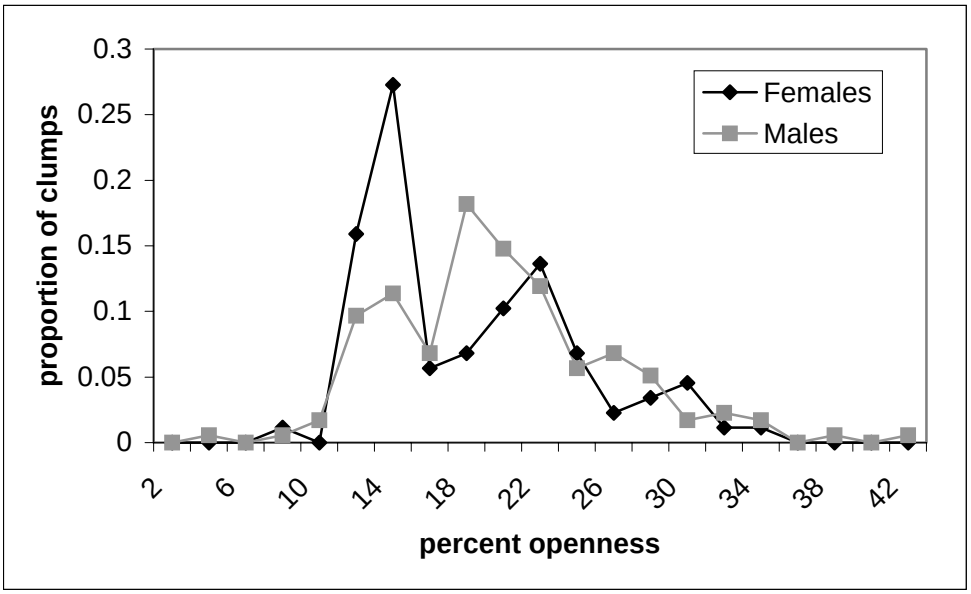
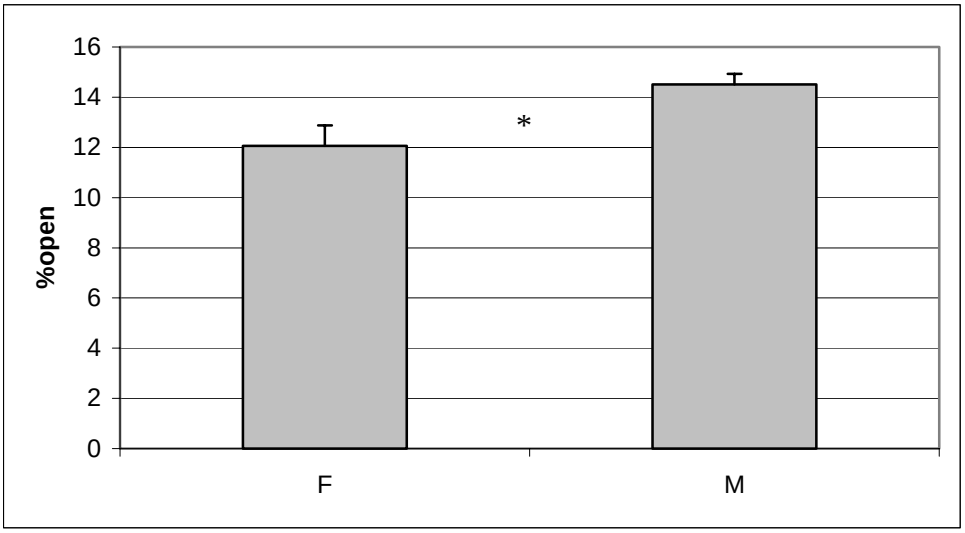


Figure 2.7 a and b. Female and male use of light environments.
Mean canopy openness (a) and light received under the canopy (b) in ppfd for both-sex USA populations of *Marchantia inflexa*. Asterisk indicates significant difference between the sexes at $P<0.05$.



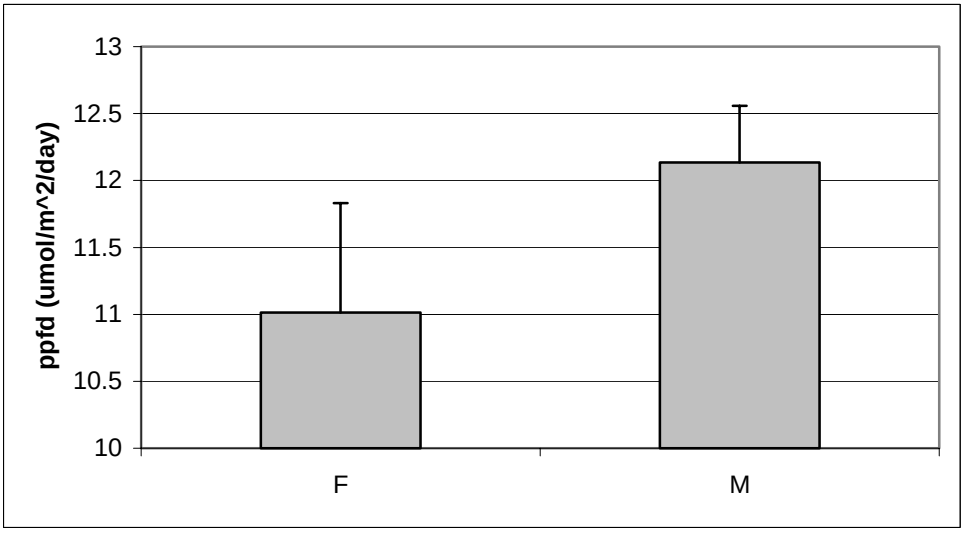
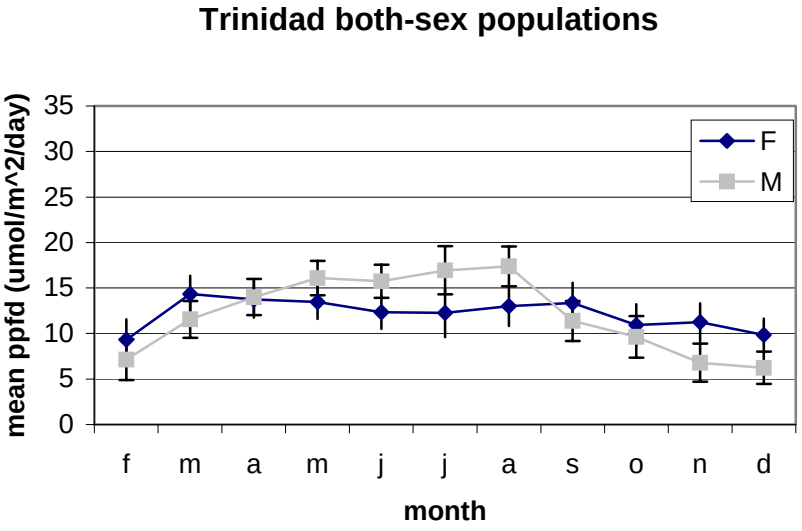


Figure 2.8a & b. Light received at patches throughout the growing season.
Amount of light (direct and indirect) received at female and male *Marchantia inflexa* patches through the growing season for three sets of populations with populations within each set combined.



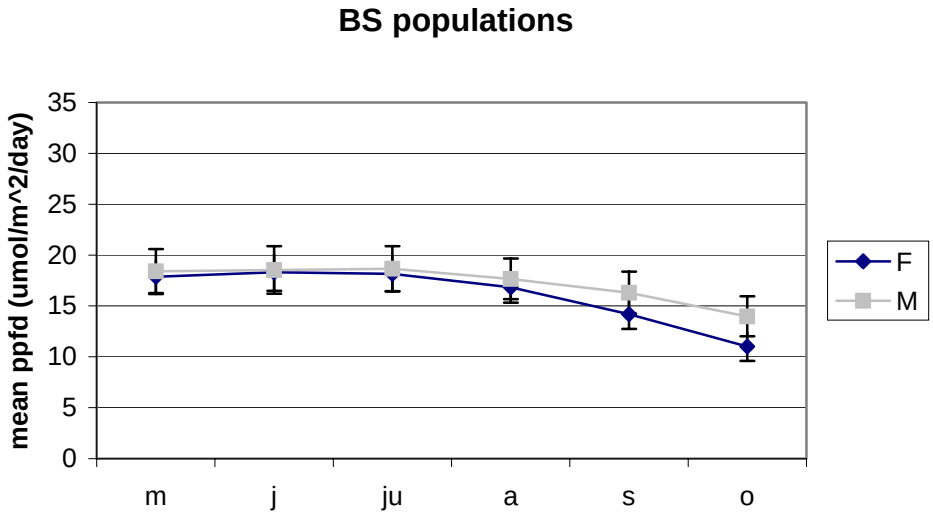
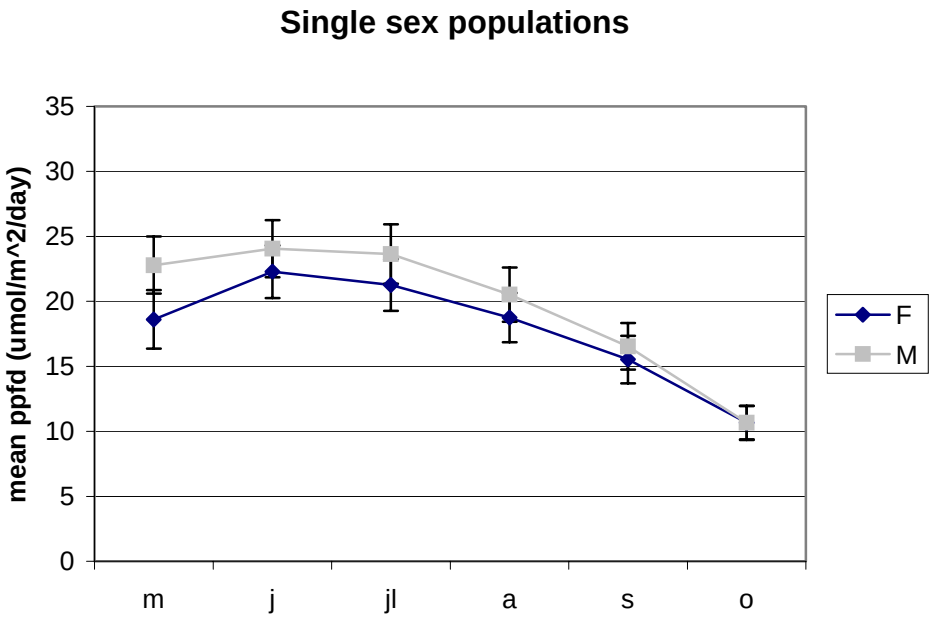


Figure 2.8 c. Light received in single-sex populations.



Chapter Three: Variation in growth, reproduction and sexual dimorphism in single-sex and both-sex populations of *Marchantia inflexa*

Summary – A common garden experiment and field observations were used to assess trait variation, and determine differences in sexual dimorphism among populations, and between single-sex and both-sex populations of *Marchantia inflexa,* a dioecious liverwort. Populations were significantly genetically differentiated, and plants from single-sex and both-sex populations differed in their life history strategies. Plants from single-sex populations invested more in growth than asexual production, and although they produced as many sex structures, they produced fewer gametangia per gametophore than plants from both-sex populations. Trait means were more variable across populations than the degree of sexual dimorphism. Plants from female and male populations displayed the same amount of sexual dimorphism as sexes within both-sex populations, indicating that local environment may be more influential than the presence of the opposite sex in maintaining sexual dimorphism. Significant among trait correlations may slow evolution of trait means, but between-sex correlations were not significant and thus, to the extent that they represent genetic correlations, may not constrain the evolution of sexual dimorphism in *M. inflexa*.

Introduction

Within population genetic architecture provides insight into relatively recent responses of a population to selection (or drift) whereas, among population genetic architecture provides a picture of the historical components that resulted in the assortment of genotypes found in particular populations (Pigliucci and Kolodynska 2002). Among population genetic architecture is also important in understanding the evolution and maintenance of sexually dimorphic traits (Delph et al 2002). Because between-sex and among-trait correlations constrain the evolution of sexual dimorphism, Lande (1980) predicted that trait means will evolve faster than sexual dimorphism across populations. However, because these correlations may change among environments (Galen, 2000; Pigliucci and Kolodynska, 2002), sexual dimorphism can vary among populations.

To provide a better picture of the influence of history on the evolution of sexually dimorphic traits, I examined among-population variation in sexually dimorphic traits in a

dioecious liverwort that exists in single-sex and both-sex populations. First I measured differences in traits among populations and, specifically, between single-sex and both-sex populations, and then examined variation in sexual dimorphism among populations, and possible constraints to the evolution of sexual dimorphism.

Application of sexual dimorphism theory to bryophytes is novel because most studies of sexual dimorphism in plants come primarily from investigations of non-clonal, dioecious angiosperms. Unlike most angiosperms, all bryophytes are clonal (During, 1990; Newton and Mishler, 1994), bryophytes have a haploid-dominant life cycle, and many occur in single-sex populations, (either females or males, separated from other populations and perpetuated solely through asexual reproduction). Further, only approximately 6% of angiosperms are dioecious (Renner and Ricklefs, 1995; Shaw, 2000) whereas, an estimated 57% of mosses and 68% of liverworts are dioecious (Shaw, 2000).

To examine factors that lead to the sorting of phenotypes into populations, and to document changes in life history strategies associated with life without sexual reproduction, I measured phenotypic differences among plants from single-sex and both-sex populations across a wide geographic range. Single-sex populations of my study organism, *Marchantia inflexa*, presumably result from expansion of plants that found refugia during the last Pleistocene sea transgression (Longton and Schuster, 1983). Genetic differences between single-sex and both-sex populations may arise for various reasons. These differences may arise because of phylogenetic relationships among populations, i.e., all single-sex populations originated from closely related plants. If successful long range dispersal is unlikely, such a relationship should also produce a pattern where geographically close populations are more closely related genetically, and are phenotypically more similar than geographically distant populations. Alternatively, plants from single-sex populations may form a distinct phenotypic group because only particular phenotypes are suitable as new colonizers or survivors in refugia. Thus, plants from single-sex populations may be more similar to each other in traits favored by the selective environment under which initial colonization or expansion from refugia occurred, and these similarities will not be correlated with geographic distribution or phylogenetic relationships. The sorting of genotypes into populations may also result from random processes, in which case, there should be no phylogenetic nor geographic pattern to genotypes found among populations

Investment in growth, asexual and sexual reproduction in single-sex and both-sex populations is expected to differ. There is a tripartite trade-off among growth, asexual reproduction and sexual reproduction in *Marchantia inflexa* (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002). Because there is no sexual reproduction in single-sex populations, and presumably sex has not occurred since isolation of these populations approximately 10,000 years ago following expansion from refugia, investment in sexual reproduction is expected to be lower in plants from single-sex than plants in both-sex populations. Consequently, lack of investment in sex is expected to result in an increased investment in either growth or asexual reproduction.

Single-sex populations of dioecious bryophytes provide a natural system in which to examine variation in sexually dimorphic traits among populations that regularly undergo sexual reproduction and those that never experience episodes of sex. This is significant because differences in sexual reproductive ecology are presumed to be the main driving force for the maintenance of sexual dimorphism (Geber, Dawson, and Delph, 1999). Thus, populations that do not experience sex may exhibit reduced sexual dimorphism. If the presence of the opposite sex in a population is involved in the maintenance of sexual dimorphism, then both-sex populations should show greater sexual dimorphism than single-sex populations. If however, single-sex populations show greater sexual dimorphism, local environmental factors may be more influential in determining the phenotype of the sexes. If the two population types do not differ in degree of sexual dimorphism, stochastic factors may be most influential in driving differences in SD among populations.

The degree to which sexual dimorphism is expressed will reflect a balance among constraining factors (Lande, 1980). Expression of dimorphic characters is constrained by: 1) the amount of genetic variation in dimorphic traits present in a population, 2) correlations among traits within a sex, and 3) between-sex genetic correlations that can prevent or slow the evolution of dimorphism, (Lande, 1980; Lande and Arnold, 1983; Meagher, 1994; Delph, Knapczyk, and Taylor, 2002).

I planted female and male *Marchantia inflexa*, a dioecious liverwort, from populations throughout its range in a common garden experiment to examine phenotypic differences between single-sex and both-sex populations, and variation in and constraints to sexual dimorphism. Use of a common garden eliminates differences that are caused by environmental differences among

populations. If there are no maternal effects, differences among phenotypes in a common garden reflect genetic differences in traits. I were unable to discern among alternative hypotheses for understanding why differences in sexual dimorphism occur, but I provide the first account of differences between single and both-sex populations, and fodder for further research into how these populations may be used to test hypotheses regarding the maintenance of sexual dimorphism in plants.

Objectives & predictions

The objectives of this experiment were to: 1) quantify phenotypic (and genetic) differences among populations in traits related to growth and asexual reproduction, 2) determine whether there is a geographic pattern to phenotypic variation among populations, 3) compare growth, asexual and sexual reproduction between single-sex and both-sex populations, 4) determine the variation in and degree of sexual dimorphism in both-sex and single-sex populations, and 5) quantify between-sex and among-trait correlations that may constrain the evolution and expression of sexual dimorphism.

I predicted that populations would show genetic variation in traits and that populations closest geographically would be most similar phenotypically. Additionally, because plants in single-sex populations may not invest in sexual reproduction to the degree that plants from both-sex populations do, plants from single-sex populations would invest more in either growth or asexual reproduction compared to plants from both-sex populations. In bryophytes, genetic variation is expected to be partitioned among populations rather than within populations, so I predicted that sexual dimorphism will differ among populations but sex differences between single-sex populaitons should be higher than tha between both-sex populations.

Methods

Common garden experiment

To monitor growth rate, asexual and sexual reproduction of *M. inflexa*, 810 thallus tips with mericells were sowed (January 26, 2003), into pots in a greenhouse and monitored for 7 months. Plants originated from fifteen populations (Table 3.1) that occur throughout the range of *M. inflexa;* Trinidad populations represent the southern, and Tennessee populations, the northern reaches of the species range. Plants of known and unknown gender were included such that the total number of isolates from a population ranged from 15 to 36 (Table 3.1). I defined an isolate as a plant collected from a distinct patch of plants within a population. A patch was defined as a

group of plants separated by neighboring groups by at least one meter, typically with an obstruction (such as unsuitable substrate) between patches. In most cases, I collected vegetative plant tips from a plant that had both expressing an non-expressing tips to be certain of the sex of the plant tip. In some single-sex patches, I could not definitively identify that the tip I collected came from the plant with the sex structures thus, there is a low probability that the plant may have been from a non-expressing plant of the opposite sex . The collected tips were presumed to be female or male, respectively. Each tip was collected from a different, randomly chosen patch along 0.5 - 3 km of stream length. Plants from Trinidad were collected from single-sex patches where sex and presumed to be the sex of the only sex-structures found in the patch. My collection method increased the likelihood of collecting individuals that were not members of the same genet. I collected plants from patches in each population, returned them to the greenhouse at the University of Kentucky and grew the plants on steam-sterilized soil from Kentucky under 55% shade in temperatures ranging from 22 ^{o}C to 25 ^{o}C. These stock plants produced cups before being used in the experiment and many had expressed sex. Thus, all isolates used in the experiment were begun from mericell-bearing tips of greenhouse stock grown under similar conditions to minimize maternal effects or any latent site-specific environmental effects. Suwannee River males were an exception to this because, although they were grown with the other stock plants, they started producing antheridiophores in the greenhouse and did not produce cups (undergo asexual reproduction) before use in the experiment. There is always the possibility that non-sex-expressing plants collected from a "single-sex" patch were plants of another sex but I did my best to minimize this probability.

Two replicates (tips) of each isolate from each population were planted into individual pots 5.9 cm diameter and 2.7 cm deep situated in trays of filtered water on one greenhouse table. Positions within trays were determined randomly, replicates were in different trays and trays were rotated on the table every four weeks. Plants were grown under 13 hr day lengths with greenhouse temperatures ranging from 16 to 20 ^{o}C for the first 42 days of the experiment (until March 10, 2003) at which point day length was increased to 14 hours and temperatures 18 – 22 ^{o}C for the remainder of the experiment. Daylength was increased because species of *Marchantia* are likely "long day" plants (Longton, 1990) and I wanted to increase the chances of sex-expression in the greenhouse. Artificial light from sodium bulbs was used to manipulate daylength.

Plants from 9 both-sex populations, 4 male and 2 female populations were included in the experiment. Although I did not include the plants in the experiment, I collected *M. inflexa* from Devil's Millhopper in Gainesville, FL. I mention this because Schuster (1992) described the population at Devil's Millhopper in Florida as a female population, but, when I collected, both sexes were present there in 2003. Schuster (1992) described the Suwannee River population as all-male, and in 2003, there was no indication that females were present. This population was considered a male population for analyses, but because the population is large and was visited only once, this may be an erroneous classification. The single-sex populations in Oklahoma were visited in numerous sequential years, at different times of the year and there has been consistently only one sex present. Sporophytes have never been detected in the female populations (LCF unpublished data). Nevertheless, the sex of plants in the field can be determined only when the plants have sex structures thus, populations designated as "single-sex" may harbor plants of the opposite sex that fail to produce sex structures.

Traits

The initial size of the mericell-bearing tips was measured 10 days after the start of the experiment and used as a covariate in analyses. Counts of cups and plant size measures were made at 10, 32, and 41 days after the start of the experiment, and counts of sex structures at 41, 61, 105, 150, and 177 days after the start. Number of days to cup and sex structure production were recorded but limited to the days on which plants were photographed and measured. Green plant area was measured using a digital image of the trays and ImageJ software, a public domain NIH program (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/nih-image/). The relationship between growth and asexual reproduction likely represents a developmental trade trade-off (Watson, 1984) because a mericell can either divide and grow into two branches, hence increasing green plant area, or produce a cup, but not both.

The length of two branches and the thallus width was measured for one replicate of each isolate 105 days after planting to assess the degree to which plants branched to form tight clusters of thalli, similar to a rosette, or long, trailing thalli, an "extender". Additionally, from photos taken on day 61, number of branches was counted and the average branch size was computed. These measures quantify the extent to which plants invest in more mericells versus growth at existing mericells. These traits were chosen because superficially, two growth forms

are visible in the field; one form with many branches and rosette-shaped growth and another form with few long branches, “extender”.

Field observations

To quantify differences in sex expression and investment in gametangia under field conditions, and assess sexual reproductive effort in populations, surveys of sex expression, and sex structures were conducted in both-sex USA populations and single-sex populations in OK in May 2002. I conducted monthly systematic surveys of sex structures in three both-sex populations (CC, LS, MS) to determine the peak of sex-expression for each sex. Degree of sex expression in populations (both-sex USA and single-sex Oklahoma) was assessed by walking down the middle of the stream, tossing a quadrat haphazardly into a patch of plants and counting the number of sex structures within the quadrat for at least 50 quadrats per population. In May, 2002, I collected sex structures from 3 male populations in Oklahoma (TF, BR, FC), the 2 female populations (BM, TC), and 3 both-sex populations (CC, LS, BD) to examine numbers of gametangia (archegonia and antheridia) per sex structure. Archegoniophores and antheridiophores were collected from patches by throwing a marker haphazardly into a patch of expressing plants and harvesting an archegoniophore and an antheridiophore nearest the marker. Wet mass of sex structures was measured with a digital balance to the nearest 0.1 mg, structures were dissected under a dissecting microscope and numbers of antheridia and archegonia were counted.

Statistical Analyses

Informative traits

To choose traits that were informative in distinguishing among populations and between sexes, I used a principal components analysis (PCA) to express traits as 2 PC axes. Sixteen variables were included in PCA: growth rate (over 51 days), plant area at days 10, 32, 41 and 61, thallus width and length, area per branch, cups per area at days 10, 32, and 41, cup counts for days 10, 32, and 41, and cup production rate (cups/area/day at day 41). Counts of sex structures were not included because very few plants expressed sex throughout the duration of the experiment. I omitted form PCA Apalachicola River (AP) males because there was only one male genotype, but I included AP females. PC scores were used in an ANOVA with contrast statements to determine if the sexes or the population types differed. The traits that were most

informative in explaining variation on the first two principal component axes were used in subsequent analyses of trait values.

Population differences

To quantify phenotypic differences among populations, trait values were used in an ANCOVA with plant area at day 10 as a covariate, and population as a main effect. All populations (n = 15) and all plants, including those of unknown sex, were included in this analysis, and growth rate, area per branch, cup production rate, and total number of cups were analyzed. The variable "total number of cups" was the total number of cups produced by day 41. Total number of cups was created and used in ANCOVA because cup counts were informative in PCA and this new variable compacted cup counts into one trait. *A posteriori* multiple comparisons tests (REGWQ option in SAS) on means were used to determine which populations differed.

Differences between both-sex and single-sex females and males were compared in an ANCOVA using a population-sex label as the main effect (e.g., SF for female plants from single-sex populations) and area at day 10 as a covariate. Contrast statements were used to determine differences between the sexes from the two population types. F-ratio tests were used to compare variation in trait values between the two population types.

Variation in sexual dimorphism

Overall patterns of sexual dimorphism were established using an ANCOVA with sex as a main effect and plant size at day 10 as a covariate. To determine patterns of variation in sexual dimorphism among both-sex populations, trait values were used in two-factor ANCOVA where plant area at day 10 was used as a covariate and population and sex were main effects. Variables examined in analyses were: growth rate, cup production rate (cups/mm^2/day), branch size and total cups produced. Apalachicola River plants were dropped from this analysis because there was only one male plant (Table 3.1), thus, the analysis included 8 both-sex populations (because Devil's Millhopper plants were not included in the PCA because of low sample size of plants that did not have negative growth rate by day 41). If there was no significant interaction between population and sex, the interaction term was removed from the model and the analysis was rerun. If sex had a significant effect, a categorical variable that combined population and sex was created and run as a one-way ANCOVA to determine which populations exhibited significant sex differences.

PCA measure of sexual dimorphism

The placement of populations and sexes on principal component axes was used to calculate a multivariate measure of sexual dimorphism and to compare sexual dimorphism between both-sex and single-sex populations. Euclidean distances between coordinates of PCA scores for sexes within a population were used to quantify sexual dimorphism in both-sex populations. For single sex populations, the average Euclidean distance between a male population (SR, TF, BR, FC) and the two single-sex female populations was used for the distance value. Mean distances between the sexes within populations for both-sex and single-sex populations were compared using a Mann-Whitney U test and rank values of Euclidean distances. I note that this test violates assumptions of non-independence of data points because sexual dimorphism values for the single-sex male populations are all based on distance to the same two female populations.

Correlation

Because most of the single-sex populations are clustered in the Arbuckle Mountain region of southern Oklahoma, USA, I examined whether the geographic distance between populations explained phenotypic differences observed among populations. To test for correlation between geographic distance and phenotypic differences, Euclidean distances among populations for scores on pc1 and pc2 for females and males separately were compared to a geographic distance matrix using Mantel's test (Leidloff, 1999). Mantel's test uses a randomization procedure to examine association between two distance matrices with an alternative hypothesis of a positive correlation between the two matrices. Geographic distances were straight line distances calculated with approximate longitude and latitude coordinates given in Table 3.1. The order that matrices were analyzed was alternated to ensure consistent results. Between-sex and among-trait correlations (within populations) were examined using Pearson product moment correlations and associated probability values with population means. To calculate between-sex correlations, I used population means as a surrogate for family means so these correlations are a conservative estimate of genetic correlations rather than phenotypic correlations. Because between-sex correlations are not based on correlations between individuals of the same genotype, they are not necessarily indicative of genetic constraints.

I used t-tests to examine differences between plants that expressed sex and those that did not express sex (produce sex structures) during the course of the experiment. Sex expression

rates were examined primarily to help explain some of the inconsistent patterns observed between my results and those of previous experiments.

All analyses were done using SAS, release 8.02 (SAS, 1990) and, for the common garden experiment, were conducted with the mean value for the 2 replicates of each plant. Plants that died or had a negative value for growth rate by day 41 were excluded from analyses. In all analyses, count data were squareroot-transformed and proportion data were arcsine square root – transformed to improve normality. On graphs and in tables, data are presented as non-transformed values.

Field

Phenology of sex expression in three both-sex populations in the USA was graphed to determine the peak of sex expression for either sex. Differences in the degree of sex expression between population types was compared using an ANCOVA with latitude as a covariate to account for any differences in degree of sex expression attributable to the influence of latitude on time of sex structure collection. Nested ANOVA with population nested within population type (either SS or BS) for each sex was used to compare number of gametangia produced per sex structure between population types.

Results

Most plants were included in analyses though some did not meet criteria and these included one QR, 2 DR, 1 SR, 1MS, 1 BM, 1 CC, and 1 FC plant. There was only one replicate used for 3 BMF, 4 DRF, 2 DRM genotypes, 1 QRF and 1 QRM genotype. With the exception of these differences, sample sizes are consistent with those given in Table 3.1.

Informative traits

The first two principal components accounted for 52.3% of the variation among genotype means (n=190) for 16 variables. The first axis incorporated 34.8% of the variance among samples and was considered a “size” axis because all variables loaded positively, and variables with large positive eigenvectors were related to growth or size of plants (Table 3.2). The traits related to cup production that loaded high on this axis were also positively correlated with growth or size characteristics. The second axis accounted for an additional 17.4% of the variation and was primarily an “asexual reproduction contrasted with growth” axis, where cupules per area had the largest eigenvector value and size variables were all negative loadings

(Table 3.2). In general, populations with high scores on PC 1 were large plants, whereas a high score on PC2 indicated a higher investment in cup production as opposed to growth (Figure 3. 2).

Subsequent analyses incorporated traits from PCA that were informative and a "composite" trait, total number of cups, as a surrogate for cup counts that were informative in PCA. Growth rate (over 51 days) was correlated with all other area measurements ($r = 0.68$ to 0.96, $P < 0.0001$), and all loaded high on the first PC axis. Cups produced per area of plant per day loaded highest, and area per branch lowest on PC2. Thus, variables examined in analyses of variance and covariance included: growth rate, total number of cups produced, cup production rate (cups/area/day), and area per branch.

Population differences

Populations were genetically differentiated in growth rate, numbers of cups produced, cup production rates and branch sizes (Table 3.3 and Figure 3. 3 a-d). Plants from the Dominican Republic (DR) and Trinidad (QR), the two tropical populations included in analyses, were among the slowest growers whereas, plants from the two female populations (BM and TC) were among the fastest growers. Plants from the four single-sex populations and the Apalachicola population had the largest branches whereas, the Trinidad, Dominican Republic and CM (Tennessee) plants had the smallest. Apalachicola River and Falls Creek plants were among the lowest cup producers, and Suwannee River plants did not produce cups.

Plants from single-sex populations ($n = 6$) were larger and had larger thalli with fewer cupules than those from both-sex populations ($n = 10$; Table 3.4). Females and males from single-sex populations had larger branches than the same sex plants from both-sex populations. Males from single-sex populations produced fewer cups per day than males from both-sex populations, and females from single-sex populations grew larger than females from both-sex populations (Table 3.4). Variance in growth rate did not differ between population types for females or males, but variance in cups produced per day, total cups produced, and area per branch was higher for females and males in both-sex populations than in single-sex populations (Table 3.5).

Principal components analysis

If my interpretation of the first two PC axes is reasonable, females invested more in growth than males, and single-sex populations invested more in growth and less in cup production than both-sex populations. In ANOVA, sexes differed in PC1 ($df = 1$, $F = 16.63$, $P < 0.0001$) and population types differed in PC2 ($df = 1$, $F = 17.75$, $P < 0.0001$) scores. Difference between the sexes on PC1 with females displaying an overall higher value than males, suggests that females invested more in growth/size than males but because the sexes did not differ on PC2 ($F = 2.42$, $P = 0.12$), the sexes were similar in relative investment in asexual reproduciton versus growth. Population types did not differ on PC1($F = 1.0$, $P = 0.31$) but did differ on PC2, indicating that single-sex populations displayed a different asexual reproduction to growth continuum of investment than both-sex populationsl. This is also supported by the trend (discussed above) for plants from single-sex populations to grow larger and produce fewer cups than plants from both-sex populations.

Sexual dimorphism

Overall, females grew larger than males and produced more cups per area per day, and a higher total number of cups than males (Table 3.6). Degree of sexual dimorphism in growth rate and total number of cups varied among populations as evidenced by significant population × sex interaction terms in ANCOVA (Table 3.7). Females grew significantly larger than males in two populations, CM and SB (see Table 3.1 for abbreviations). Total number of cups produced differed between the sexes in two populations, CM and DR, but these populations showed opposite trends. In CM, males produced more cups and in DR female plants produced more cups. Cup production rate differed between the sexes in only one population, CC, where females produced more cups than males (Table 3.7).

Sexual dimorphism, measured as the Euclidean distance between sexes of the same population in PC space, varied among populations. Plants from Trinidad displayed the least and plants from CM, the greatest differences between the sexes (Figure 3. 4). The difference in principal components space between sexes within populations was not correlated with latitude ($R = 0.15$, $P = 0.64$). The average distance between the sexes within populations in PC space was

not significantly different for single-sex and both-sex populations (P = 0.23) though, there was a trend for single-sex populations to display higher dimorphism.

Geographic differences

Phenotypes of female plants were positively related to geographic distance of populations (r = 0.77, P < 0.05) whereas, there was no correlation between phenotype and geographic distance (r = 0.19, P > 0.05) for male plants. These patterns held when Florida populations (Suwannee River males and Apalachicola River females) were excluded from analyses.

Correlations

There were no significant between-sex correlations (n = 11) for growth rate (r = 0.31, P = 0.34), cup production rate (r = 0.49, P = 0.12), total number of cups (r = 0.35, P = 0.27) or area/branch (r = -0.07, P = 0.83). Both females and males displayed significant among-trait correlations. In both sexes, growth rate was positively correlated with total cups produced and, in males only, total cups produced was positively correlated with cup production rate and branch size, negatively correlated with cup production rate (Table 3.8).

Sex

Plants that produced sex structures delayed cup production. A total of 33 plants, 21 males (16 isolates), 12 females (9 isolates), from four populations (DM, TT, DR, SR) expressed sex by day 215. Males started sex expression earlier (mean = 51 days) than females (mean = 90 days). However, females tended to produce more sex structures than males (females mean = 0.63, males mean = 0.48 sex structures per plant on average over six census periods). Sex-expressing plants did not produce cups until day 32 (with the exception of one female that produced at day 21) whereas, 47% of the non-expressing female, and 39% of the non-expressing male isolates produced cups prior to day 32.

Only one DR and one DM male produced sex structures so, no statistical comparisons within these populations were possible, but neither produced cups before day 32. No SR males produced cups, but those that produced sex structures (n = 6) tended to be larger than the SR males that did not sex express (n = 19, d.f. = 22, t = -1.64, P = 0.11). Plants from Trinidad that expressed sex did not differ in growth rate or cup production from plants that did not express sex (t-test with unequal variances for females, n = 12.5, equal variance for males, n=15, t values ranged from -0.59 to 1.23 and P values from 0.24 to 0.36). However, expressing females tended to be larger and produce more cups, and expressing males tended to be smaller and produce

fewer cups than non-expressing plants of the same sex. The same percentage of isolates, 28.5%, produced cups by day 41 for both expressing and non-expressing females and males from Trinidad.

Field Results

Monthly systematic surveys of sex structures in both-sex USA populations indicated a phenology of sex expression such that in April, few archegoniophores were present and in July, few antheridiophores were present in populations (Figure 3. 1). Single-sex populations produced as many sex structures as both-sex populations. In ANCOVA, after correcting for differences in latitude, numbers of archegoniophores ($F = 1.26$, $P = 0.26$, $df = 1$) and antheridiophores ($P = 0.97$) in single-sex populations did not differ from numbers of sex structures in both-sex populations. Males from single-sex populations in Oklahoma produced fewer antheridia per mg of antheridiophore than males from both-sex USA populations (nested ANOVA, $df = 7$, $P < 0.0001$; Figure 3.5). Likewise, females from single-sex populations produced fewer archegonia per archegoniophore ($P = 0.04$, $df = 4$) than females from both-sex populations.

Discussion

Plants from populations of *M. inflexa* were genetically differentiated in traits related to growth and asexual reproduction, and single-sex and both-sex populations differed in growth rate and reproductive strategies. Similarities among single-sex populations, male populations in particular, indicated that plants from these populations are either genetically related or that similar phenotypes have been favored by selection in geographically distant populations. Although plants were sexually dimorphic, and sexual dimorphism varied among populations, single-sex and both-sex populations showed comparable levels of sexual dimorphism. Thus, the local environment plays a greater role in the expression of sexual dimorphism than does the presence of the opposite sex in a population.

My prediction of greater genetic variation among than within populations was supported. Also, as I predicted, single-sex and both-sex populations differed but they did not differ in apparent levels of investment in sex-expression in the field. Surprisingly, phenotypic similarity was not related to geographic proximity for males as I had predicted. Genetic variation, as

predicted, was partitioned among populations rather than within populations, and, consequently, sexual dimorphism between single-sex populatiosn was higher than within both-sex populations.

Population differences

Bryophyte populations often occur in isolation throughout their geographic range, and there is little opportunity for long range dispersal of gametes or diaspores among populations (Bischler and Boisselier-Dubayle, 1997). Once established, small founder populations are exposed to genetic drift where, because of a haploid-dominant life cycle, mutations are immediately expressed. Thus, genetic variation in bryophytes is hypothesized to be partitioned among, rather than within, populations (Bischler and Boisselier-Dubayle, 1997). My observations are consistent with this hypothesis (and my prediction), and differences between single-sex and both-sex populations implicate local environmental factors as important in sorting phenotypes among populations.

Populations were genetically differentiated in growth rates and cup production, and plants exhibited two general growth forms: large, wide branches with few cups and a smaller, highly branching, rosette-like plants with many cups. Single-sex populations and Apalachicola River plants tended to express the "extender" growth form, whereas plants from both-sex populations, expressed the "rosette" growth form. To the extent that differences in a common garden represent genetic variation, this indicates that the Apalachicola and Suwannee River plants from Florida, and the Oklahoma plants are closely related. Or, if these populations are relic populations of a once larger distribution, that only particular phenotypes, i.e., those with the fast growth, few cup form, were best at surviving the conditions that led to relict population expansion and persistence.

In species with little long range dispersal, geographically close populations should be more closely related, and result in a positive correlation between genotypes and geographic distance. I found that phenotypes of male plants were not correlated with geographic distance but, phenotypes of females plants were correlated with geographic distance. Successful long-range dispersal is unlikely in *M. inflexa* because of habitat specificity (isolated suitable habitat patches), and data are lacking on the efficiency of dispersal by either spores or gemmae in the species. The reason that phenotypes of females were correlated with genetic distance may be a sampling artifact because the only single-sex female populations I've found were in Oklahoma and these female populations were phenotypically distinct from plants from both-sex

populations. Whereas, in the analysis of male plant phenotypes and geographic distance, I were able to include plants from single-sex male populations from both Florida and Oklahoma. It is possible that if all-female populations were found in regions outside of the Arbuckle Mountains in Oklahoma, that the correlation between female phenotypes and geographic distance would no longer be significant. Because the analysis with male plants includes a better sampling distribution, and may be more reliable, this indicates that the colonization and evolution of genotypes in isolated populations may be more complicated than directional dispersal from nearby source populations.

Single and Both-sex populations

I predicted that plants from single-sex populations would differ in growth or asexual reproductive rates from plants from both-sex populations. In the greenhouse, plants from single-sex populations tended to grow larger and produce fewer cupules than plants from both-sex populations. Sexual reproduction aside, plants from single-sex populations invested in growth at the expense of asexual propagule production. The lower variation in some trait values observed in plants from single-sex populations is indicative of lower genetic variability in those populations. If single-sex populations originated from plants that survived in refugia and later expanded, genetic variation is expected to be low, barring somatic mutation, which has been implicated as an important force for creating variation in populations of clonal organisms (Les and Gabel, 1996). However, variation within single-sex populations was not zero, and variation in growth rate did not differ from that observed in plants from both-sex populations, indicating that single-sex populations are not genetically homogeneous. This is consistent with previous studies that have found bryophyte populations more genetically variable than expected (Mishler, 1988; Newton and Mishler, 1994; Wyatt, 1994).

In the field, single-sex populations invested in sex structure production to the same extent as both-sex populations. However, plants from single-sex populations may invest less in gametangia or have a there may be a developmental constraint to gametangia production because plants from single-sex populations had fewer gametangia per unit mass in their sex structures.

Sexual dimorphism

Female *M. inflexa* were larger and produced more cupules than males. Growth rate differences between the sexes were consistent with previous greenhouse and field experiments (Fuselier et al., sel chapter; McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002)

however, differences in cup production were contrary to patterns previously observed. In previous studies, males produced more cups than females, whereas, here the opposite pattern was observed. However, females grew larger than males and neither sex showed typical rates of sex structure production, i.e., by day 215, most plants still had not produced sex structures whereas in previous greenhouse experiments most plants produced sex structures before 180 days (Fuselier and McLetchie, 2002).

One explanation for the incongruent result is that, lack of sex expression means that plants lack the physiological trade-off between cup production and gametophore production. If environmental conditions were not right for gametangial initiation for either sex, females, because they were larger than males, could produce more cups without the cost of sex expression later. Of the plants that did produce sex structures, females produced more than males, and males started producing sex structures before females, patterns consistent with previous studies. Compared to all other plants, the small proportion of sex-expressing plants delayed cup production, produced fewer cups and grew larger than the non-expressing plants. Suwannee River males did not produce cups, started sex expression early in the experiment, and the plants that expressed sex were larger than those that did not express sex. All of these patterns are indicative of a trade-off between cup and sex structure production. However, among Trinidad plants there were no differences between plants that expressed sex and those that did not, suggesting that there may be more factors involved than were measured here. The greenhouse in which this experiment was conducted was different from the one used in previous experiments. Evidently, cup production by females is more plastic than previously realized, and influenced by environmental conditions and investment in sex expression.

Variation in trait means among populations occurred more readily than variation in sexual dimorphism in *M. inflexa*. Most populations were similar in their degree of sexual dimorphism although there were a few outliers to this pattern. Thus, as would be expected with constraints to the evolution of sexual dimorphism, trait means are evolving more readily than sexual dimorphism in traits (Lande, 1980). Variations in the degree of sexual dimorphism may be a result of local environmental factors or stochastic events. Sexual dimorphism was just as high in both-sex populations as between sexes in single-sex populations. Thus, the local environment was more influential in determining the degree of sexual dimorphism than the presence of sex in a population.

Constraints

Between-sex correlations (overall all populations) were not significant however, the sexes did exhibit among trait correlations, and these may constrain the response to selection for some characters. Branch size was negatively correlated with cup production rate in males, indicating a trade-off between mericell investment in asexual reproduction versus growth (McLetchie and Puterbaugh, 2000). The results of this trade-off were seen in single-sex populations and Apalachicola female plants that invested more in growth than cup production. The sexes showed the same pattern for among trait correlations though, more correlations were significant for males than females. If among trait genetic correlations are truly significant in males and not significant in females, males may have constraints to the response to selection that females do not, and this pattern may maintain sexual dimorphism. A larger sample size of plants and examination of actual family means (and genetic correlations) in future experiments may reinforce this pattern.

For both sexes, faster growing plants produced more cups, and dimorphism in traits such as cup production, may result from correlative selection on growth (Delph, Knapczyk, and Taylor, 2002), a pattern that may be important to the evolution of sexual dimorphism in bryophytes. Sexual size dimorphism has been observed in many other liverworts (Voth and Hamner, 1940) and may evolve if body size is heritable, the sexes respond differently to selection, and between-sex correlations are less than 1.0 (Lande, 1980; Lande and Arnold, 1983; Slatkin, 1984; Geber, 1999). The sexes of *M. inflexa* were significantly variable in growth rate across populations and did not show significant between-sex correlations for the traits, indicating that the sexes may be able to respond independently to selection (Lande, 1980). However, the plants in this experiment behaved inconsistently with those from previous experiments. Any bias resulting from the greenhouse environment would tend to skew correlations in the positive direction thus, the negative correlations detected are those most likely to present true constraints. The only negative correlation detected in this experiment was between branch size and cup production, underlining the phenotypic differences described for the two growth forms detected (Houle, 1991; Davis, 2001). Additionally, selection may act with different magnitudes and directions among life stages of individuals and the adaptive significance of sexual dimorphism at the asexual stage may be confounded with the action of selection on the sexual stage (Schluter and Smith., 1986; Andersson, 1994; Purrington and Schmitt., 1998; Preziosi and Fairbairn,

2000). For bryophytes, constraints to the expression and evolution of sexual dimorphism may be heavily dependent on the local environment and further research is necessary to examine the importance of selection acting at different life stages in different environments.

In summary, by examining sexual dimorphic characters in a common garden, I demonstrate that, barring maternal effects, there is a genetic basis to the phenotypic variation observed among populations. Females are larger than males and single-sex and both-sex populations differ. However, the degree to which sexual dimorphism is expressed is not associated with the presence of the opposite sex in a population but likely influenced by local environment. Further, unlike in some angiosperms (Meagher, 1999; Delph, Knapczyk, and Taylor, 2002), between-sex correlations are low and may not be the primary constraint on the expression of sexual dimorphism in *M. inflexa.* From here, the next step is to see if the phenotypic differences between the sexes and among populations are adaptive.

Table 3.1. Population locations and sample sizes.
Population locations and numbers of females (F), males (M) and unknown sex isolates (U) of *Marchantia inflexa* grown in greenhouse. Population types are B=both-sex, F=female, M=male and are listed in order of latitude. Latitude and longitude are approximate.

Population and abbreviation	Location	Latitude N /Longitude W	Population type	n	F, M, U
Quare River (QR)	Trinidad	10.40/ 61.12	B	32	15, 17, -
Madrigal (DR)	Dominican Republic	18.48/69.9	B	20	15, 5, -
Suwannee River (SR)	Hamilton Co., FL	30.42/83.15	M	25	-, 25, -
Apalachicola River (AP)	Gadsden Co., FL	30.56/84.94	B	26	13, 1, 12
Clark's Creek (MS)	Woodville, MS	31.017/91.506	B	30	16, 14, -
Little Schultz (LS)	West Blocton, AL	33/87	B	33	16, 11, 6
Turner Falls (TF)	Turner Falls, OK	34.425/97.148	M	24	-, 24, -
Travertine Creek (TC)	Sulphur, OK	34.508/96.968	F	25	25, -, -
Stinking Bear Creek (SB)	Russellville, AL	34.59/87.69	B	15	4, 7, 4
Byrd's Mill Creek (BM)	Fittstown, OK	34.615/96.634	F	25	25, -, -
Cooks Creek (CC)	Russellville, AL	34.62/87.719	B	18	4, 8, 6
Blue River (BR)	Connerville, OK	34/96	M	25	-, 25, -
Falls Creek (FC)	Dougherty, OK	34/97	M	21	-, 21, -
Fourth Creek (BD)	Bearden, TN	35.5/84	B	32	16, 16, -
Carter's Mill (CM)	Carter, TN	36.01/83.71	B	24	6, 7, 11

Table 3.2. Eigenvectors from PCA.
Eigenvectors for traits included in principal components analysis for sexes of *Marchantia inflexa* within populations. PC1 was interpreted as a size axis whereas, PC2 was an asexual reproduction axis. Sexes were significantly different on PC1 and population types different on PC2. Values are sorted in ascending order of PC2.

Trait	PC1	PC2
Area per branch	0.164	-0.260
Growth	0.344	-0.172
Area day 32	0.355	-0.164
Area day 61	0.356	-0.160
Area day 41	0.372	-0.153
Thallus width	0.028	-0.086
Thallus length	0.045	-0.079
Area day 10	0.046	-0.017
Cups day 10	0.235	0.081
Cups day 41	0.360	0.194
Cups per day	0.360	0.194
Cups day 32	0.343	0.199
Cups per area per day	0.009	0.496

Table 3.3. Population and sex differences.
Differences among 16 populations of *Marchantia inflexa* for four traits that were informative in distinguishing among populations and sexes in PCA. Results are from an ANCOVA where area day 10 (iinitial plant size) was used as a covariate in the model.

Trait	Source	DF	Mean Square	F Value	Pr > F
Growth	Population	14	317795.485	8.00	<.0001
	Area day 10	1	721919.883	18.17	<.0001
Total Cups	Population	14	128.22	24.08	<.0001
	Area day 10	1	165.34	31.05	<.0001
Cup Production	Population	14	0.00000671	3.35	<.0001
	Area day 10	1	0.00000421	2.11	0.1476
Area/branch	Population	14	151313.71	14.63	<.0001
	Area day 10	1	32579.73	31.13	<.0001

Table 3.4. Population type differences.
Females and males from single-sex and both-sex populations of *Marchantia inflexa* differ. Contrast statement results are from ANCOVA with sex as main effect and size at day 10 as a covariate. SS= single-sex population, BS = both-sex population. For all contrasts, d.f. = 1

Trait	Contrast SS vs. BS	Contrast Mean Square	F Value	Pr > F
Growth				
(mm^2/day)	Female	1228495.271	23.61	<0.0001
	Male	34768.778	0.67	0.414
Cup production				
(cups/area (mm^2)/day)	Female	3.50E-06	2.17	0.142
	Male	9.80E-06	6.06	0.014
Total cups				
	Female	0.3084878	0.04	0.849
	Male	276.0210084	32.33	<0.0001
Branch size				
(mm^2/branch)	Female	19335.35391	11.92	0.001
	Male	60817.25621	37.48	<0.0001

Table 3.5. Trait variation.
F-ratio tests for female and male *Marchantia inflexa* from both sex (BS) and single-sex (SS) populations show that variation in cup production and branch size was higher in both-sex than in single-sex populations.

Sex	Trait	Numerator, denominator d.f.	BS variance	SS variance	F Value	Pr > F
Females	growth	48,101	63813.610	85915.420	1.35	0.21
	total cups	104,48	12.370	6.634	1.86	0.02
	cups/day	104,48	0.008	0.004	1.99	0.01
	branch size	92,47	1818.960	1078.430	1.69	0.05
Males	growth	85,92	41654.090	37189.730	1.12	0.59
	total cups	85,94	8.820	5.047	1.75	0.01
	cups/day	85,94	0.005	0.003	1.74	0.01
	branch size	78,78	1265.220	2071.730	1.64	0.03

Table 3.6. Sex differences in traits.
The sexes of *Marchantia inflexa* differed in growth, cup production and total number of cups produced. Means and standard errors for the sexes for each trait are given and significance values are from ANCOVA with size at day 10 as a covariate. *P < 0.0001.

	F (n=154)		M (n=181)	
Trait	Mean	Std Err	Mean	Std Err
Growth (mm^2)	368.63	22.68	245.51	14.83 *
Cup production ($cups/mm^2Day$)	0.0010	0.0001	0.0007	0.0001 *
Branch size (area (mm^2)/branch)	81.77	3.48	77.79	3.60
Total cups	4.20	0.26	2.82	0.21 *

Table 3.7. ANCOVA results.
Results of 2-way analysis of covariance testing for effects of population, sex and the covariate plant size 10 days after planting *Marchantia inflexa* in greenhouse. The interaction term was removed if not significant (at $P<0.20$) and analysis re-run. These results are for both-sex populations without Apalachicola River plants.

Source	d.f.	F	P
Growth			
Population	7	4.28	0.0002
Sex	1	3.95	0.0486
Population × sex	7	2.68	0.0119
Area day 10	1	22.67	<0.0001
Number cups			
Population	7	11	<0.0001
Sex	1	1.42	0.2344
Population × sex	7	4.04	0.0004
Area day 10	1	23.43	<0.0001
Cup production			
Population	7	2.30	0.0289
Sex	1	4.15	0.0432
Population × sex	7	1.47	0.1826
Area day 10	1	3.15	0.0776
Branch size			
Population	7	5.53	<0.0001
Sex	1	1.69	0.1951
Area day 10	1	11.69	0.0008

Table 3.8. Among trait correlations.
Among-trait genetic correlations within a sex for *Marchantia inflexa* from Pearson produce moment correlations. The correlation coefficient is above and the probability value below. Females are above the diagonal, males are below the diagonal. For females, n=12, for males, n=13.

	Growth (mm^2/day)	Cups/mm^2/day	Area/branch (mm^2)	Total cups
Growth		0.03	0.44	**0.59**
(mm^2/day)		0.93	0.16	0.04
Cups/mm^2/day	0.44		-0.24	0.26
	0.12		0.45	0.42
Area/branch	0.14	**-0.50**		-0.17
(mm^2)	0.65	0.08		0.59
Total cups	**0.68**	**0.78**	-0.27	
	0.01	0.001	0.38	

Figure 3.1. Principal components analysis.
First two principal components from PCA with Female (diamond) and male (square) *Marchantia inflexa* population means plotted. Upper case letters indicate both-sex and lower case letters indicate single-sex populations. The first 2 letters of the abbreviations are for the population and the third for the sex; F=females, M=males, population abbreviations are given in

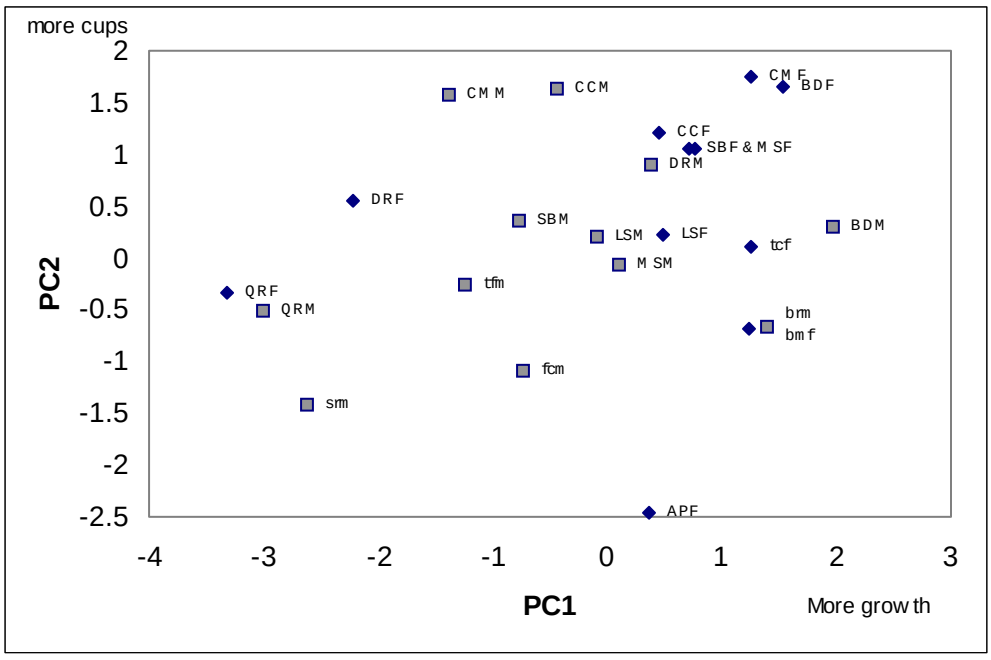
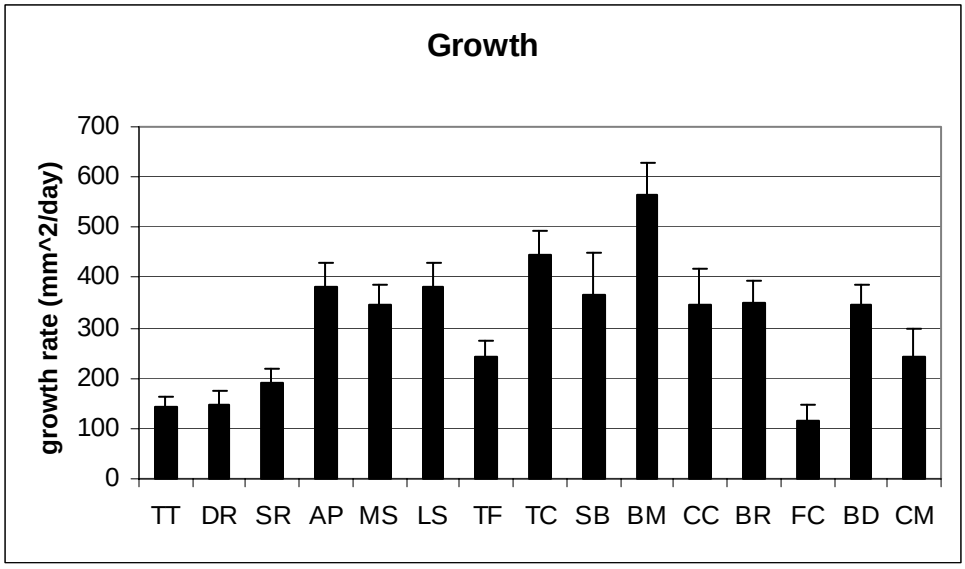


Figure 3.2 a - d. Trait and population differences.
Populations of *Marchantia inflexa* differed in growth, branch size, cup production rate and numbers of cups produced. Results are from analyses of covariance; error bars are standard error and lines indicate no significant differences within a group. Sample sizes (n) were as follows: AP=14, BD=32, BM=24, BR=25, CC=12, CM=13, DR=18, FC=20, LS=27, MS=29, SB=11, SR=24, TC=25, TF=24, QR=31.

a.



b.

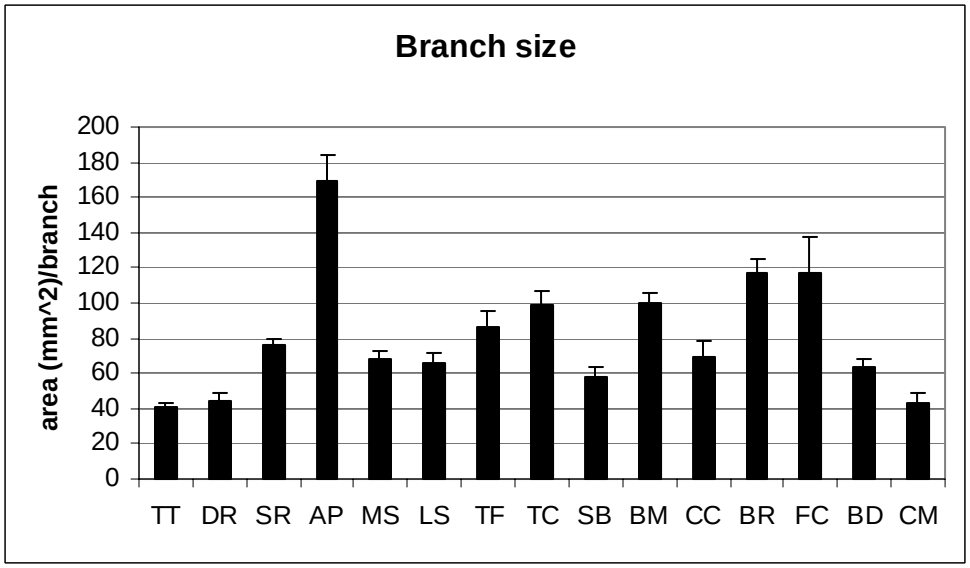
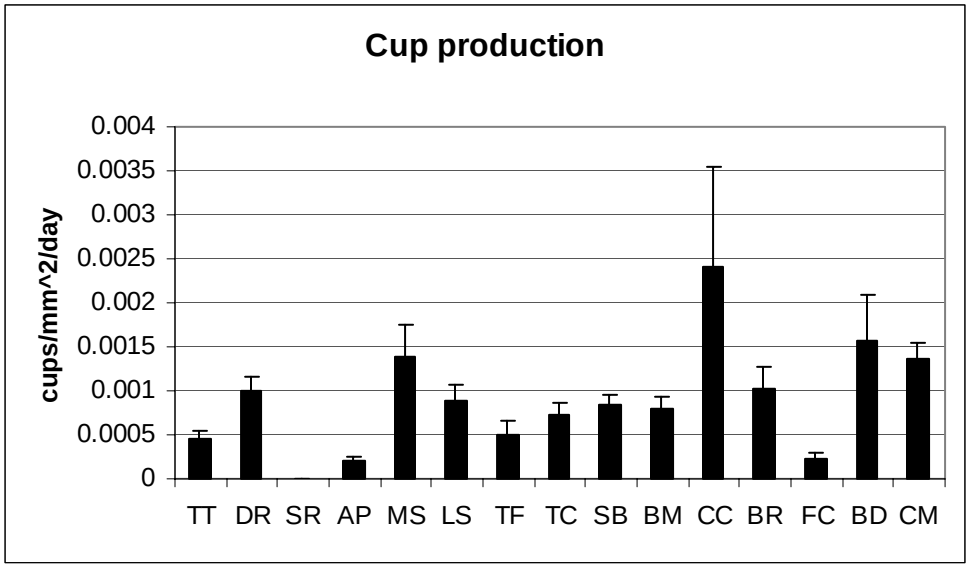


Figure 3.2c and d.



d.

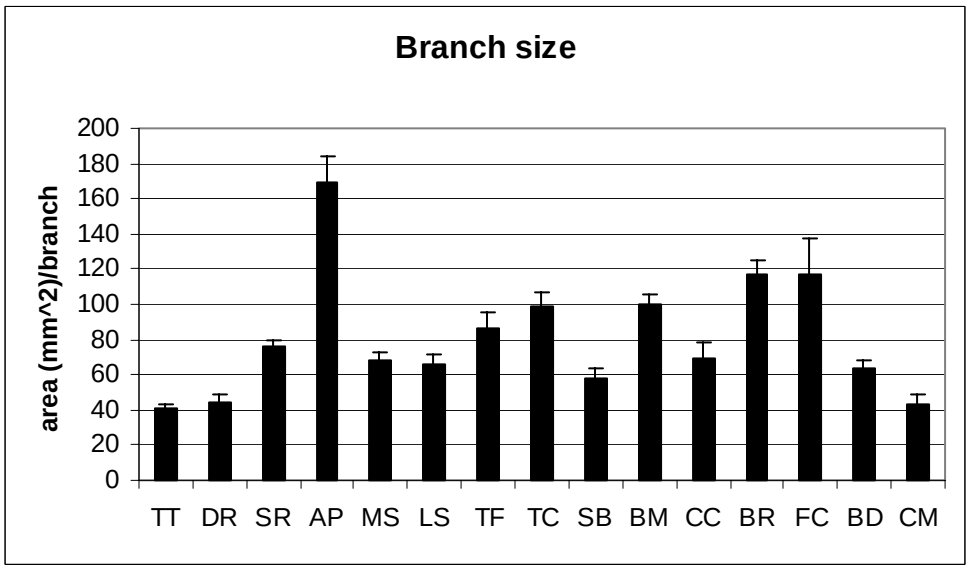


Figure 3.3. Sexual dimorphism.
Sexual dimorphism in *Marchantia inflexa* from populations throughout its range as the Euclidean distance between points for females and males in principal components space. Distance measures for single-sex populations were the distance from the male population to the mean distance to the two female populations. Populations are in order of increasing latitude and light colored bars indicate single-sex populations.

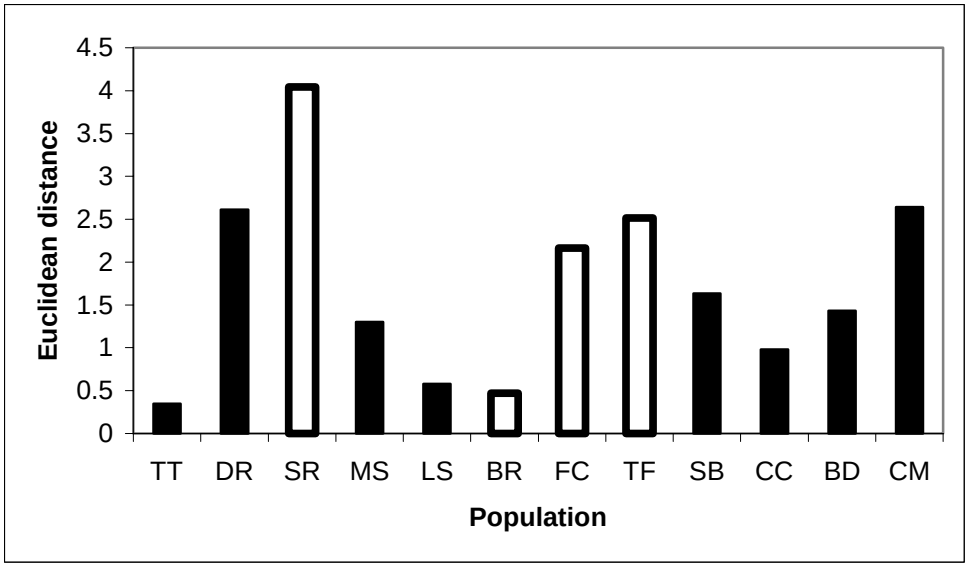


Figure 3.4. Seasonal production of sex structures.
Proportion of antheridiophores per total number of sex structures in three populations (CC=Cooks Creek, LS=Little Schultz, MS=Mississippi) over three months; n = 50 for each point.

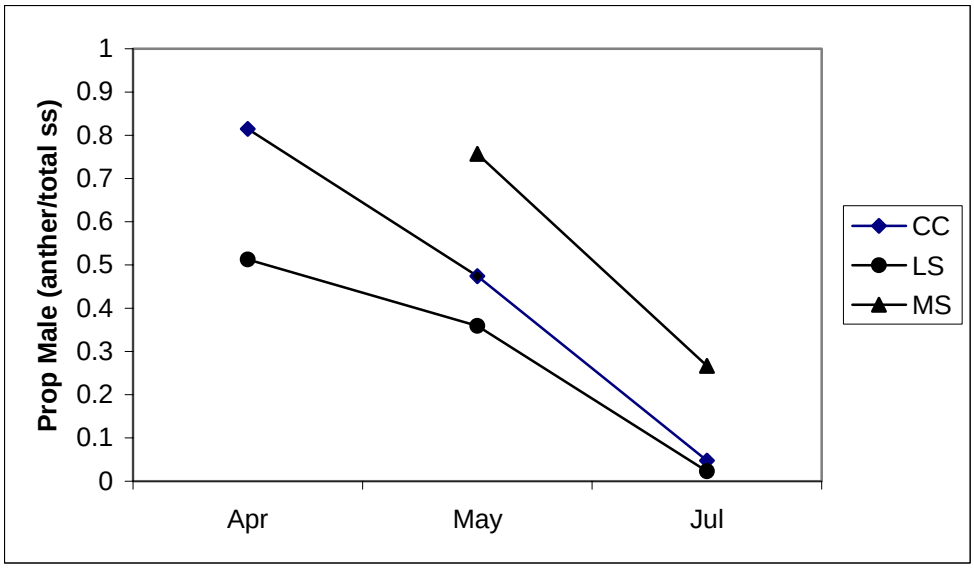
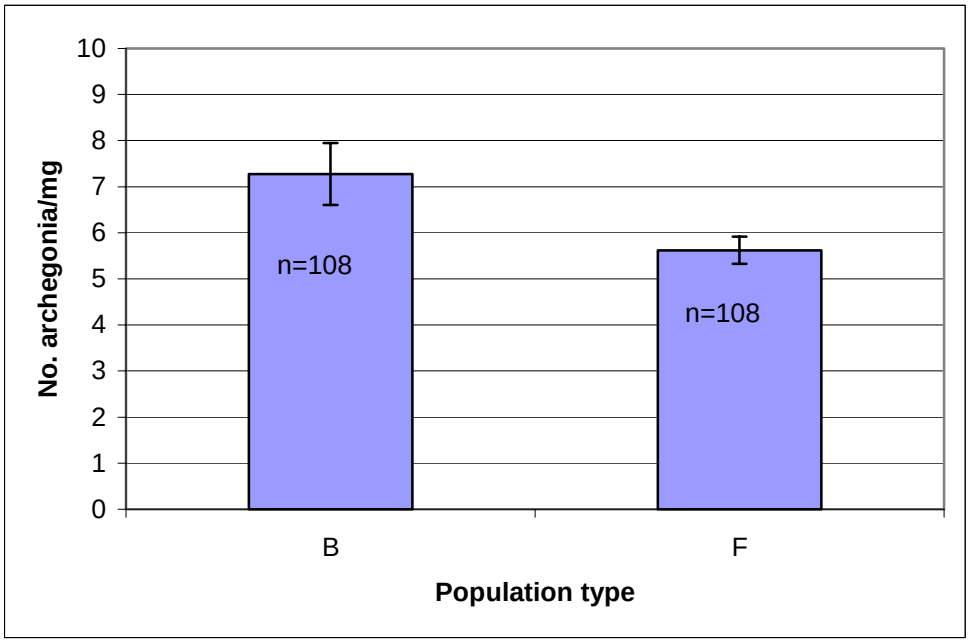
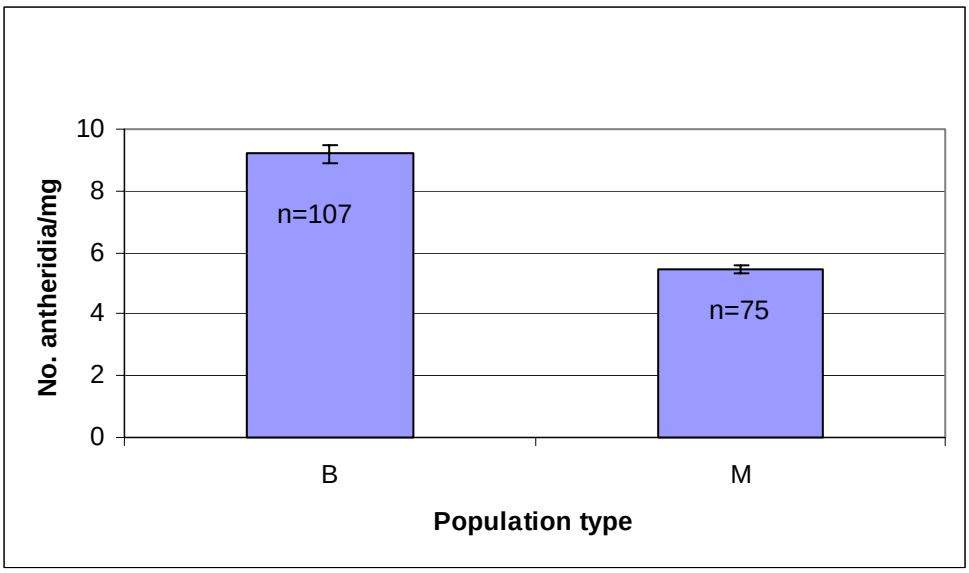


Figure 3.5. Gametangia produced per sex structure.
Numbers of gametangia produced per sex structure for male and female *Marchantia inflexa* in both-sex (B), female (F) and male (M) populations. Error bars are standard error. Both-sex populations included CC, LS, BD, and single-sex populations included BR, TF, trib, TC and BM.





Chapter Four: Sex-specific and environment-dependent selection on *Marchantia inflexa* in nature

Summary

Sexual dimorphism may evolve and be maintained when selection favors different phenotypic trait optima for the sexes. Additionally, selection acting differently in different environments may promote phenotypic and genetic variation within populations. To examine selection in nature and quantify how selection on traits associated with early colonization of patches may result in sexually dimorphic life history strategies, I planted female and male *Marchantia inflexa* into four light/moisture treatments at a field site where the species historically occurred. I used genotypic selection analyses to quantify natural selection and examine patterns of sex-specific and environment-dependent selection. Selection favored larger plants that produced more asexual propagules but selection was stronger on males than females, resulting in different phenotypic optima for the sexes. Additionally, selection was environment-dependent, primarily on males. Selection favored larger males than females under closed canopy but sexes of similar phenotypes under open and wet conditions. The importance of early sexual dimorphism is discussed in relation to competition for space in early patch establishment. Because selection favored different phenotypic maxima for males under different conditions, the combination of sex-specific and environment-dependent selection may promote the persistence of both sexes in a population.

Introduction

The evolution of dioecy is presumed to be followed by sex-specific selection that results in sexually dimorphic traits commonly observed in dioecious plants (Lloyd and Webb, 1977; Geber, Dawson, and Delph, 1999). Hypothetically, sexual dimorphism evolves and is maintained because the sexes of a dioecious species differ in reproductive ecology (Lloyd and Webb, 1977; Charnov, 1982a; Lloyd, 1982; Meagher and Antonovics, 1982; Meagher, 1984; Shine, 1989; Eppley, Stanton, and Grosberg, 1998; Delph, 1999; Geber, 1999). Sex-specific costs of reproduction result in sex-specific patterns of selection that are manifest as different phenotypic optima for the sexes (Putwain and Harper, 1972; Dawson and Geber, 1999; Geber, 1999). In plants, sexually dimorphic traits are typically not identifiable until the sexes are discernable through flowering, (Delph, 1999; Geber, Dawson, and Delph, 1999). However,

expression of sexually dimorphic traits prior to sexual reproduction has been reported in both seed plants and bryophytes (Allen, 1919; Voth and Hamner, 1940; Godley, 1964; Lloyd, 1973; Shaw and Gaughan, 1993; McLetchie and Puterbaugh, 2000). Early sexual dimorphism in growth rates may be especially important in clonal plants, such as bryophytes, that compete for space during colonization and whose competitive early growth rates later influence population sex ratios (McLetchie, Garcia-Ramos, and Crowley, 2002; Crowley, Stieha, and McLetchie, submitted).

Investigation of the early expression of sexually dimorphic traits in bryophytes is evolutionarily significant because sexually dimorphic life history strategies may be the driving force behind the commonly observed female-biased sex ratios (McLetchie, Garcia-Ramos, and Crowley, 2002) which, in turn, may lead to the lack of sexual reproduction in bryophyte populations (Gemmel, 1950). Bryophytes have been underrepresented in the literature on plant sexual dimorphism (which comes mainly from studies of non-clonal angiosperms), despite the fact that over half of mosses and liverworts are dioecious (Shaw, 2000). Sexual dimorphism in bryophytes runs the gamut from subtle differences in growth rates (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002) to the presence of dwarf males that live on relatively large females (Une, 1984, 1985b). All bryophytes can reproduce clonally (During, 1990; Newton and Mishler, 1994), and although sex is determined by sex chromosomes (Ramsay and Berrie, 1982), bryophytes often exhibit female-biased adult population sex ratios (Longton and Schuster, 1983; Longton, 1990; Wyatt, 1994; Bowker et al., 2000).

The distribution of the sexes in bryophytes is unusual in that in many dioecious species, the two sexes never occur together, and single-sex populations persist via asexual reproduction alone (Schuster, 1992). In some cases the geographic disjunction between the sexes is extreme (Schuster, 1989). Dioecious bryophytes have lower rates of sexual reproduction than monecious species (During, 1990). Successful fertilizations require close proximity of the sexes, yet, within populations, both-sex patches are rare (Bowker et al., 2000; McLetchie and Puterbaugh, 2000; Chapter 3).

Adult population sex ratios in bryophytes may be influenced by sex-specific life history strategies such as clonal expansion ability, or investment in asexual and sexual reproduction (McLetchie, Garcia-Ramos, and Crowley, 2002; Stark et al., 2004; Crowley, Stieha, and McLetchie, submitted; Stark et al., submitted). Male-biased sex ratios in some seed plants are

correlated with greater clonal expansion abilities of males than females (Stark et al., 2004). However, the female-biased sex ratios of bryophyte populations have not been clearly associated with sexually dimorphic life history strategies (Stark et al., 2004). Sexual dimorphism of growth and asexual reproductive characters was invoked to explain patch level sex ratios of *Marchantia inflexa*, a dioecious thallose liverwort, in a model parametized for patches in a homogeneous environment (McLetchie, Garcia-Ramos, and Crowley, 2002; Stark et al., 2004). However, in natural populations, the microhabitats inhabited by *M. inflexa* differ among patches and populations (Chapter 3).

If selection is environment-dependent as well as sex-specific, selection may favor different phenotypes or sexes in different environments during early colonization of patches and ensure the establishment of both sexes in a population. To determine the influence of sex-specific and environment-dependent selection in nature, I measured phenotypic natural selection on sexually dimorphic traits important to early establishment of fragments of a dioecious, thallose liverwort, *M. inflexa*. Because the extent to which traits respond to selection depends on existing genetic variation as well as the variance-covariance structure of the traits within and between the sexes (Lande, 1980), I also measured phenotypic correlations among traits between and within the sexes to examine the constraints to the response to selection. Female and male plants were planted in four light-moisture treatments and survival, growth and levels of asexual reproduction were measured. Based on previous greenhouse studies (Fuselier and McLetchie, 2002) I expected some traits to be under sex-specific selection and that both males and females would experience environment-dependent selection. Further, patterns of environment-dependent selection are expected to be shaped by trade-offs among life history characters. In particular, in *M. inflexa*, there is a tripartite tradeoff among growth, asexual and sexual reproduction (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002) that likely influences the outcome of selection on traits important to early establishment of plants.

My focus on traits important to early establishment, and my use of viability as a measure of fitness provides only part of the picture of how selection acts on the sexes of a dioecious bryophyte. Selection on traits associated with early establishment of fragments of *M. inflexa* may differ from selection on gemmae and spores, the asexual and sexual propagules of the species. Sexual fecundity may play a major role in shaping traits of pre-adults (Geber, Dawson, and Delph, 1999; Preziosi and Fairbairn, 2000; Fuselier and McLetchie, 2002). However,

models of this system have indicated that the frequency of disturbance and early strategies in the competition for space via clonal expansion were traits most influential on within-patch sex ratios (McLetchie, Garcia-Ramos, and Crowley, 2002). Many patches within a population of *M. inflexa* are non-sex-expressing (Chapter 3), indicating that sexual reproduction may not readily occur in most patches, and that costs associated with sexual reproduction may not be as important as factors related to space occupation and clonal reproduction. Further, even given its limitations, this is the only published experiment that I know of that measures genotypic selection in nature on sexually dimorphic characters in a bryophyte.

Methods

Study location

Buffalo Springs, a spring-fed stream in Grainger County, Tennessee,was home to a population of *M. inflexa*, the last documented collection of which was in 1936. A hatchery building was constructed on top of the original creek in 1939, and the creek was diverted around the hatchery building. In 2000, although the stream was intact, there were no *M. inflexa* found in the areas where they were recorded in decades previous. However, the stream appeared suitable for growth of *M. inflexa*. Patches of *Conocephalum conicum* occur along the stream banks and *M. inflexa* readily occurs with *C. conicum* in other USA populations (LCF, personal observation).

Collection of stock plants

I defined an isolate as a plant collected from a distinct patch of plants within a population. A patch was defined as a group of plants separated by neighboring groups by at least one meter, typically with an obstruction (such as unsuitable substrate) between patches. In most cases, I collected vegetative plant tips from a plant that had both expressing an non-expressing tips to be certain of the sex of the plant tip. In some single-sex patches, I could not definitively identify that the tip I collected came from the plant with the sex structures thus, there is a low probability that the plant may have been from a non-expressing plant of the opposite sex . The collected tips were presumed to be female or male, respectively. Each tip was collected from a different, chosen patch along 0.5 - 3 km of stream length. This collection method increased my likelihood of collecting individuals that were not members of the same genet. I collected plants from patches in different populations, returned them to the greenhouse at the University of

Kentucky and grew the plants on steam-sterilized soil from Kentucky under 55% shade in temperatures ranging from 22 ^{o}C to 25 ^{o}C. Thus, all isolates used in the experiment were begun from greenhouse stock grown under similar conditions to minimize maternal effects or any latent site-specific environmental effects. Plants used on artificial patches were from greenhouse stock plants that had undergone numerous bouts of cupule (cup) production over at least one year. Because it is impossible to discern individuals in the field, plants were collected from patches, separated by at least one meter and by unsuitable substrate, were termed "isolates". To the extent that plants from spatially separated patches are different individuals, isolates from within a population may be considered different genotypes.

Field Experiment

I planted 13,000 mericell-bearing tips of *M. inflexa* into 260 artificial patches into a stream reach at Buffalo Springs. Artificial patches were made of rectangular pieces of capillary matting, with a central area for plants surrounded by 2.2 cm margin. Mats were 15.4 cm x 30.8 cm and mericell bearing tips of *M. inflexa* were placed 1 cm apart on a 5 row x 10 column grid with 50 spaces within a 11cm x 26.4 cm rectangle on the mat. Each patch had a 1:1 sex ratio and plant placements on the mat were random.

Genotypes used in this experiment were chosen in a systematic random fashion from a pool of genotypes that were collected from 10 populations within the USA (populations are described in Chapter 4). Genotypes were randomly chosen from among both-sex populations, but I limited genotypes from single-sex populations to one per population, hence the systematic random sample. Plants underwent several asexual generations in the greenhouse, thus removing effects of home environment. Fifty genotypes (25 F, 25 M) were used for each patch, with some substitutions (Table 4.6). The same 23 female and 22 male genotypes were on all 260 mats, and an additional 4 female and 8 male genotypes were used as substitutes to keep the total number of genotypes on all mats the same (Table 4.6). For those genotypes that did not have 65 replicates, numbers of replicates ranged from 12 – 47. Genotypes were randomly assigned to a location within the patch and the patches randomly assigned to one of four treatments. Tips were grown under artificial light (florescent and incandescent) in a growth room for 5 days to allow rhizoid growth and anchorage into the mat before placement in the field (July 22, 2002).

Field treatments consisted of two light environments (high and low) crossed with two moisture availability levels (wet and "dry"). The light environment was quantified using hemispherical photographs, taken at low sun angles (to avoid scattering of light), through a 180^{o} fish-eye lens (Nikon FC-E8) on a tripod-mounted digital camera (Nikon 950, Nikon Corporation, Tokyo, Japan) at each mat placement site. Images were analyzed using Scanopy software (Reagent Instruments Inc, Quebec, Canada) to estimate the total amount of photosynthetically active photon flux density (PPFD, mol/m^2/day) reaching a mat from May through October (approximately the period of canopy fullness), and percent canopy openness. Irradiance measures (PPFD) were used to classify sites into treatment categories of high and low light. Most sites were covered with canopy thus, to create the high light treatment, I opened the canopy by removing overhanging vegetation. Total irradiance at open canopy sites ranged from 10.99 to 28.65 umol/m^2/day, and at closed canopy sites, from 4.34 to 10.01 umol/m^2/day. Four sites had a mid-range irradiance value and were classified according to percent canopy openness. Open canopy sites had significantly higher mean total irradiance (direct and indirect light) than closed canopy sites ($df = 159$, $t = -19.90$, $p < 0.0001$; Figure 4.1a). The range of light environments used in this study are within the range found in natural populations of *M. inflexa* (Chapter 2).

Within each light treatment and at each site, patches were placed either near the stream edge, in contact with the water, or further up the stream bank. Wet patches had one entire edge of the patch contacting stream water whereas, dry patches had two strap-like extensions of matting that contacted the water and wicked moisture up to the plant tips. This created one very wet mat and one with lower moisture than the wet patch but still provide enough moisture for the plants to survive. Mats were anchored to soil substrate. The difference in amount of moisture held by wet and dry mats was estimated by weighing test mats and examining the difference in grams of moisture. Test mats, 8 in each moisture treatment, were placed at the field site in randomly selected locations near mats with plants (under open and closed canopy and anchored to soil substrate). Wet mats held significantly more water than dry mats after one week ($df = 30$, $t = -14.48$, $p < 0.0001$; Figure 4. 1b).

The light treatment levels used here were chosen because previous experiments indicated that females and males invest in growth and asexual reproduction differently in different light environments (Fuselier and McLetchie, 2002). Moisture levels were manipulated because field observations indicate that periods of drought within and among patches may be an important

factor in structuring metapopulations of *M. inflexa*. Additionally, some studies on mosses indicate that females may have a higher tolerance for extreme temperatures (Une, 1985b) or desiccation stress (Newton, 1972) than males.

Traits

I measured growth and asexual reproduction because of the importance of these strategies to patch establishment, expansion and persistence. Early establishment and invasion of unoccupied space within patches may be important in determining ultimate patch sex ratio for clonal bryophytes (McLetchie, Garcia-Ramos, and Crowley, 2002).

Growth of plants (area increase in 21 days) was estimated by taking digital photos of mats and measuring green area of plants using ImageJ software (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/nih-image/). I made two area measurements, one right before field placement and one 21 days after field placement. Growth was the size of the plant at day 21 minus the initial size of th plant before field placement. To measure investment in asexual reproduction, I counted cups on each plant at 21 and 54 days after field placement. No plant had cups before field placement. Survival of isolates was scored as 0 or 1 for each of the field visits. Most plants had grown to overlap neighboring plants, and many new gemmae were growing on mats by day 54. Counts of cups at day 21 are referred herein as “cup 1” or early cup production and the second cup count at day 54 is referred to as “cup 2”. The variable, maximum cups or “max cup”, was created and is the maximum number of cups counted out of the two cup count days. Isolates were overgrown and not distinguishable by the onset of sexual reproduction so, results presented here are limited to growth and asexual reproduction.

An additional trait, area/branch, was added to analyses although it was not measured in this study. It was measured on the same genotypes in a greenhouse study (Chapter 3), and included here as a descriptor of the genotype. It is an interesting character because it varies among genotypes and populations when plants are grown in a common garden (Chapter 3), and represents different growth forms. A plant with many small branches produces a rosette-like form that has more mericells available for cup or sex structure production than a plant with few large branches. A plant with few large branches extends further from the original growth point than the rosette plant, but does so as long single thalli extensions with few growth regions.

Analyses

To examine patterns in trait means and survival across treatments and between sexes, I used trait values and proportion of plants surviving (arcsin-squareroot transformed) in an ANCOVA (analysis of covariance) with initial size as a covariate and gender and treatment as a main effects. Differences among treatments were examined using the REGWQ multiple comparisons test in SAS (statistical analysis system; SAS, 1990). To assess constraints to the evolution of sexual dimorphism, correlations among standardized traits and between sexes over all treatments were examined using PROC CORR in SAS (1990).

Genotypic selection analyses

I used standardized measures of growth, cup 1, max cups and area/branch in genotypic selection analyses to determine the strength and direction of selection on these traits in the sexes in each treatment (Lande and Arnold, 1983; Rausher, 1992). Unlike phenotypic selection analyses, genotypic selection analysis reduces the likelihood that results are biased by environmentally induced correlations that impact fitness (Rausher, 1992; Scheiner et al., 2002). To measure non-linear selection, I used squared terms for the standardized traits in quadratic regression. A negative value for the quadratic selection coefficient indicates stabilizing selection whereas a positive value indicates disruptive selection (Lande and Arnold, 1983; Brodie, Moore, and Janzen, 1995).

I used standardized trait values and relative fitness measures in selection analyses. Genotype mean trait values were standardized by sex and treatment by subtracting the mean and dividing by the standard deviation of the measure. Fitness was measured as survival to day 21, and genotype mean fitness values were made relative within each sex and treatment by dividing by mean fitness in each sex-treatment group. Residuals from regressions were normally distributed, and all selection analyses used genotype mean values.

Graphic analyses

To visualize fitness functions, I used the cubic spline with initial size (mm^2) as a covariate (Schluter, 1988; Brodie, Moore, and Janzen, 1995; Schluter, 2000) to fit curves to fitness functions. The cubic spline is a non-parametric method of fitting a curve to data without an a priori curve shape, and it uses bootstrapping to provide confidence intervals for the curve (Schluter, 1988). From these curves, if significant stabilizing selection was detected, I used a polynomial equation to fit the data and calculated maxima as the point at which the slope of the

line equals zero, and graphically compared maxima between the sexes within treatments. This is instructive in determining the meaning of quadratic selection coefficients because in some cases a negative coefficient is not sufficient to demonstrate stabilizing selection (Mitchell-Olds and Shaw, 1987).

Sex-specific and environment-dependent selection

To determine whether selection was sex-specific, I used ANCOVA within each treatment with relative fitness as the dependent variable, sex as a main effect, and the standardized plant traits as covariates. A significant sex × trait interaction indicated sex-specific selection (Donohue et al., 2001). To determine if selection was environment-dependent, I used similar ANCOVAs within each sex with either moisture or light as a main effect; a significant treatment × trait interaction indicated environment-dependent selection. I did this within canopy and moisture treatments to determine whether selection differed across moisture and light treatments separately. To determine if selection acted differently on the sexes in different environments, I used fitness made relative over all treatments and genotypes (female and male), and traits standardized over all treatments in an ANOVA with sex and treatment as main effects, the standardized traits as covariates and relative fitness as the dependent variable. A significant sex × treatment × trait interaction indicated that selection differed on the sexes in different treatments. Correlations, regression analyses and ANCOVA were conducted using SAS (SAS, 1990).

Results

Plants survived better, grew more and produced more cups in the open-wet treatment. Sexes showed the same pattern of survival among treatments, with significantly higher survival in open-wet than any other treatments, and higher survival in closed-wet conditions than either of the dry treatments (total df = 227, treatment df = 3, $F = 72.42$, $P < 0.0001$). Survival was lowest in the open-dry treatment though, survival in this treatment was not significantly lower than in the closed-dry treatment (Figure 4.1). On average, under wet conditions of both canopy types, females and males experienced 81% survival whereas, under dry conditions, males had a 66%, and females a 63% survival rate. There were no significant differences in survival between the sexes for any treatment (Females, total df = 107, $F = 0.06$, $P = 0.61$, Males, total df = 119, $F = 0.35$, $P = 0.79$). Both sexes grew larger and produced more cups in the open-wet treatment

(females: growth, F = 14.04, P <0.001; cup 1 and max cups, F = 10.77, 12.42, P <0.0001; males: growth F = 26.38; cup1 F = 11.39, max cup F = 17.77, P < 0.0001; Figure 4. 1). There were no significant differences in traits between the sexes in any treatment.

Correlations

Plants that grew larger also produced more cups in their first month of growth and had a higher maximum number of cups (Table 4. 2). Plants with large branches produced fewer cups in their first month. Maximum number of cups produced was significantly positively correlated with growth for males in four treatments, and females in one treatment, and was positively correlated with cups 1 for males in four treatments and females in three treatments. Area per branch was negatively correlated with cups1 for females and males in one treatment and negatively correlated with maximum cups for males in open-dry (Table 4.2). The sexes were significantly positively correlated in number of cups produced in the first month (df = 27, r = 0.5, P = 0.01) and maximum number of cups produced (r = 0.46, P = 0.01), but not correlated for growth rates (r = 0.22, P = 0.26).

Genotypic selection analyses

Growth and cup production were under significant total selection in one or more treatments, and, in most cases, linear selection was accompanied with significant stabilizing selection (Table 4. 3). Across all treatments except open-dry, both males and females experienced significant direct and total linear selection, and significant stabilizing selection on growth, and in all cases, selection favored larger plants. There was significant stabilizing selection on growth in females in the open-dry treatment. In the open-dry treatment, selection on growth in males was influenced by other traits not measured in this experiment as evidenced by nonsignificant direct selection but significant total selection. Males in the closed-dry treatment were under significant direct and total selection for more cups in the first month, and males in the open-dry treatment were under significant direct and total selection for more total cups. Cups 1 and max cups were likely influenced by selection acting on other, unmeasured traits as evidenced by non-significant direct selection but significant total selection on these traits for most treatments.

Among trait correlations (Table 4. 2) explained cases where there was direct selection but no total selection on a trait. Females in the closed-dry treatment were under significant direct selection on area/branch but no total selection (Table 4. 3). Area/branch for females in this treatment was negatively correlated with cups 1, and cups 1 was under positive total selection.

Because selection favored higher values of cups 1, and cups 1 was negatively correlated with area/branch, area/branch did not experience total selection. Similarly, in the open-dry treatment, there was positive direct selection on growth but no significant total selection. Growth was correlated with area/branch and although there was no significant selection on area/branch both direct and total selection coefficients were negative for females in this treatment, implying a possible negative interaction between the variables, though not a significant one. Females in the open-wet treatment were under significant direct selection for smaller area/branch but there was no total selection on this trait. For females in this treatment, area/branch was correlated with both cups1 and max cups, both of which experienced positive total selection.

Males in the closed-dry treatment were under negative direct selection on maximum cups, but there was no total selection on the trait. However, max cups was strongly positively correlated with both growth and cup1, and there was significant direct and total selection on both of these traits. Thus, the direct selection against high maximum cups was muted by strong positive selection for more growth and higher cup production in the first month of growth.

Sex-specific selection

The strength, type or direction of direct selection differed between the sexes in closed-dry, closed-wet and open-dry treatments (Table 4. 4). Three of four traits examined showed significant sex-specific selection under the harshest conditions (open-dry treatment) whereas, there was no sex-specific selection detected in the most benign treatment, open-wet. In general, where sex-specific selection was detected, selection was stronger on males than females. A trade-off between growth and cup production was evident in the pattern of selection on males in the closed-wet treatment. In closed-wet conditions, fitness was maximized at a higher growth and lower cup production in the first month for males.

Growth

Selection for increased growth was stronger on males than females in the closed-wet and open-dry treatments (Table 4.4). In the closed-wet treatment, stabilizing selection was strong on males but both sexes experienced directional and stabilizing components to selection. In the open-dry treatment, selection on females was purely stabilizing whereas on males there was a directional and stabilizing component to selection. Additionally, fitness was maximized at larger size for males than females in closed-wet and open-dry treatments (Figure 4.3). Under closed-dry conditions, females and males exhibited the same growth maxima on their fitness curves.

Cup 1

Males were under significantly stronger selection for producing more cups in their first month than females in the closed-dry treatment (Table 4.4; Figure 4.4). Both sexes experienced significant directional selection in this treatment, but males also experienced stabilizing selection, and fitness was maximized at a higher cup production value for females than males. Under closed-wet conditions, females were under directional selection for more cups produced in the first month whereas, males were under both directional and stabilizing selection for higher cup 1 values. Thus, males had a maxima on the fitness function graph whereas, in females, selection favored a much higher value for cup 1.

Max cups

Selection for a higher maximum number of cups was stronger on males than females in the closed-wet and open-dry treatments (Table 4. 4). Males were under stabilizing selection for maximum number of cups in closed-wet conditions whereas, selection was purely directional on females in this treatment. Thus, fitness was maximized at a lower maximum number of cups for males than females. In the open-dry treatment, selection was neutral on females but strongly directional on males, thus, selection favored higher maximum cup production in males than females. Although there was no sex-specific selection detected in the open-wet treatment, the fitness functions for females and males differed in that there was a peak for fitness at very low maximum cups for males (Figure 4.5). Thus, to some extent, selection may favor both low and high max cup production, however, no disruptive selection was detected for males in this treatment in quadratic selection analyses.

Branch size

In the open-dry treatment, selection favored females with lower area/branch and males with a higher area/branch. The fitness functions for this character in the four treatments revealed a pattern of consistently negative sloping fitness curves for females, and positive sloping curves for males (Figure 4.6). There may be biological significance to this trend, however, there was no significant total selection on branch size for either sex in any treatment.

Environment-dependent selection

Selection acted differently in different environments on both sexes (Table 4. 5). The strength and direction of selection did not differ for females across moisture treatments within canopy treatments. However, within the closed canopy treatments, there was stronger selection

on females for higher growth rates in the dry areas than in the wet areas. Also within the closed canopy treatments, selection on males was stronger in dry than wet conditions for increased number of cups in the first month and higher maximum number of cups. Within open canopy treatments, selection on max cups was stronger in males in the dry treatments compared to the wet treatments. In dry treatments, selection for more cups early was stronger under closed canopy than open canopy whereas, selection for higher max cups was stronger under the open canopy than the closed. In wet conditions, selection for higher growth in males was stronger under closed canopy than open canopy.

Selection on growth differed between the sexes in the different treatments (Figures 4. 7 & 4.8), as indicated by a significant sex × treatment × growth interaction ($F = 3.41$, $P = 0.002$). There was no significant difference between the sexes among treatments for either max cup ($F = 1.77$, $P = 0.09$) or early cup production ($F = 1.14$, $P = 0.34$).

Discussion

The combination of sex-specific and environment-dependent selection on *M. inflexa* may be the driving force for maintaining sexually dimorphic life history strategies, as well as maintaining both sexes in a population during early establishment of patches. Selection differed in strength, direction and type for the sexes but, overall, selection was stronger on males than females. The sex-specific strength and type of selection resulted in different fitness maxima for the sexes in growth and cup production, and promoted early sexual dimorphism in *M. inflexa.* Further, environment-dependent selection favored different phenotypes of the sexes in different environments, and this may maintain variation and ensure the persistence of both sexes within populations.

Although I used survival as a fitness metric and cup production as a trait, in previous studies of *M. inflexa*, cup production and sex structure production were used as fitness metrics. Cup production may be considered a fitness metric, but in females, the expressed phenotype was more consistent with patterns of selection on sexual fitness (sex structure as a fitness metric; Fuselier and McLetchie, 2002).

Early sexual dimorphism

Phenotypic differences between the sexes of *M. inflexa* are often subtle and difficult to detect without large sample sizes because of the extreme phenotypic plasticity of the species. In

previous experiments, where gemmae (rather than fragments) were grown to sexual maturity to examine early sexually dimorphic traits in *M. inflexa* (Fuselier and McLetchie, 2002), females were larger than males under 55% shade, and males started cup production earlier and produced more cups than females (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002). The open-wet field treatment was most like 55% shade greenhouse conditions. In the open-wet treatment, selection favored larger plants and, although selection was not sex-specific, fitness was maximized at a larger size for females than males.

Sexual dimorphism in vegetative traits, such as early growth and cup production in bryophytes, may result from correlated selection on adult sexual reproductive traits (Kohorn, 1994; Geber, 1995). Sexually dimorphic traits expressed during early establishment of *M. inflexa* females and males may be correlated with traits not measured in this experiment but associated with sexual reproduction, e.g., numbers of sex structures produced and timing of sexual reproduction. Such correlated selection may produce sexual dimorphism in pre-adult traits that is not necessarily adaptive for pre-adults (Geber, 1995). However, in the light of the importance of competition for space in early establishment of patches of *M. inflexa* (Crowley, Steiha and McLetchie, submitted), early differences in growth and cup production may be adaptive if they encourage the formation of both-sex patches necessary for successful sexual reproduction.

Whether early sexually dimorphic traits in *M. inflexa* are adaptive is a question for further research. Additionally, expression of early sexual dimorphism in sporelings, sexual propagules of the species, and the relative frequency of sporeling versus asexual propagule survival in different environments are also topics for further investigation.

Sex-specific selection

Sexual dimorphism may evolve via natural selection when viability or growth are maximized at different optima for the sexes (Lloyd and Webb, 1977; Wallace and Rundel, 1979; Meagher, 1984; Geber, Dawson, and Delph, 1999; Delph, Knapczyk, and Taylor, 2002). The response to selection depends on the strength and direction of selection as well as the variance-covariance structure of the traits and their heritabilities (Falconer, 1981; Lande and Arnold, 1983). Significantly stronger selection on one sex indicates a closer association of the trait under selection with fitness for that sex. All else equal, and barring significant constraints, if the

strength of selection on the sexes differs, the sex under stronger selection should respond to selection at a faster rate than the other sex.

In *M. inflexa*, natural selection under field conditions favored larger genotypes of both sexes, but the strength of selection was greater on males, resulting in different fitness maxima for the sexes for most traits (Figure 4.8). Selection favored fast growing females and males but, selection was stronger on males in the open-dry and closed-wet treatments. In the open-dry treatment, fitness was maximized at a high growth rate for males but, in this treatment, males tended to be smaller than females. The fitness maxima for males in the closed-wet treatment was higher than that for females, and males in that treatment tended to be larger than females. In both cases, fitness was maximized at a growth rate above the mean growth expressed for each sex in each treatment. Although there was stabilizing selection, the mean phenotypes expressed by both sexes were not the optimal phenotype for viability selection.

It is interesting that sex-specific selection acted on the sexes at the time of early patch establishment. In a models parametized for *M. inflexa* and designed to elucidate factors influencing patch sex-ratios, asexual reproduction and growth played important roles in colonization and occupation of space (McLetchie, Garcia-Ramos, and Crowley, 2002; Crowley et al., submitted). Males were expected to secure space within a patch during early establishment because they produce more gemmae than females whereas, females were expected to expand more rapidly than males and overtake space occupied by males because they have a higher growth rate (McLetchie, Garcia-Ramos, and Crowley, 2002). I found that in early establishment, selection is stronger on males than females for increased growth and cup production. Although selection favored larger plants for both sexes, when there was a significant stabilizing selection component, differences in the strength of selection resulted in different phenotypic maxima for the sexes. The different maxima were most obvious in the closed-wet treatment where selection favored larger males with fewer cups than females. This pattern of sex-specific selection only under certain environmental conditions in a highly phenotypically plastic plant may result in a wide range of phenotypic variation for the sexes and different degrees of sexual dimorphism under different environmental conditions.

At an equilibrium level of selection, selection coefficients are not significantly different from zero (Lande and Arnold, 1983) and the sexes express the optimum phenotype for the environment (Geber, 1999). In the closed-dry treatment, females and males expressed the same

fitness maxima for growth, and non-significant selection coefficients, though, for both sexes, the fitness maxima were slightly higher than the mean growth.

In a greenhouse experiment (Fuselier and McLetchie, 2002), selection favored larger males and smaller females under low light but selection did not differ between the sexes under high light (55% shade). Results reported here corroborate those from the greenhouse study in that selection favored larger males than females in the closed-wet treatment, but fitness was maximized at a larger size for females than males in the open-wet treatment. However, in this experiment, selection favored larger plants of both sexes.

Sex-specific selection in growth and cup production was detected under three of the four treatments but was inconsistent across treatments. For example, in the closed-dry treatment, the sexes differed only in the strength of selection on early cup production whereas, in the closed-wet treatment the sexes differed in strength of selection only on growth. When sex-specific selection is also environment-dependent, I expect to see differences in the degree of sexual dimorphism among natural populations if the environmental conditions at the populations differ. Indeed, sexual dimorphism varies among populations (Chapter 3) and the microhabitat among populations also differs (Chapter 1). Thus, differences in the way selection acts on the sexes within these populations, and the frequency of microhabitats within populations may explain the variation in the degree of dimorphism among populations.

Environment-dependent

Environmental heterogeneity may result in the persistence of both sexes in a population if selection acts differently on the sexes in different environments. I found that selection acted differently on growth rate between the sexes in different environments, a sex × environment × growth interaction. In wet areas, selection for more growth was stronger on males under closed canopy than under open canopy. If males respond to selection in concordance with the relative strength of selection in these different environments, different phenotypes of males may persist in populations. This is significant because, for males to persist during early establishment, they must out compete females for space, they must grow faster or produce fast-growing gemmalings. My data suggest that even though the sexes overlap in distribution among microhabitats (Chapter 2), closed canopy areas may provide a refuge for males where selection strongly favors larger males. This, of course, remains to be tested.

Consideration of a heterogeneous landscape may be very important to understanding the dynamics of population sex ratios in *M. inflexa*. Populations of *M. inflexa* differ in microhabitat availability (Chapter 3) and male *M. inflexa* inhabit a wider range of light environments than females in natural populations (Chapter 2). With the combined influence of environment-dependent and sex-specific selection in some environments, populations with heterogeneous habitat may promote the persistence of both sexes within populations.

Constraints

The traits monitored in this experiment likely have a genetic basis (Fuselier unpublished data) but, heritable traits may not respond to selection even where significant selection is detected (Kruuk et al., 2001). Not all sexually dimorphic traits are adaptive but may be expressed simply as a result of correlated responses to other traits under selection (Lande, 1980; Geber, 1995). Selection on growth, in particular, may be the driving force for the maintenance of sexually dimorphic traits observed in *M. inflexa*. Faster growth and higher cup production were strongly positively associated with higher fitness in both females and males during early patch establishment. Growth, cup production in the first month, and maximum cup production were significantly positively correlated so, selection on growth can result in correlated selection on cup production traits. Cup production is also related to the degree of sex structure production because in *M. inflexa*, there is a trade-off between asexual and sexual fitness (Fuselier and McLetchie, 2002).

Response to selection of sexually dimorphic traits is also constrained by between-sex genetic correlations (Lande, 1980). Between-sex trait correlations are also environment-dependent. In greenhouse experiments there were no significant between-sex character correlations for a similar suite of traits measured in *M. inflexa* (Chapter 3), whereas under field conditions, sexes showed positive between-sex correlations for all characters except growth. In greenhouse experiments, growth is the most consistently dimorphic character, with females larger than males (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002), and this same pattern is observed in many sexually dimorphic bryophytes (Chapter 1). This lack of between-sex correlational constraint may allow growth rates to evolve differently in the sexes, and characters correlated with growth to appear dimorphic. If selection for growth is sex-specific, this may result in a suite of characters correlated with growth that are sexually dimorphic.

Influence of sex

Patterns of sex-specific selection may change throughout the life cycle of bryophytes and produce a very different pattern of lifetime fitness correlates (Schluter and Smith., 1986; Schluter, Price, and Rowe, 1991). I present only part of the picture for lifetime fitness correlates; what happens after colonization and in relation to sexual reproduction may be influential on ultimate adult population sex ratios. For example, in a greenhouse experiment, selection on asexual fitness and sexual fitness acted in opposite directions on female *M. inflexa*, and female plants expressed phenotypes consistent with response to selection on sexual fitness (Fuselier and McLetchie, 2002).

Because of positive between-sex correlations, strong selection favoring early cup production in males may influence cup production in females, and indirectly select for females with high early cup production. However, females that produce fewer cups earlier are favored by selection on sexual fitness. Thus, the between sex correlation and selection for more cups on males may constrain female ability to produce few cups early and more sex structures later. On the other hand, if selection on sexual fitness is stronger and selection favors females with low cup production and high growth, increased cup production in males may be constrained by similar indirect selection but on the sexual fitness component of the life cycle.

Distribution of the sexes

Single-sex populations of *M. inflexa* are found in the Arbuckle Mountain region of Oklahoma, and in Florida, USA, where plants are hypothesized to have found refuge during the last Pleistocene sea transgression and later expand (Longton and Schuster, 1983). Plants from single-sex populations grow larger and produce few, large branches and few cups compared to plants from both-sex populations when grown in a common garden (Chapter 3). Additionally, there are more all-male populations than all-female populations documented for *M. inflexa.* Selection favored males and females that grew faster and produced more cups during early establishment. Also, fitness in females was maximized at a lower area/branch in most treatments whereas, either the opposite trend or no trend was observed for males. If a larger area/branch is beneficial in some way for expansion after a period in refugia, (barring phylogeographic influences among populations), and females with higher area/branch have lower fitness, it follows that males may be more likely to survive and expand after periods in refugia. This

combined with the trend for males to survive better than females in the harshest of treatments may explain the greater number of male than female populations.

Some caution must be used in interpretation of these patterns. Single-sex populations may indeed harbor both sexes, but only one sex is recognized because the other fails to produce sex structures, which are necessary for definitive sex determination in the field. Also, currently there are no phylogeographic studies that examine the genetic relationships among populations, and phylogeny may explain the phenotypic differences observed between population types.

Table 4.1. Trait values in four treatments.
Mean trait values for female and male *Marchantia inflexa*under four light - moisture treatments in the field. Sample size is the number of genotypes, relative fitness is survival for 21 days, cup1 = number of cups produced in 21 days, max cups is the number of cups produced after 54 days.

	Females n = 27				Males n = 30			
Treatment	proportion surviving	growth (mm^2)	cup1	max cups	proportion surviving	growth (mm^2)	cup1	max cups
Closed-dry	0.67	12.471	0.276	0.492	0.67	12.609	0.321	0.528
Closed-wet	0.79	16.886	0.300	0.542	0.77	17.326	0.322	0.543
Open-dry	0.63	17.785	0.294	0.522	0.64	16.264	0.314	0.549
Open-wet	0.83	21.808	0.482	0.785	0.83	22.537	0.490	0.851
Means	0.73	17.237	0.338	0.585	0.73	17.184	0.362	0.618

Table 4.2. Among trait correlations in 4 treatments.
Trait correlations using genotype mean standardized traits; females above diagonal, males below. * $P < 0.05$, **$P < 0.01$ Growth is in mm^2 for 21 days, cup 1 is the number of cups produced by day 21, max cups is the total number of cups produced by day 54 for both female and male *Marchantia inflexa* grown under four light/moisture treatments at a field site.

Treatment	Trait	growth	cup 1	max cups	area/branch
Closed-dry	growth		0.329	0.477*	0.129
	cup 1	0.287		0.807**	-0.534 *
	max cups	0.584 *	0.797 **		-0.321
	area/branch	0.192	-0.007	0.101	
Closed-wet	growth		0.120	0.225	0.192
	cup 1	0.507*		0.883 **	-0.399 *
	max cups	0.529 *	0.854 **		-0.480 *
	area/branch	0.316	0.006	0.129	
Open-dry	growth		-0.236	0.002	0.551 *
	cup 1	0.521 *		0.860 **	-0.442 *
	max cups	0.634 **	0.797 **		-0.286
	area/branch	0.315	0.206	0.053	
Open - Wet	growth		0.277	0.408 *	0.240
	cup 1	0.291		0.933 **	-0.495 *
	max cups	0.523 *	0.793 **		-0.478 *
	area/branch	0.279	-0.135	-0.030	

Table 4.3. Genotypic selection analysis results.

Genotypic selection analysis results for female and male Marchantia inflexa in four light/moisture treatments at a field site. Beta = coefficient of selection for direct selection; s = coefficient of selection for total selection on four traits. Growth is in mm^2 for 21 days, cup 1 is the number of cups produced by day 21, max cups is the total number of cups produced by day 54. Non-linear (quadratic) selection analyses (g) were conducted only for traits that showed total selection in genotypic selection analyses.

			Direct Selection			Total Selection			Non-linear Total Selection		
Treatment	Sex	Trait	Beta	Stderr	Pr > |t|	s	Stderr	Pr > |t|	g	Stderr	Pr > |t|
Closed-dry	Female	growth	0.55	0.11	<0.0001 *	0.50	0.10	<0.0001 *	-1.53	0.34	0.000 *
		cup 1	0.14	0.15	0.347	0.33	0.11	0.005 *	-0.68	0.38	0.087
		maxcups	-0.13	0.16	0.418	0.34	0.12	0.012 *	-0.54	0.49	0.289
		area/branch	-0.06	0.02	0.027 *	-0.05	0.03	0.089			
	Male	growth	0.54	0.16	0.003 *	0.39	0.13	0.006 *	-1.25	0.54	0.028 *
		cup 1	0.81	0.30	0.012 *	0.44	0.19	0.028 *	-3.84	1.00	0.001 *
		maxcups	-0.54	0.26	0.046 *	0.25	0.14	0.092	-0.83	0.80	0.306
		area/branch	0.00	0.02	0.865	0.00	0.02	0.873			
Closed-wet	Female	growth	0.19	0.09	0.043 *	0.23	0.10	0.023 *	-0.86	0.38	0.033 *
		cup 1	0.11	0.13	0.422	0.25	0.06	0.001 *	-0.29	0.28	0.310
		maxcups	0.15	0.16	0.371	0.31	0.07	0.0002 *	0.00	0.38	0.992
		area/branch	-0.01	0.02	0.677	-0.03	0.02	0.183			
	Male	growth	0.62	0.17	0.001 *	0.64	0.13	<0.0001 *	-1.30	0.41	0.004 *
		cup 1	0.33	0.27	0.242	0.35	0.17	0.048 *	-2.38	0.59	0.000 *
		maxcups	-0.33	0.26	0.211	0.27	0.17	0.115	-1.61	0.57	0.009 *
		area/branch	0.02	0.03	0.383	0.05	0.03	0.108			
Open-dry	Female	growth	0.56	0.22	0.017 *	0.28	0.20	0.165	-3.87	0.66	<0.0001 *
		cup 1	0.22	0.23	0.365	0.26	0.11	0.030 *	0.18	0.47	0.701
		maxcups	0.03	0.22	0.879	0.28	0.11	0.017 *	-0.03	0.33	0.926
		area/branch	-0.05	0.03	0.116	-0.03	0.03	0.277			
	Male	growth	0.09	0.11	0.438	0.41	0.10	0.0003 *	-0.85	0.42	0.052 *
		cup 1	-0.11	0.14	0.437	0.40	0.11	0.001 *	-0.75	0.44	0.095
		maxcups	0.59	0.17	0.002 *	0.56	0.09	<0.0001 *	-0.66	0.41	0.121
		area/branch	0.02	0.02	0.206	0.03	0.02	0.233			
Open-wet	Female	growth	0.31	0.08	0.001 *	0.30	0.07	0.000 *	-0.63	0.17	0.001 *
		cup 1	0.10	0.18	0.592	0.28	0.08	0.002 *	-0.27	0.30	0.383
		maxcups	0.00	0.17	0.994	0.27	0.06	0.000 *	-0.62	0.17	0.001 *
		area/branch	-0.05	0.02	0.034 *	-0.04	0.02	0.107			
	Male	growth	0.37	0.14	0.014 *	0.43	0.11	0.001 *	-1.26	0.57	0.037 *
		cup 1	0.14	0.16	0.403	0.27	0.11	0.020 *	-0.27	0.41	0.525
		maxcups	0.04	0.18	0.837	0.31	0.10	0.005 *	-0.36	0.40	0.377
		area/branch	0.00	0.02	0.809	0.01	0.02	0.710			

Table 4.4. Sex-specific selection.
Analysis of covariance interaction terms that reveal sex-specific selection in *Marchantia inflexa* grown in four light/moisture treatments (df = 1). Growth is in mm^2 for 21 days, cup 1 is the number of cups produced by day 21, max cups is the total number of cups produced by day 54.

Treatment	interaction	Type III SS	F Value	Pr > F	
Closed-dry	sex × growth	0.0000	0.01	0.981	
	sex × cup 1	0.0422	4.43	0.041	*
	sex × max cups	0.0185	1.94	0.170	
	sex × area/branch	0.0248	2.61	0.113	
Closed-wet	sex × growth	0.0588	4.95	0.031	*
	sex × cup 1	0.0064	0.54	0.467	
	sex × max cups	0.0241	2.03	0.161	
	sex × area/branch	0.0083	0.7	0.406	
Open-dry	sex × growth	0.0404	4.27	0.044	*
	sex × cup 1	0.0146	1.54	0.221	
	sex × max cups	0.0364	3.84	0.055	*
	sex × area/branch	0.0454	4.79	0.034	*
Open-wet	sex × growth	0.0010	0.13	0.718	
	sex × cup 1	0.0002	0.03	0.866	
	sex × max cups	0.0001	0.02	0.893	
	sex × area/branch	0.0148	1.92	0.173	

Table 4.5. Environment-dependent selection.
Analysis of covariance interaction terms that reveal environment-dependent selection on female and male *Marchantia inflexa* in four light/moisture treatments. Growth is in mm^2 for 21 days, cup 1 is the number of cups produced by day 21, max cups is the total number of cups produced by day 54.

			Direct Selection		Total Selection	
Treatment	Sex	Source	F	Pr > F	F	Pr > F
Closed	Females	growth × moist	4.64	0.037	5.72	0.02
		cup 1 × moist	0.23	0.637	0.01	0.93
		max cups × moist	0.55	0.464	0.10	0.75
	Males	growth × moist	1.67	0.203	1.91	0.17
		cup 1 × moist	3.95	0.052	0.19	0.66
		max cups × moist	0.51	0.479	0.40	0.53
Open	Females	growth × moist	1.43	0.238	1.45	0.23
		cup 1 × moist	0	0.982	0.10	0.75
		max cups × moist	0.08	0.776	0.21	0.65
	Males	growth × moist	0.26	0.615	0.16	0.69
		cup 1 × moist	2.57	0.115	0.11	0.74
		max cups × moist	4.62	0.037	0.85	0.36
Dry	Females	growth × light	0.01	0.906	4.23	0.04
		cup 1 × light	0.01	0.935	0	0.99
		max cups × light	0.51	0.478	0.08	0.78
	Males	growth × light	3.09	0.085	0	0.97
		cup 1 × light	5.91	0.019	0.69	0.41
		max cups × light	12.35	0.001	6.54	0.01
Wet	Females	growth × light	0.07	0.786	1.83	0.18
		cup 1 × light	0.11	0.740	0.24	0.63
		max cups × light	0	0.982	0.40	0.53
	Males	growth × light	6.31	0.015	1.12	0.30
		cup 1 × light	1.4	0.242	0	0.95
		max cups × light	1.12	0.295	0.19	0.67

Figure 4.1a. Light treatments.
Mean light received under the canopy and at two treatment levels C = closed, O = open canopy.
Bars are standard error, diamonds are maximum and circles, minimum values.

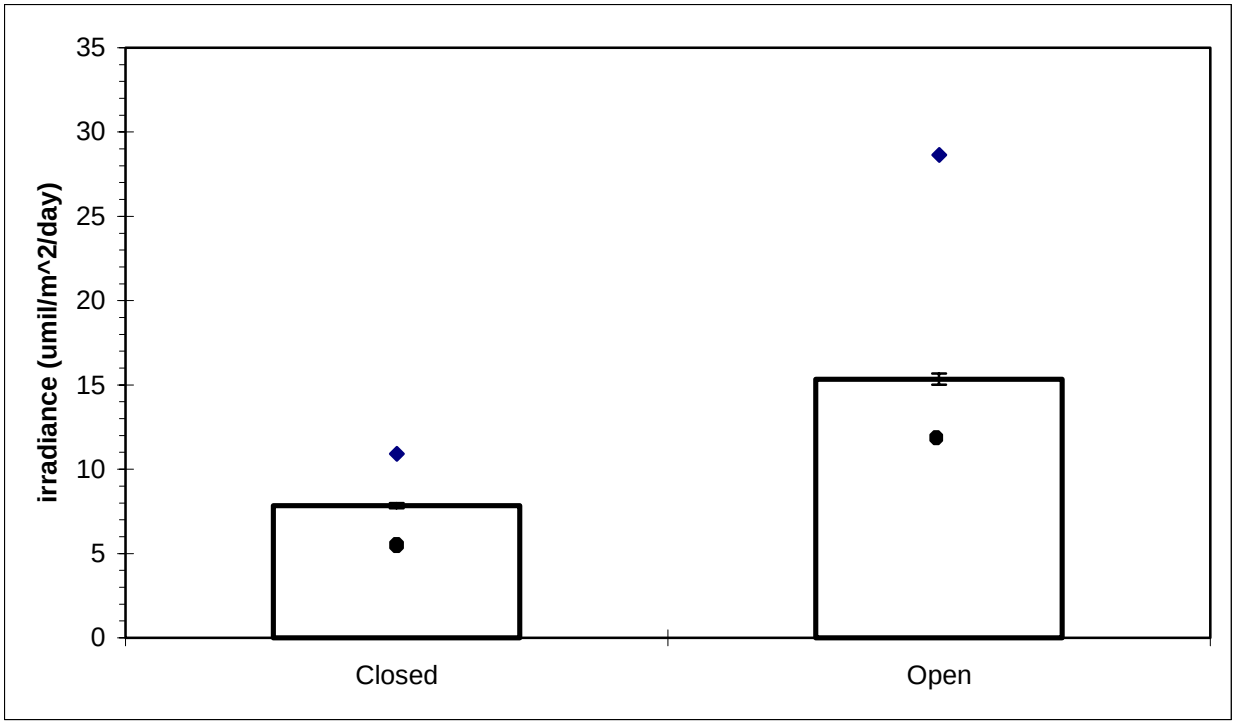
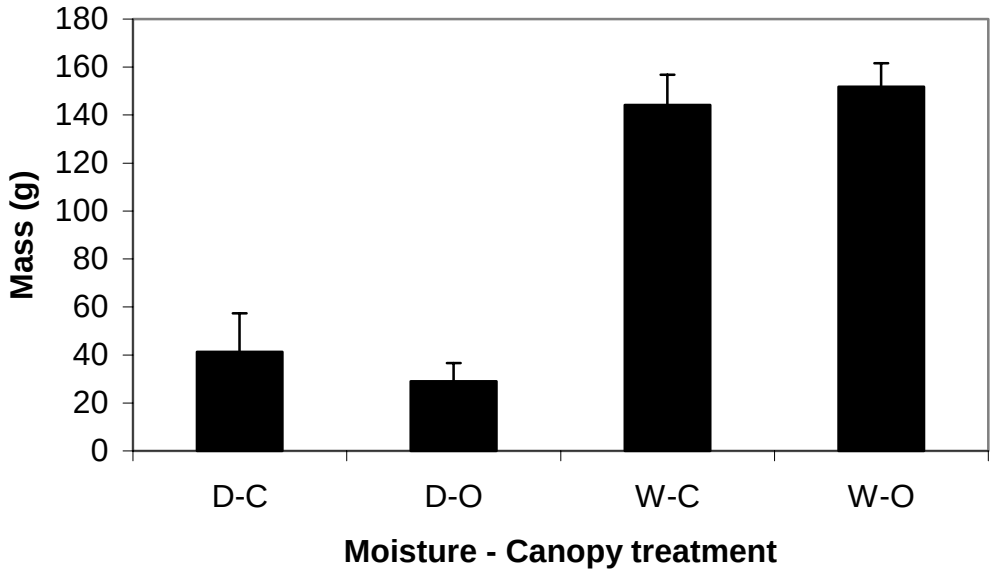


Figure 4.1b. Moisture treatments.
Moisture in mats in four different canopy - moisture treatments given as mass of water gain in mat after one week in the field. D=dry, W=wet, C=closed canopy, O=open canopy. Bars are standard error.



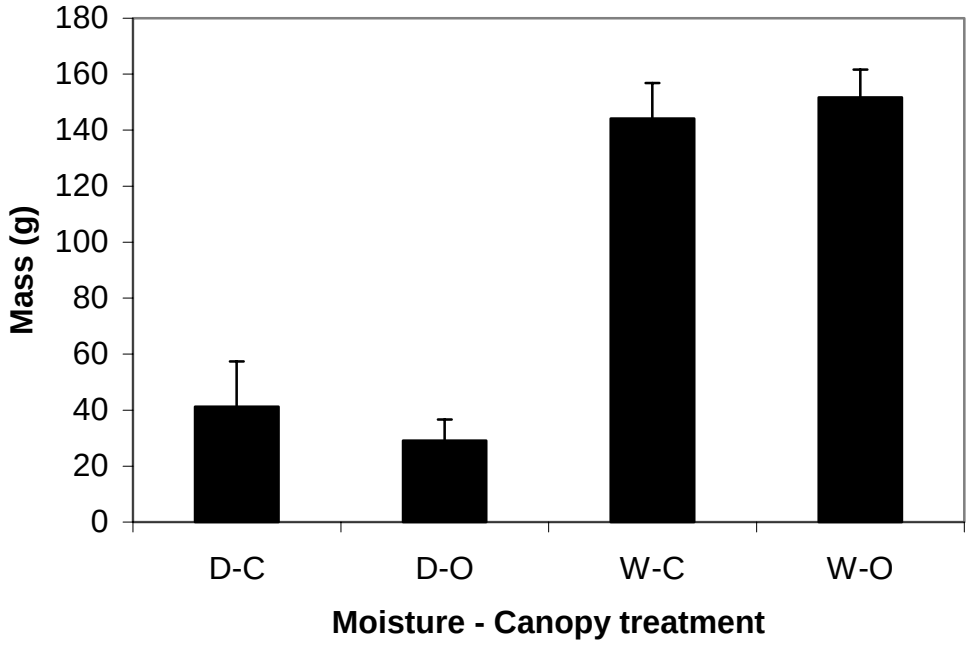
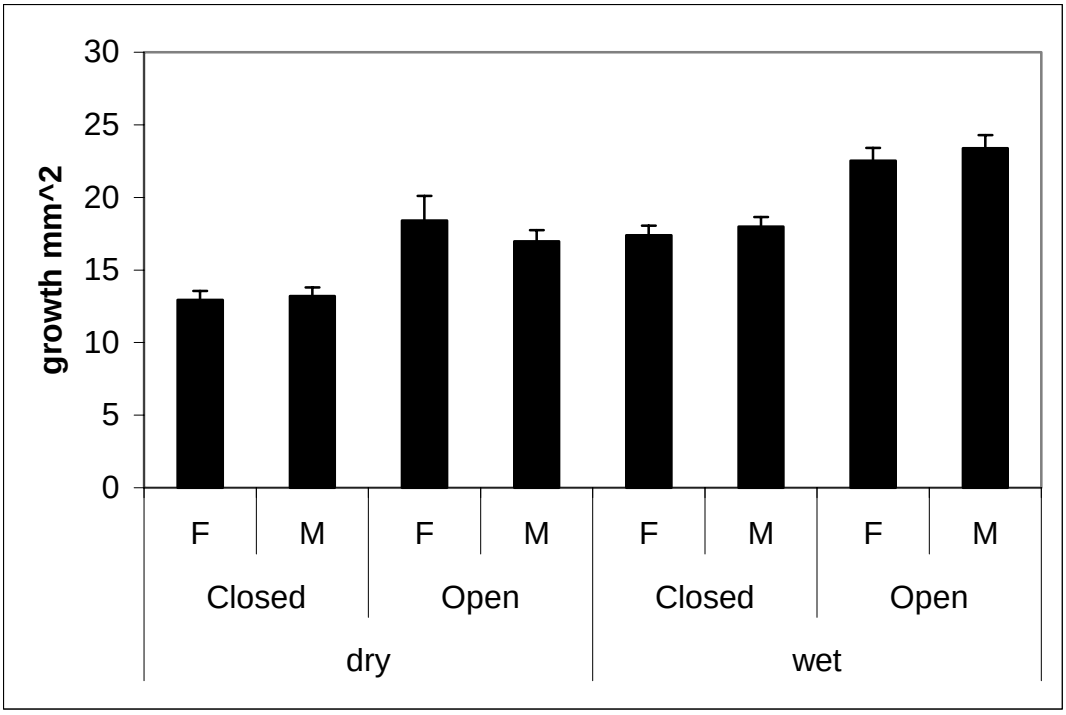


Figure 4.2 a - b. Trait values in four treatments.
Mean trait values and mean proportion of genotypes surviving for female and male *Marchantia inflexa* in four moisture/light treatments in the field. Sample size is the number of genotypes (females=27, males=30), relative fitness is survival for 21 days, cup1 = number of cups produced in 21 days, max cups is the number of cups produced after 54 days.



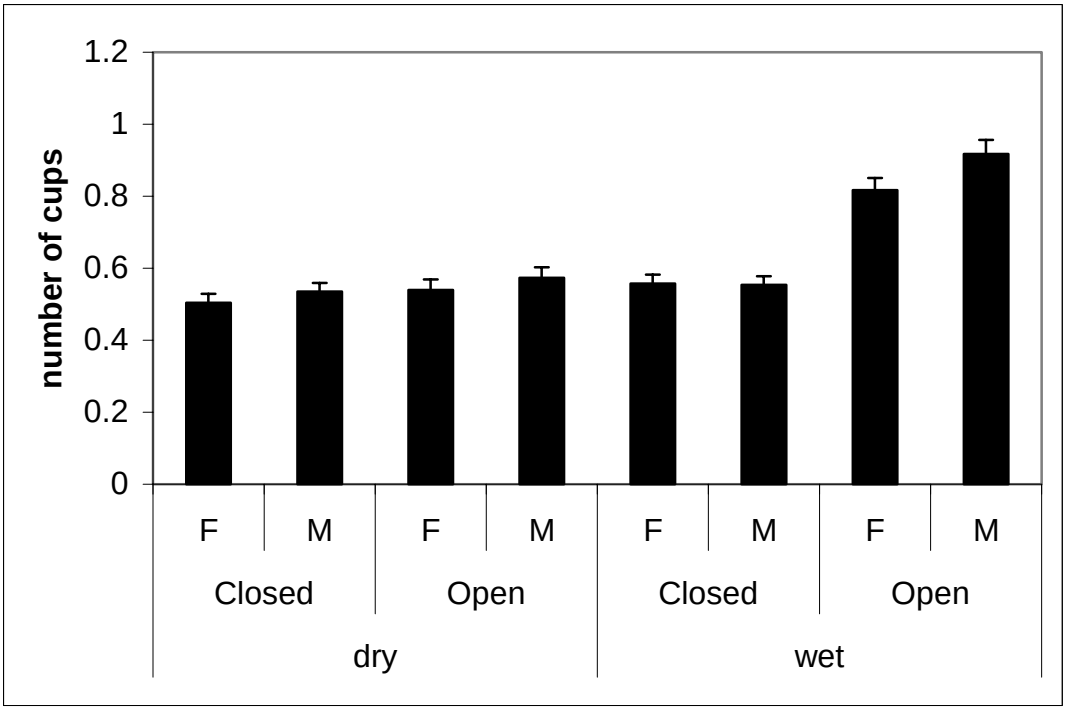


Figure 4.2c.Proportion surviving in four treatments.

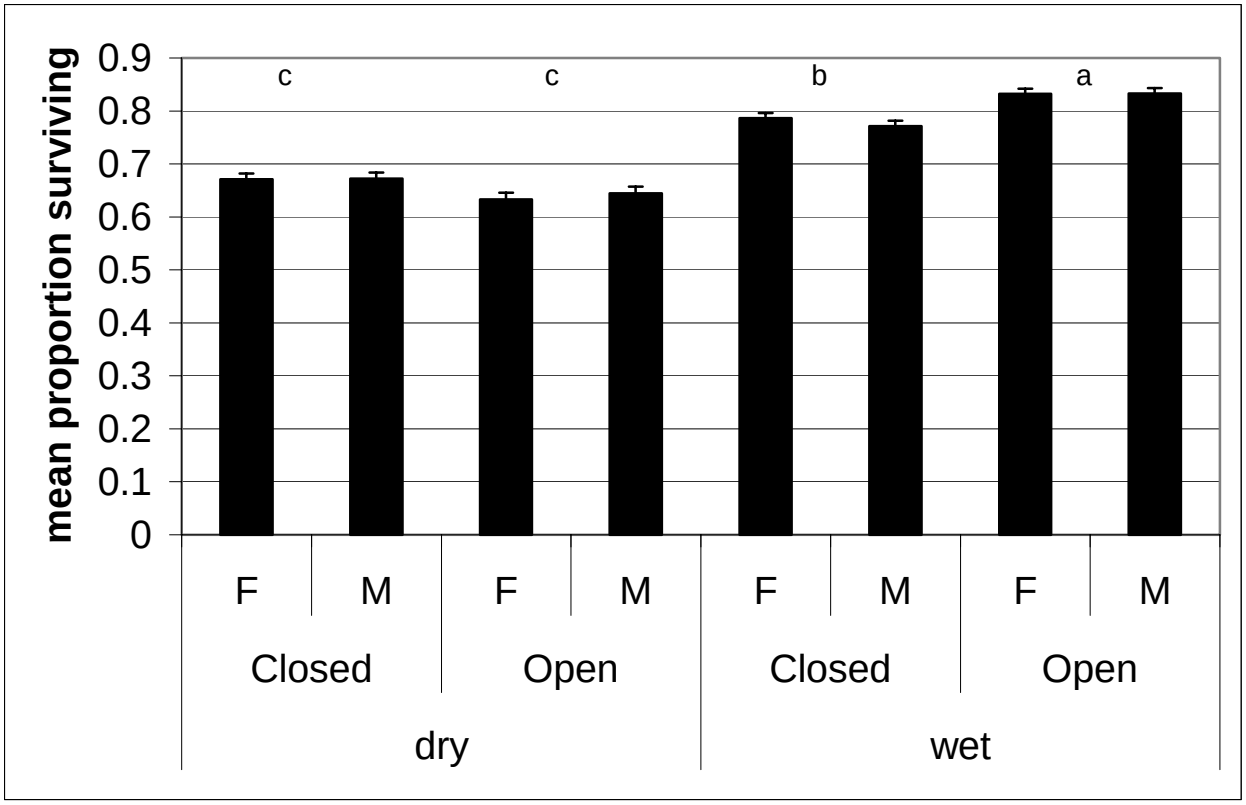
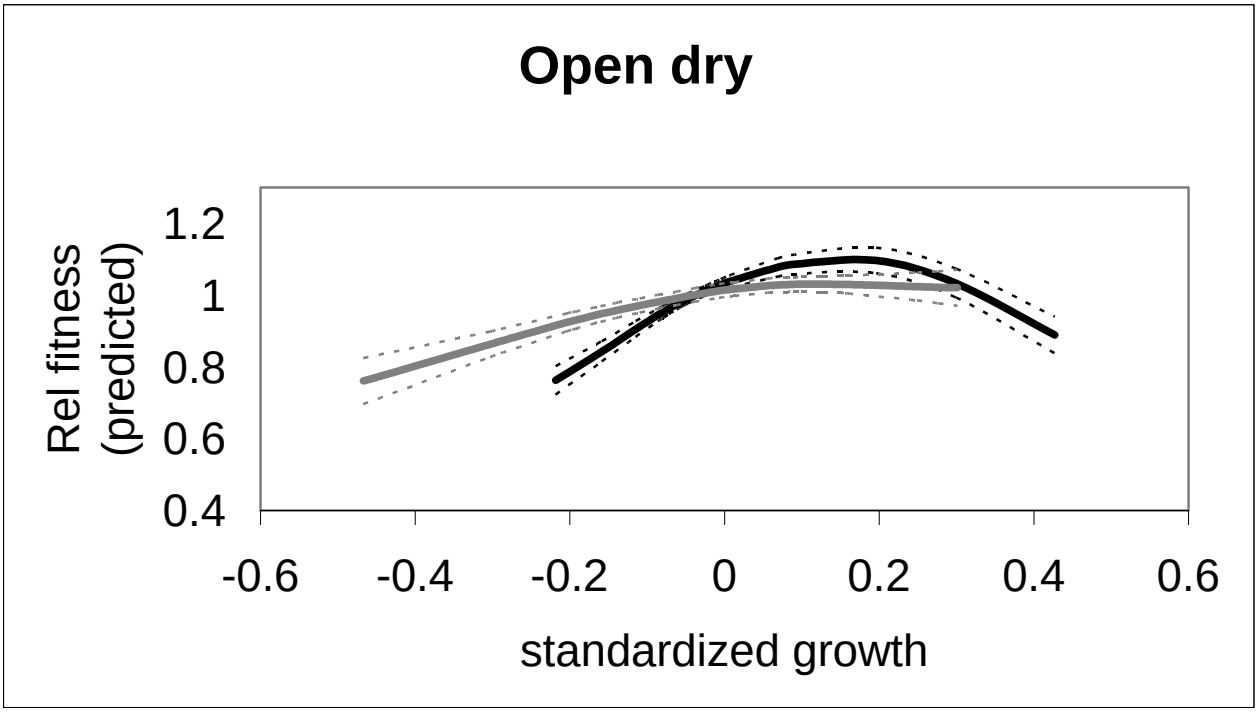


Figure 4.3. Fitness curves: growth.
Relationship between standardized growth values and predicted relative fitness as calculated by cubic spline fit to fitness data for female (black line) and male (grey line) *Marchantia inflexa* in four moisture/light treatments in a field site. Dotted lines are upper and lower 95% confidence intervals.



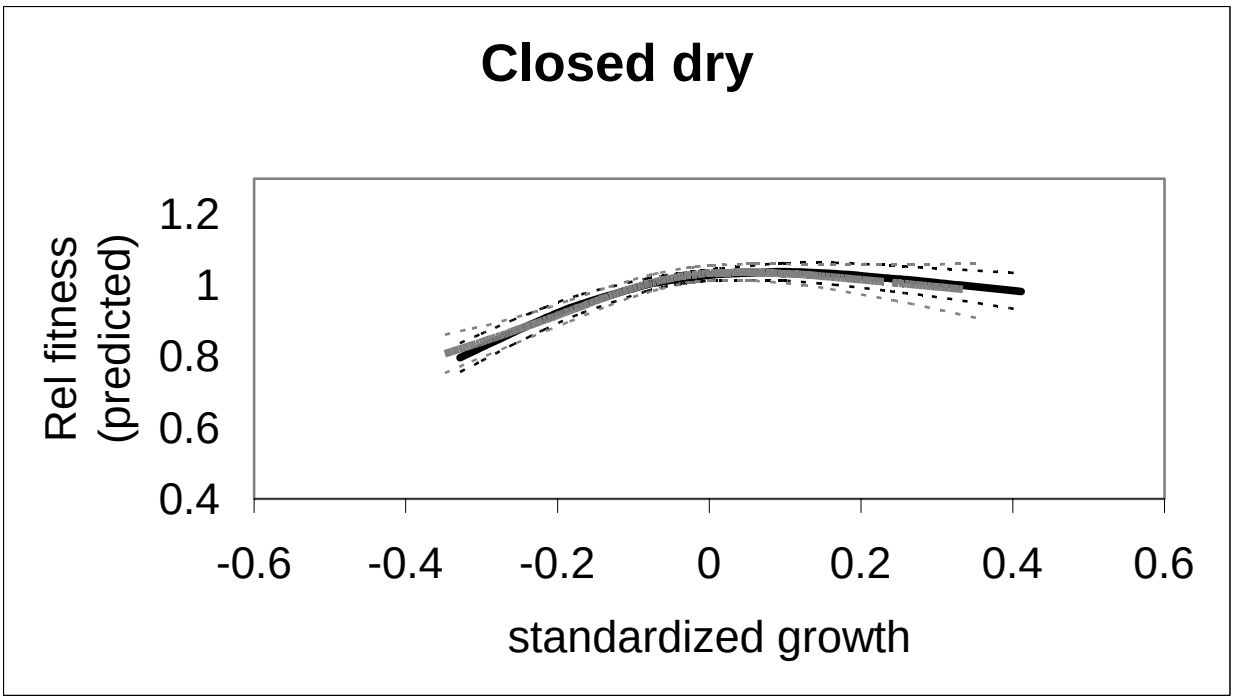
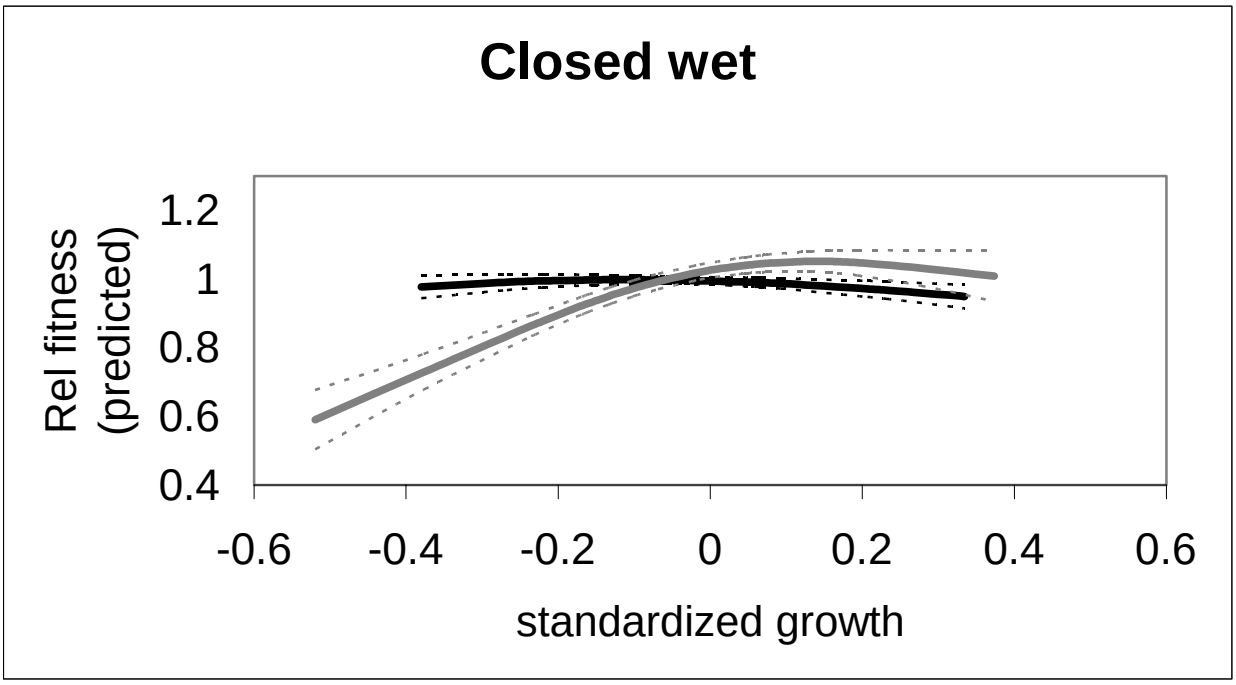


Figure 4.3 continued.



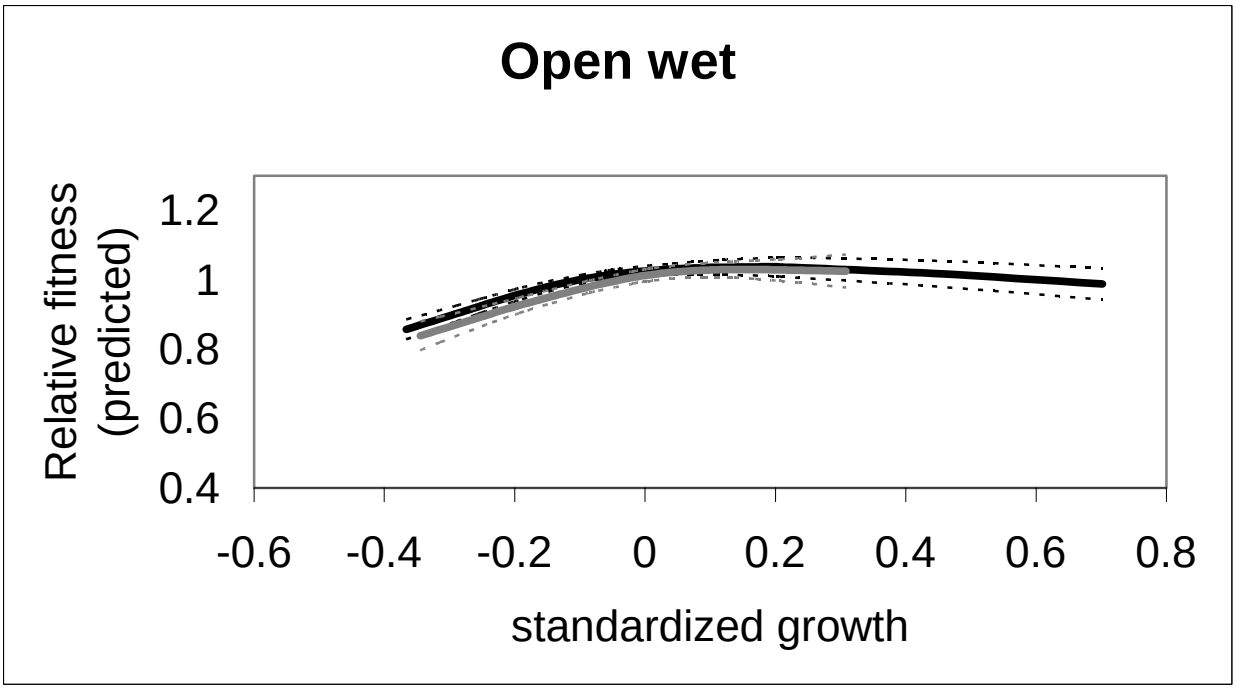
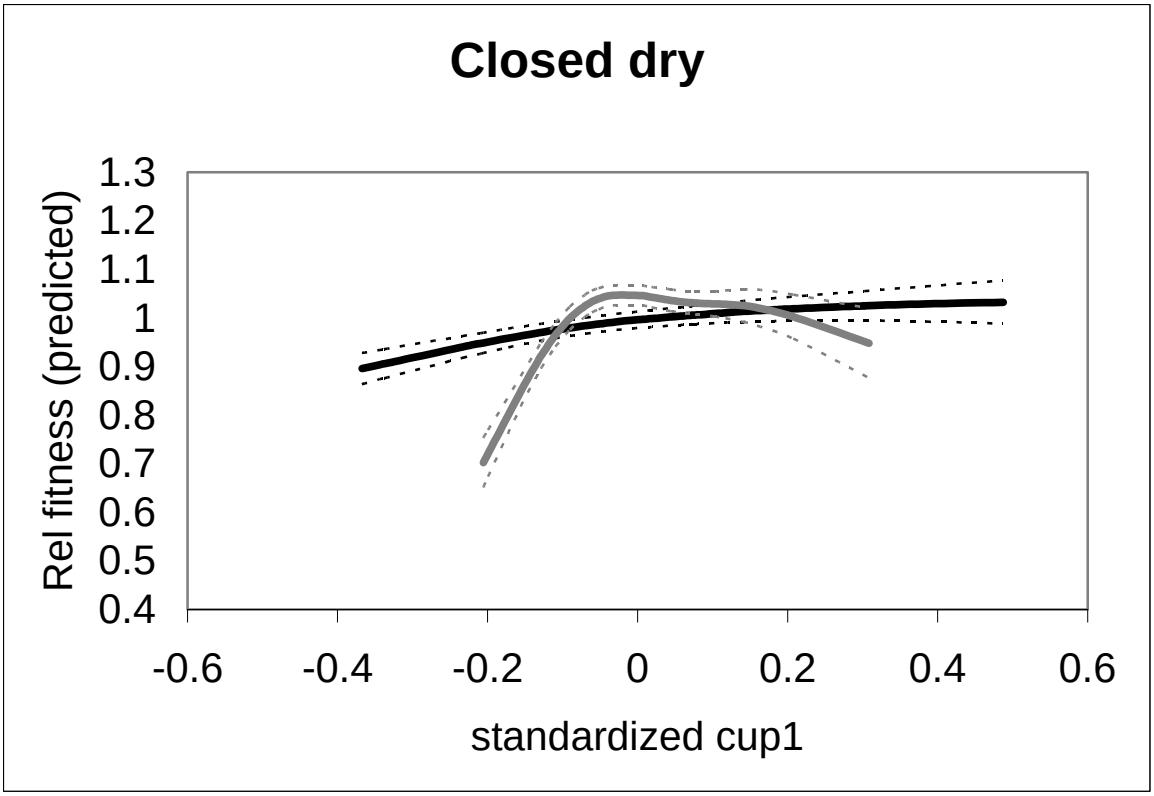


Figure 4.4. Fitness curves: early cup production.
Relationship between standardized early cup production values and predicted relative fitness as calculated by cubic spline fit to fitness data for female (dark line) and male (light line) *Marchantia inflexa* in four moisture/light treatments in a field site.



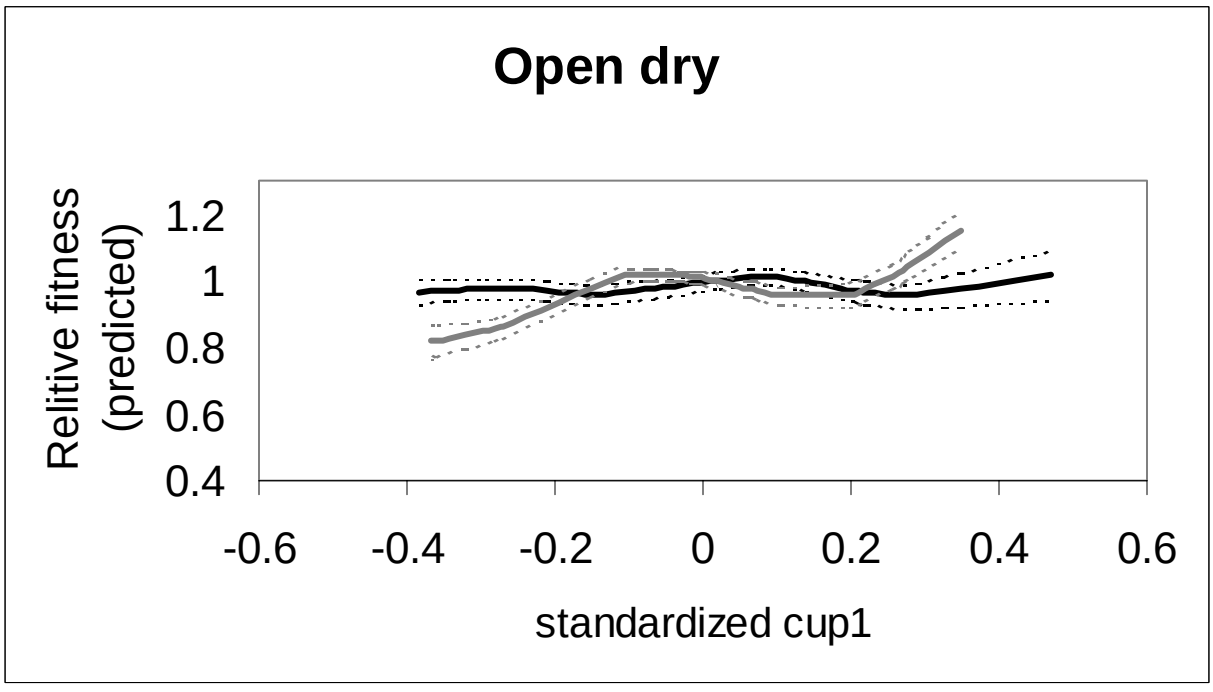
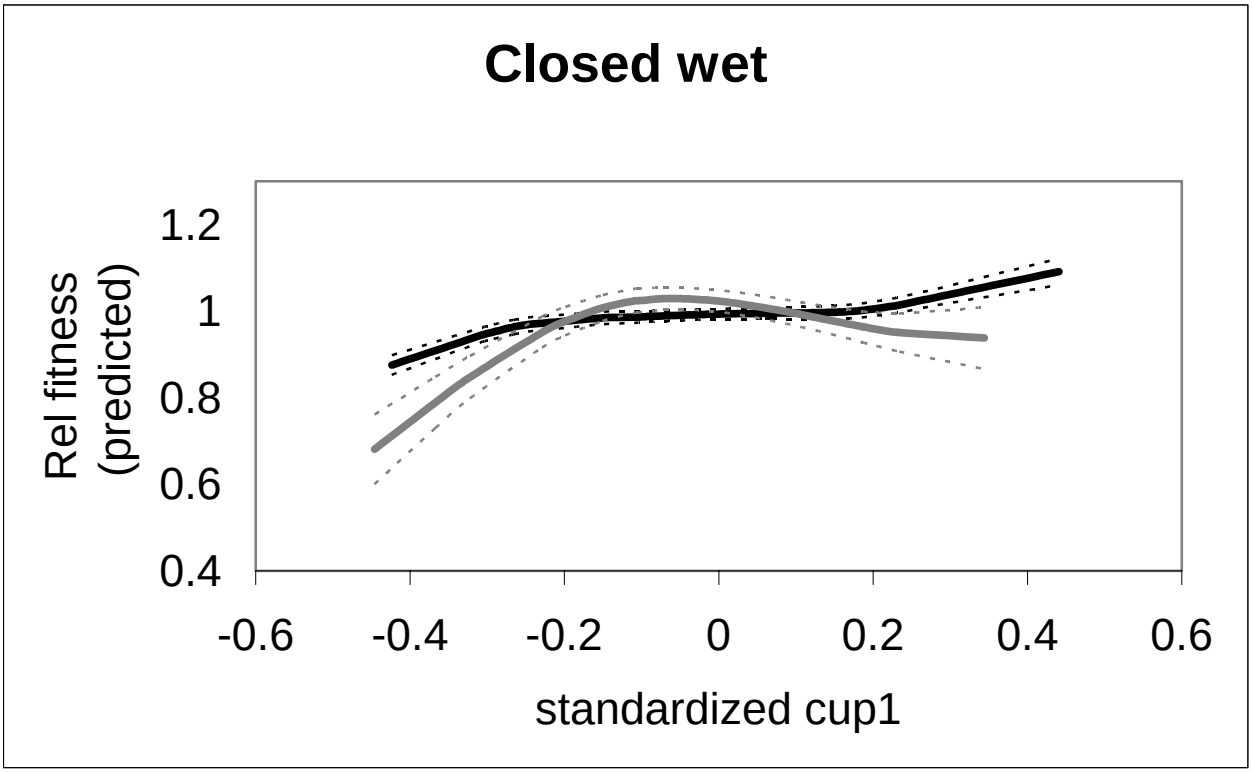


Figure 4.4 continued.



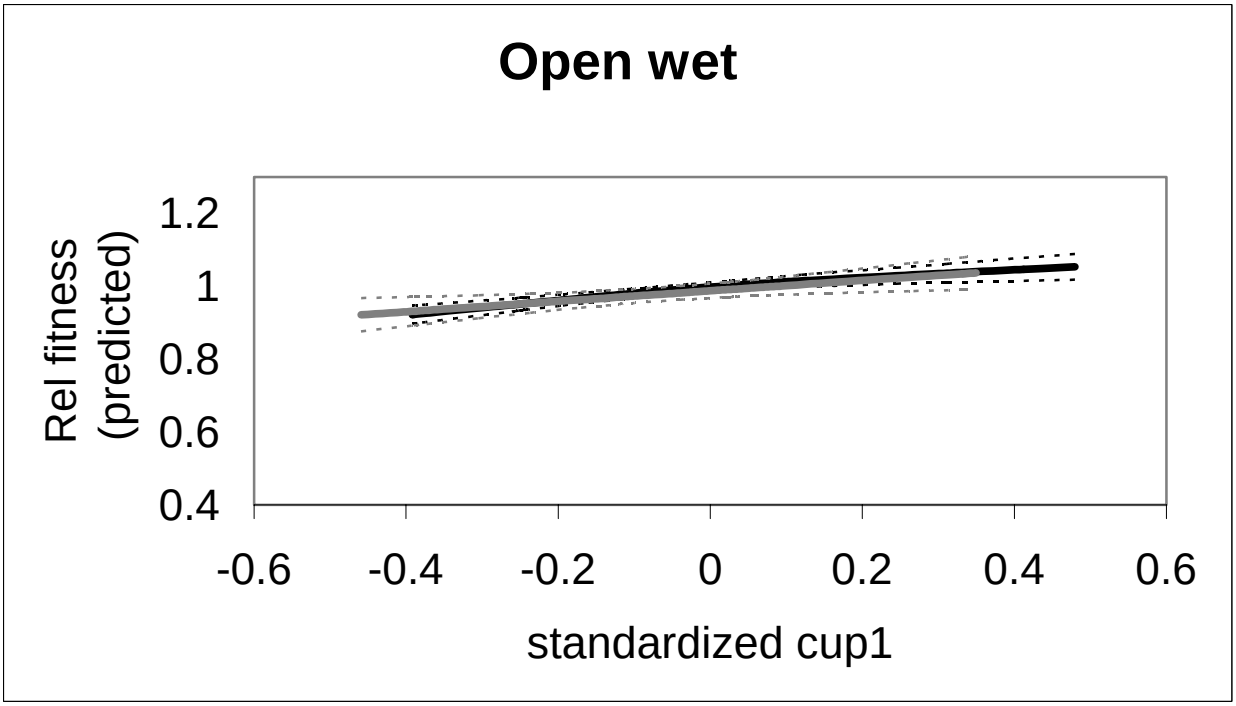
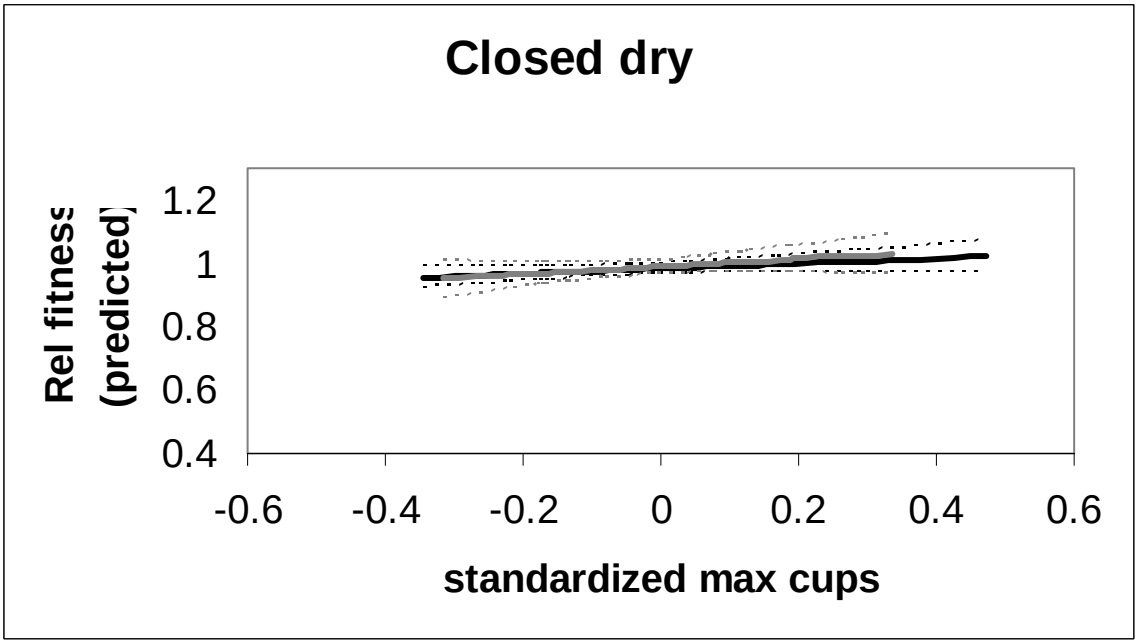


Figure 4.5. Fitness curves: max cups.
Relationship between standardized maximum number of cup values and predicted relative fitness as calculated by cubic spline fit to fitness data for female (black) and male (grey) *Marchantia inflexa* in four moisture/light treatments in a field site.



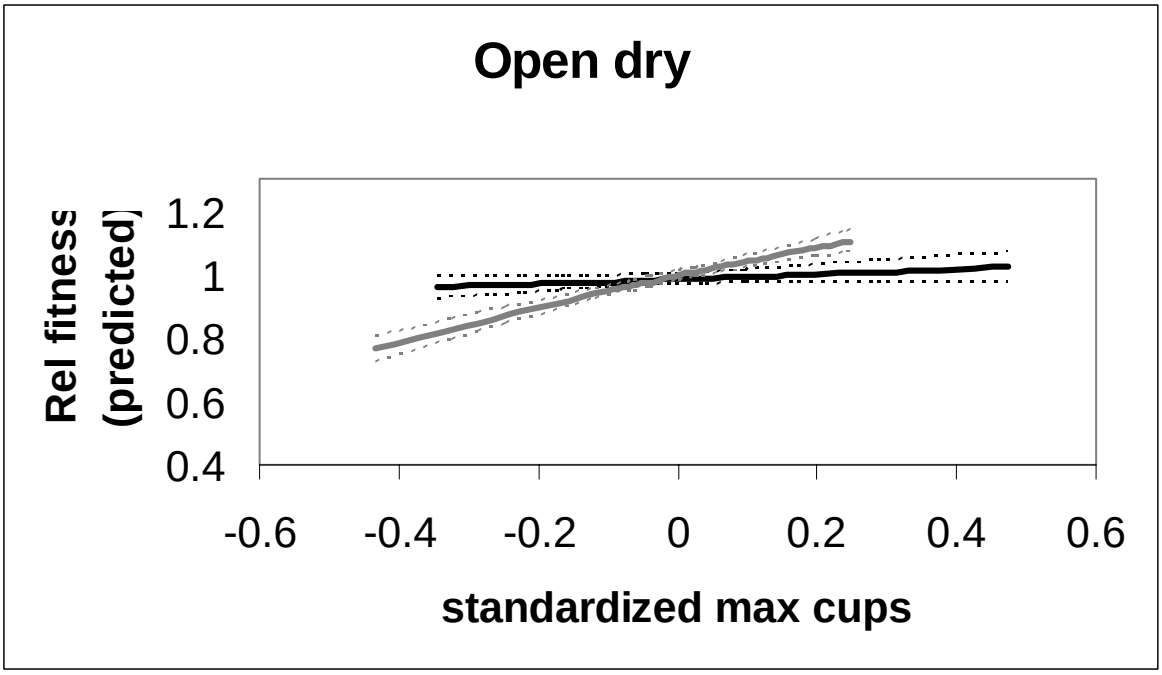
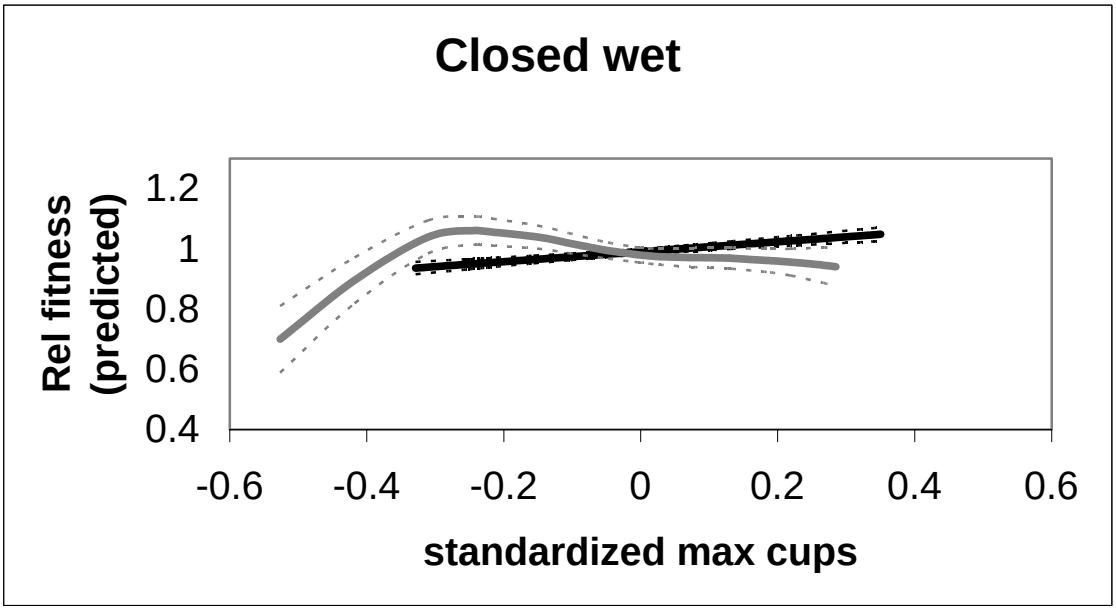


Figure 4.5 continued.



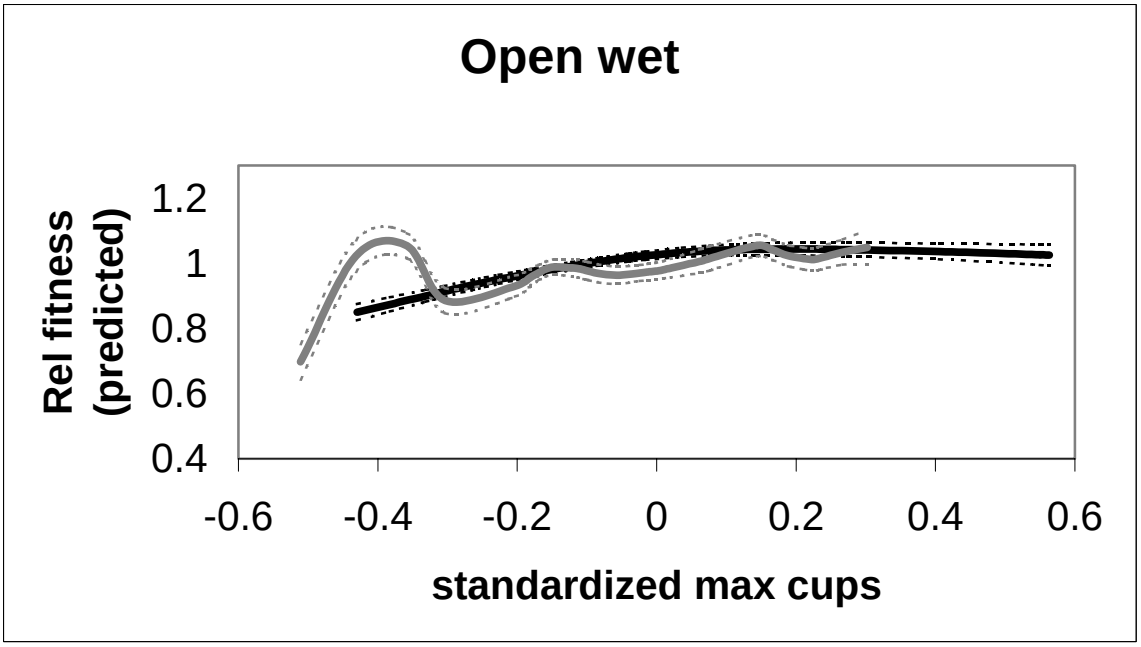
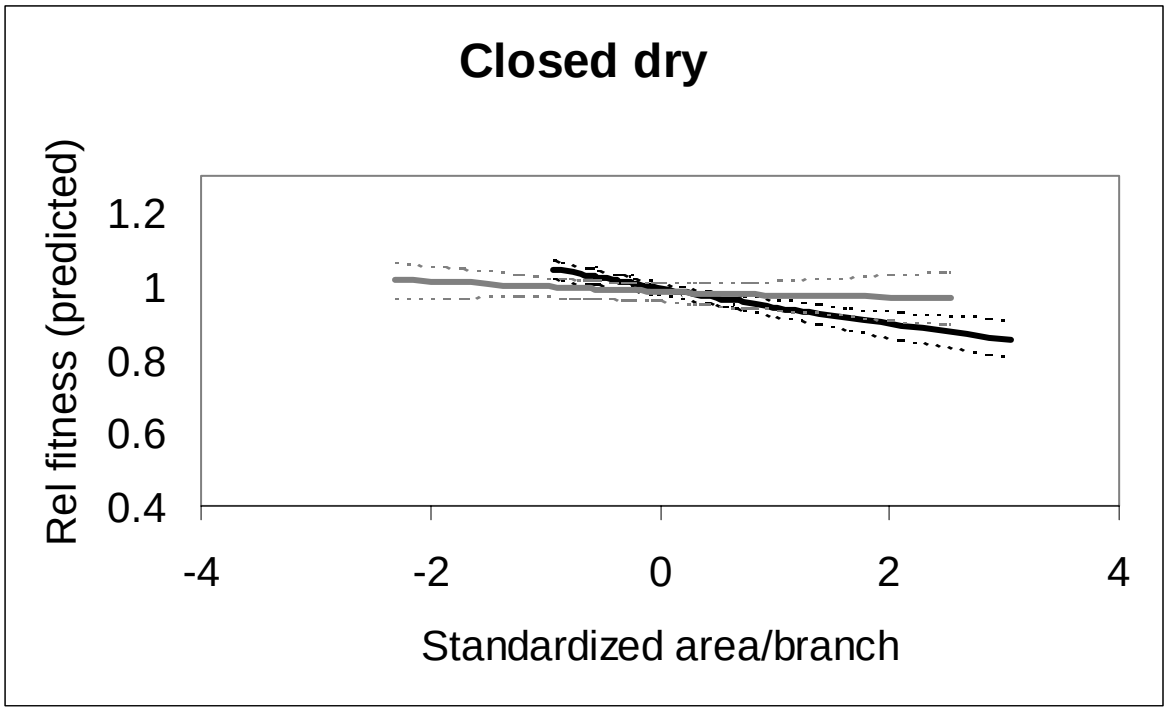
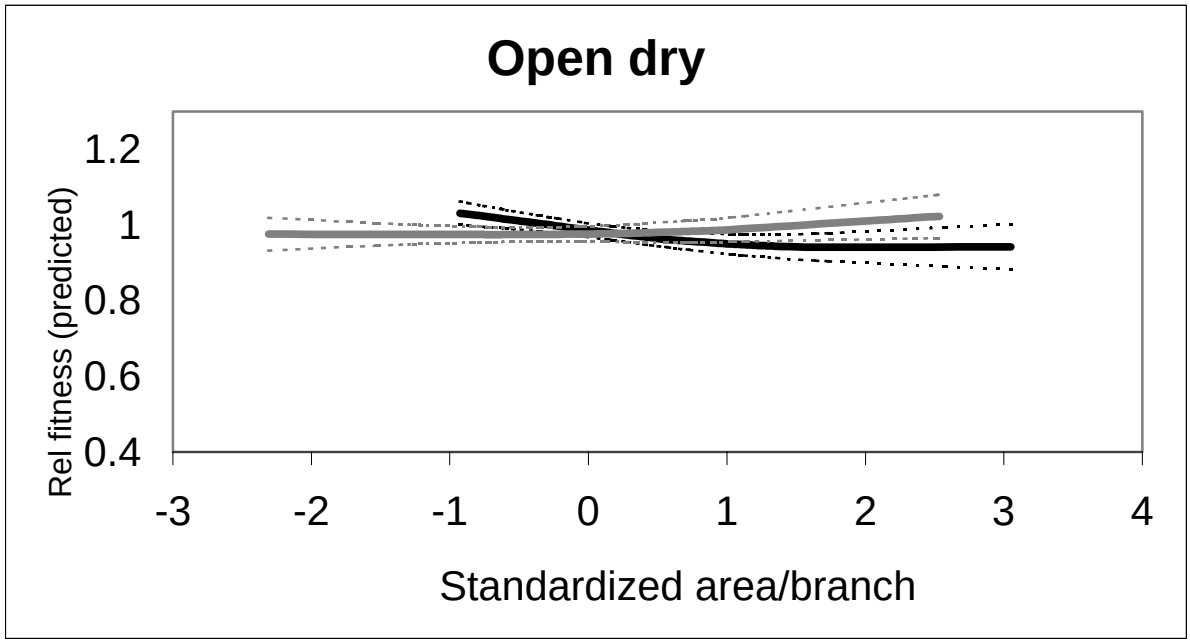


Figure 4.6. Fitness curves: branch size.
Relationship between standardized area (mm^2)/branch and values and predicted relative fitness as calculated by cubic spline fit to fitness data for female (black) and male (grey) *Marchantia inflexa* in four moisture/light treatments in a field site.





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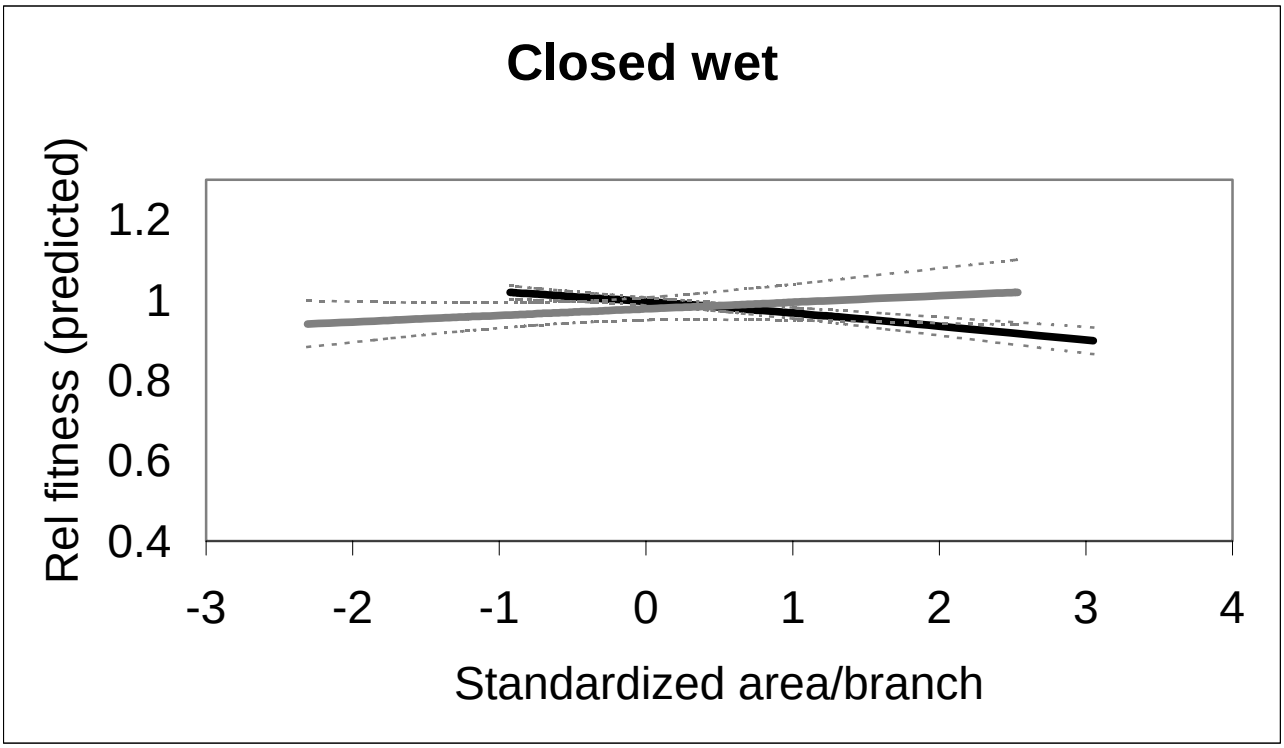


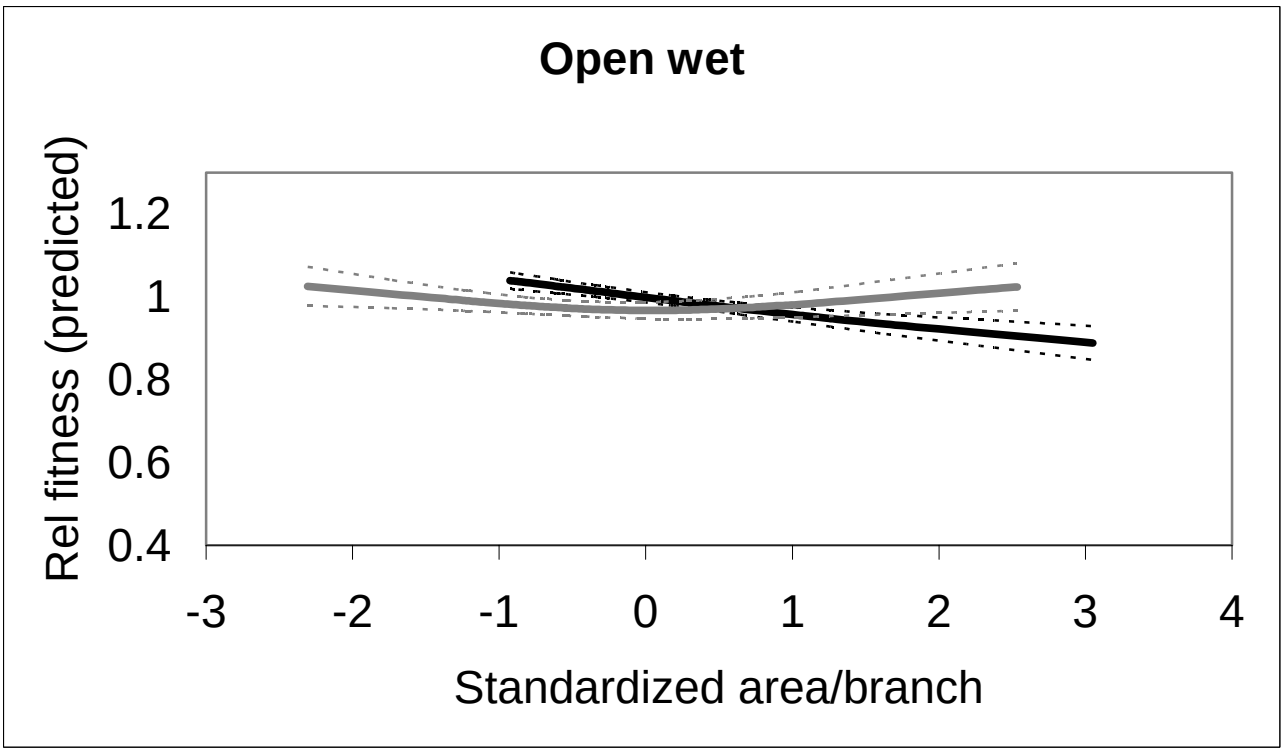


Figure 4.7. Selection across environments for the sexes.
Selection differed across environments and differed on the sexes among environments.

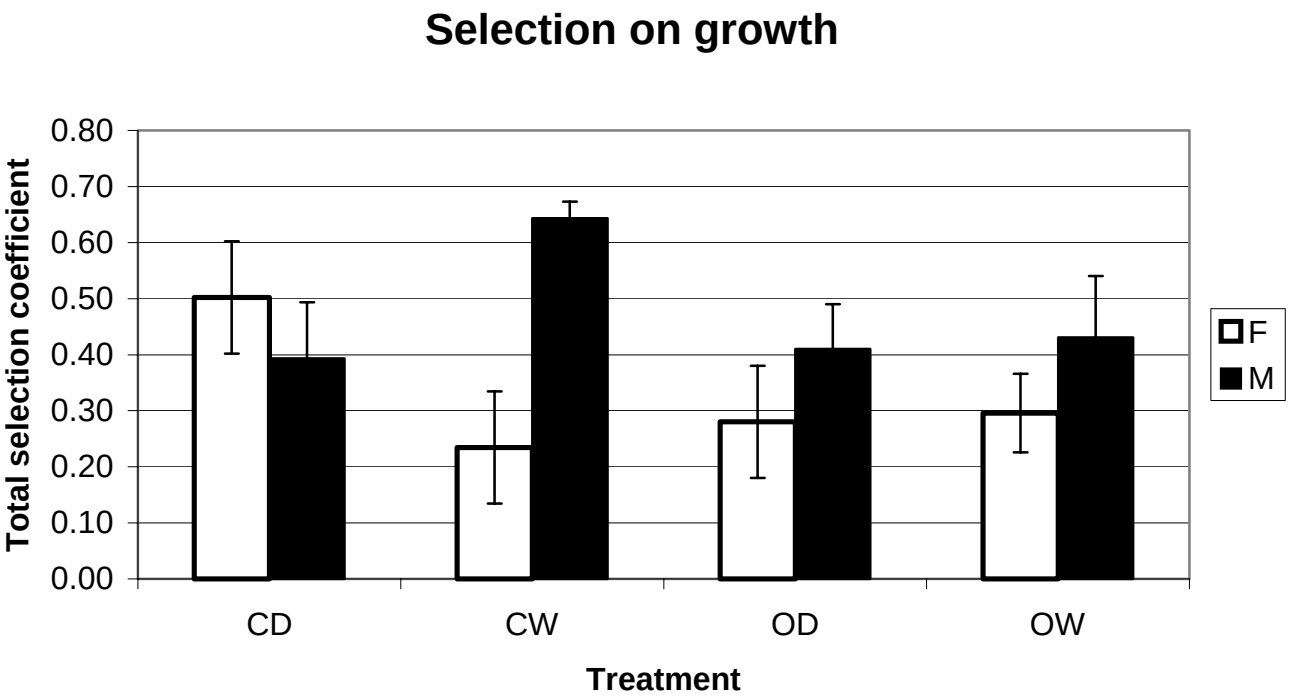


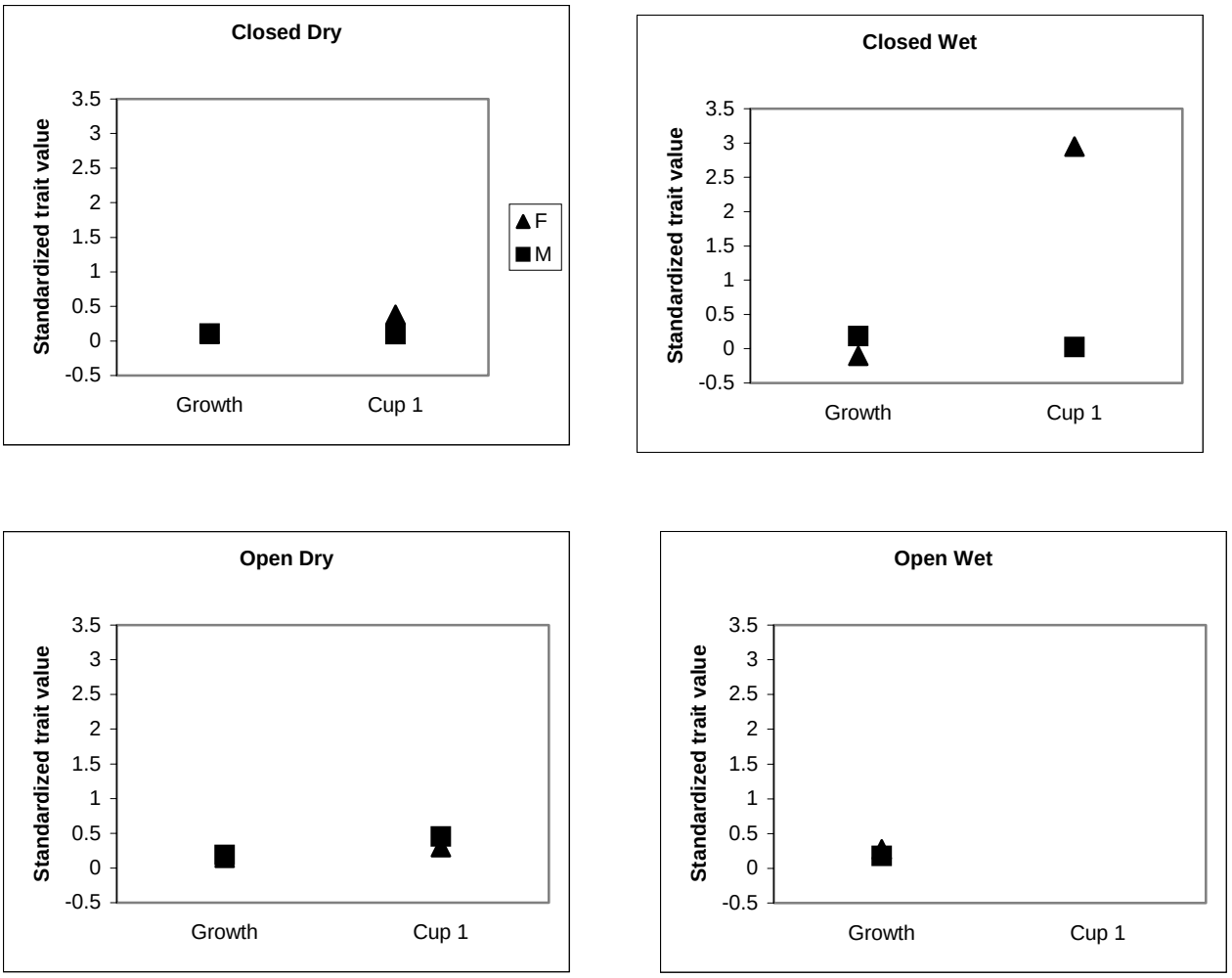
Table 4.6. Population locations.

Population locations and numbers of isolates (genotypes) of *Marchantia inflexa* used in field study; listed in order of increasing latitude. Population type refers to either single-sex female (F) or male (M), or both-sex (B). Numbers in parentheses behind number of substitute plants indicate number of genotype replicates per treatment.

Population	Location	Latitude N /Longitude W	Population type	Female	Male	Substitute Female	Substitute Male	Total Genotypes
Clark's Creek	Woodville, MS	31.017/91.506	B	3	1	1(12)	3 (18, 45)	8
Little Schultz	West Blocton, AL	33/87	B	4	4			8
Turner Falls	Turner Falls, OK	34.425/97.148	M		1			1
Travertine Creek	Sulphur, OK	34.508/96.968	F	1				1
Stinking Bear Creek	Russellville, AL	34.59/87.69	B	1	3	1 (47)	2 (47)	7
Byrd's Mill Creek	Fittstown, OK	34.615/96.634	F	1				1
Cooks Creek	Russellville, AL	34.62/87.719	B	1	5	1 (18)	1(45)	8
Blue River	Connerville, OK	34/96	M		1			1
Fourth Creek	Bearden, TN	35.5/84	B	7	6			13
Carter's Mill	Carter, TN	36.01/83.71	B	5	1	1(47)	2 (46)	9

Figure 4.8. Summary of sex-specific selection.

Trait maxima for female (F) and male (M) *Marchantia inflexa* grown in four field treatments. Trait maxima are fitness maxima from cubic spline analyses and are peak fitness for standardized traits.



Chapter Five: Sexual dimorphism in the plasticity of traits associated with early establishment of a dioecious plant, *Marchantia inflexa*

Summary

Bryophytes are notoriously plastic and many dioecious species are also sexually dimorphic. *Marchantia inflexa*, a liverwort with sexually dimorphic life-history processes, exhibits disjunct distribution of the sexes within and among populations. Because sex-specific trait plasticity may evolve and influence the distribution of sexes of dioecious species I examined trait plasticity of female and male *M. inflexa* across light and moisture environments to determine whether sex-specific selection on plasticity of traits had the potential to influence the distribution of the sexes. There was no sexual dimorphism in plasticity of traits detected across environments but selection was environment-dependent and selection on plasticity of traits, outside of the traits themselves, was evident across light and moisture environments. Females tended to respond in non-adaptive manner across environments and selection favored less plastic female genotypes. Selection on trait plasticity in males was often neutral or favored less plastic genotypes. There is some indication that growth-plastic females should be less prevalent under closed-canopy conditions, but there is not a strong case for sex-specific differences in plasticity of traits to explain the disjunct distribution of the sexes in *M. inflexa*.

Introduction

In clonal plants, selection on sexually dimorphic traits may influence the distribution of sexes, population sex-ratios, and the maintenance of sexual reproduction in populations (McLetchie, Garcia-Ramos, and Crowley, 2002; Crowley, Stieha, and McLetchie, 2004). Dioecious plants have been described as sexually dimorphic in life history traits, vegetative morphology, physiology, competitive ability and susceptibility to herbivores or pathogens (reviewed in Geber, 1999). Further, the distribution of the sexes of dioecious plants may be related to sex-specific microhabitat specializations along moisture, nutrient, light, temperature or salinity gradients (Freeman, Klikoff, and Harper, 1976; Cox, 1981; Bierzychudek and Eckhart, 1988; Lovett-Doust and Lovett-Doust, 1988; Dawson and Bliss, 1989; Korpelainen, 1993; Ramadan et al., 1994). The evolution, maintenance and identification of sexually dimorphic characters has been a recent focus of vascular plant research, but sexual dimorphism in the plasticity of traits and how sex-specific differences in plasticity influence the distribution of the

sexes has not been addressed. Examining plasticity on this scale should provide insight into the influence of plasticity of traits on the distribution of the sexes within and among populations.

Studies of plasticity have focused on plasticity among populations, within-species (Byers and Quinn, 1998; Donohue et al., 2001; Pigliucci and Kolodynska, 2002) and among species (Sultan, 1995). Phenotypic plasticity is non-genetic variation observed in the phenotype of a known genotype across environments (Bradshaw, 1965; Schlighting and Levin, 1986; Stearns, 1989). The set of phenotypes displayed by a genotype across environments is a genotype's reaction norm (Stearns, 1992). The best way to describe phenotypic expression of organisms is through the reaction norm because estimated heritabilities can vary depending on which individuals are observed and in which environments (Bradshaw, 1965; Falconer, 1981; Grupta and Lewontin, 1982). Research on the evolution of reaction norms and plasticity suggests that plasticity of traits, independent of the traits themselves, can respond to selection (Bradshaw, 1965; Van Tienderen, 1991), and plasticity does evolve (Scheiner, 1993).

Sexual dimorphism in the plasticity of life history traits may be especially influential in the distribution of the sexes of dioecious bryophytes because (a) bryophytes are notoriously phenotypically plastic (Hatcher, 1967; Longton, 1974; Mishler, 1985; Shaw and Bartow, 1992; Newton and Mishler, 1994), (b) over half of moss and liverwort species are dioecious (Shaw, 2000), and many of those are sexually dimorphic (Une, 1984, 1985b), (c) despite the importance of spatial proximity, sexes of dioecious bryophytes often occupy separate patches in populations (Longton and Schuster, 1983; Longton, 1990), (d) although sex determination occurs by a sex chromosome system (Ramsay and Berrie, 1982), bryophytes often have female-biased population sex ratios (Longton, 1990; Wyatt, 1994; Shaw, 2000; Stark, Mishler, and McLetchie, 2000; Bowker et al., 2000) and, in some species, widely disjunct distribution of the sexes (for examples see Schuster, 1992). Because phenotypic plasticity is known to influence the distribution of plants, and has been associated with the expansion of taxa into new areas (Byers and Quinn, 1998), sexual dimorphism in plasticity of traits in dioecious bryophytes may influence the distribution of the sexes of these plants.

I examined plasticity of traits in the sexes of a dioicous liverwort, *Marchantia inflexa*, to determine if patterns of sex-specific selection on plasticity of traits explain the distribution of the sexes of this plant. *Marchantia inflexa* (Chapter 1) has population sex ratios ranging from 100% female to 100% male (Chapter 2), although Bischler (1984) reports them as female-biased, and

McLetchie and Puterbaugh (2000) report 1:1 sex ratios. The species ranges from Venezuela to the southern USA, and all-male populations are known from Oklahoma and Florida whereas all-female populations are found only in Oklahoma. Although additional single-sex populations may be discovered, the current pattern indicates that males are more widely distributed than females. Additionally, within populations, males inhabit a wider range of light environments than do females (Chapter 2). If a wider distribution is related to higher phenotypic plasticity (Byers and Quinn, 1998), I expect that males are the more plastic of the sexes of *M. inflexa* and that the distribution of the sexes is related to differences in trait plasticity between the sexes.

Plasticity in traits that are involved in physiological trade-offs within individuals may help the genotype to fine-tune energetic investments in response to the environment. In clonal, dioecious bryophytes, there is tripartite trade-off among growth, asexual and sexual reproduction (McLetchie and Puterbaugh, 2000; Fuselier and McLetchie, 2002). For example, in *Marchantia inflexa*, and many other liverworts (Chapter 1), males typically invest in asexual reproduction over growth whereas, females invest more in growth (McLetchie and Puterbaugh, 2000). Females tend to produce more gametangia-bearing structures than males, but begin their "sex season" later than males (Chapter 3). Plastic genotypes may exhibit more flexibility in traits involved in these trade-offs and have a fitness advantage in particular habitats if selection is environment-dependent.

When selection is environment-dependent and there is genetic variation in plasticity of traits, then selection may influence sex-specific levels of trait plasticity. Plasticity is favored by selection when a genotype that expresses different phenotypes across environments expresses the favored phenotype, rather than an alternate phenotype, in each environment. If the females and males differ in levels of trait plasticity and selection acts differently on the sexes across environments, the combination of sex-specific and environment-dependent natural selection may enforce sexual dimorphism in plasticity and influence the distribution of sexes within and among populations.

I measured differences in plasticity of sexually dimorphic traits between the sexes of *M. inflexa* and examined the potential for trait plasticity to respond to selection and influence the distribution of the sexes. I characterized phenotypic plasticity in growth and asexual reproduction for the sexes across light and moisture environments to answer the following questions:

1. Do the sexes experience sex-specific selection on plasticity of traits that may influence the distribution of the sexes in and among populations?
2. Which environmental factors elicit plasticity and which exert selection on plasticity?
3. What constraints are there to the expression and evolution of plasticity of growth and asexual reproductive traits?

Methods

I planted genotype replicates of *M. inflexa* into four field treatments, two moisture and two light levels fully crossed, and measured growth and asexual reproduction in female and male plants in the four treatments. Plants used in this experiment were randomly selected from a group of stock plants from 12 populations housed in the greenhouse at the University of Kentucky. In collecting plants, I defined a genotype as a plant collected from a distinct patch of plants within a population. A patch was a group of plants separated by neighboring groups by at least one meter, typically with an obstruction (such as unsuitable substrate) between patches. I returned plants to the greenhouse and grew them on steam-sterilized soil under 55% shade in temperatures ranging from 22 ^{o}C to 25 ^{o}C. Stock plants were housed for approximately one year and all plants underwent at least one bout of asexual reproduction before being used in the experiment. Thus, all genotypes used in the experiment were begun from greenhouse stock grown under similar conditions to minimize any maternal or latent site-specific environmental effects. Initial size of stock plants ranged from 6 to 12 mm^2. Plants from both-sex populations used in this experiment were randomly chosen from among stock plants from 6 populations in the USA. I chose only one genotype from single-sex populations (two female and two male) because these populations may have very low genetic variation because of the lack of sexual reproduction (Bischler and Boisselier-Dubayle, 1997).

Mericell-bearing tips from 25 female and 25 male genotypes were planted on water-wicking mats (n = 260) in a 1:1 sex ratio with 50 plants per mat. Plants were grown on mats under artificial light in a growth chamber for five days before placement in the field. Plants were grown under four treatment levels at a field site in Grainger, County, Tennessee on a stream where the species occurred historically, but is no longer found (see chapter 4). Mats were randomly assigned to a treatment and location with 65 replicate mats per treatment (approximately 65 replicates of each genotype). Some genotypes were not replicated 65 times

because of lack of stock but these were substituted with genotypes from the same population. For replicate numbers see Chapter 4. Treatments were two light levels, open and closed canopy, fully crossed with two moisture levels, wet and "dry". Light treatments differed significantly in amount of light, measured as photon flux density (umol/m^2/day), reaching the patch, and moisture treatments differed significantly in amount of water (g) held in a mat after one week in the field. Details of the field experiment are presented in chapter 4.

Light environment and moisture regime are factors that are important in the distribution of plant species. The light is a fundamental component of a plant's habitat that can induce adaptive responses (Schmitt, Dudley, and Pigliucci, 1999). In *Marchantia inflexa*, the sexes overlap in the type of substrate, substrate composition, pH, wind speed and humidity (Chapter 2). However, males use a wider range of light environments than do females (Chapter 2). Species of *Marchantia* are not reviviscent and die when they completely dry out (Bischler, 1984). In the USA, *M. inflexa* is found only along permanent streams and does not grow very far above water level on stream banks (Fuselier, personal observations), indicating that moisture is an important component to suitable habitat.

Traits

I measured growth and asexual reproduction because of the importance of these strategies to patch establishment, expansion and persistence. Growth measured as the change in area of plants over 21 days, was estimated by taking a digital photos measuring green area of plants using ImageJ software (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/nih-image/). Growth measurements and counts of cups were made before field placement, and on 21 and 54 days after field placement. Most plants had grown to overlap neighboring plants, and many new gemmae were growing on mats by day 54, thus, growth measurements used here are from day 21. Counts of cups at day 21 are referred herein as "cup 1" or cup formation in the first month whereas, "max cup" is the maximum number of cups counted out of the two cup count days. Isolates were overgrown and not distinguishable by the onset of sexual reproduction so, results presented here are limited to growth and asexual reproduction.

Survival over 21 days in the field was averaged over the genotype replicates and used as the dependent variable in analyses. At day 21 most plants had produced cups and were still recognizable as individuals, i.e., not overgrown by other plants. Despite the short time, this is a

reasonable measure of viability fitness for *M. inflexa* because, survival at this early stage is important in initial patch colonization. McLetchie, Garcia-Ramos and Crowley (2002) found that growth and asexual reproduction were the most influential traits for determining patch sex ratios in a model parametized for *M. inflexa*. Additionally, a model of overgrowth competition in *M. inflexa* (Crowley, Stieha, and McLetchie, submitted) showed that different patterns of growth provide early advantages to plants colonizing new space. Thus, the traits and fitness measurements represent characteristics crucial to the early establishment of the sexes of *M. inflexa*, and are important because they may ultimately determine patch sex ratios, and influence the probability of sexual reproduction.

Analyses

I examined phenotypic differences and selection across light environments within moisture treatments and across moisture environments within light treatments to examine which environmental factors elicit plasticity and which exert selection on the sexes of *M. inflexa*. Because not all populations that were available were used in the experiment, and genotypes were randomly chosen (except those from single-sex population which were systematically randomly chosen) from among a pool of potential plants, genotype was considered a random rather than a fixed variable, and population was not considered a main effect in any model. Trait plasticity and genetic variation in plasticity was assessed using analyses of variance (ANOVA) with treatment as a fixed effect and genotype as a random effect. A treatment effect indicated that traits were plastic across environments, and a genotype x treatment interaction indicated genotypic differences in phenotypic plasticity (Schlichting and Levin, 1986; Shaw and Bartow, 1992). If the genotype x treatment interaction term was significant, treatment was tested over the mean square for the interaction term, otherwise, treatment was tested over the error mean square for the model. Sexual dimorphism in plasticity of traits was identified using genotype means in ANOVA.

Selection analyses

Genotypic selection analyses were used to examine the strength, direction and type of selection trait plasticity within the sexes. Unlike phenotypic selection analyses (Lande and Arnold, 1983), genotypic selection analysis reduces the likelihood that results are biased by environmentally induced correlations that impact fitness (Rausher, 1992; Scheiner et al., 2002). Environment-dependent selection was measured using traits as covariates and treatments in an

ANCOVA as described in Chapter 4. Results from ANCOVA detecting environment-dependent selection were used here for comparisons with reaction norms for the sexes across environments. To determine whether the plastic response by a plant was favored by selection, I compared the reaction norm across light and moisture environments separately to patterns of selection in the two environments within light and moisture treatments.

To measure the magnitude and direction of selection on plasticity of traits outside of the traits themselves, I used standardized traits and trait plasticity values in genotypic selection analyses. Values of trait plasticity for the light environments were calculated for each genotype as the absolute value of the difference in genotype means for the open minus the closed treatment trait value. Trait plasticity values for moisture treatments were calculated as the absolute value of the difference in genotype means for the wet minus the dry treatment trait value. Survival was made relative over two treatments within a sex, and traits and trait plasticity values were standardized over two treatments within a sex (e.g., for females in the open canopy treatment, values were standardized over females in open-wet and open-dry by subtracting the mean and dividing by the standard deviation of trait values for females in these 2 treatments). Genotypic selection analyses for females and males in light and moisture treatments were run separately. A negative selection coefficient for the plasticity of a trait indicated a cost to plasticity, in that selection favored less plastic genotypes. Whereas, a positive selection coefficient indicated that selection favored more plastic genotypes. Residuals from multiple regression were normally distributed. Because among-trait can constrain a trait's response to selection, I measured correlations between traits and trait plasticity. I used Pearson product moment correlations for all female and male genotypes, and ran correlations for each sex.

Results

Growth and cup production were highest in open and wet treatments compared to closed or dry treatments (Table 4.1). Females and males grew larger and produced more cups in the open-wet treatment than any other treatment (Figure 4.1). Both sexes showed significant plasticity for all traits across light environments and for most traits across moisture environments (Table 5.1). However, the plasticity was not genetic as evidenced by non-significant genotype by treatment interaction terms for most traits. A significant genotype x light environment interaction was found for early cup production (cup 1) in females, indicating genetic differences

in cup production plasticity in females across light environments, but there were no genetic differences in trait plasticity detected for males. There were no significant differences between the sexes in plasticity of traits across any treatment pairs (Figure 5.1).

There were significant positive correlations between growth and growth plasticity exhibited by females across most environment except within the closed canopy treatments (Table 5.2), but growth and growth plasticity in males were uncorrelated across all treatment pairs. Both sexes exhibited significant positive correlations between cup 1, max cup and their plasticity values within wet treatments (across open and closed canopy areas) and within open treatments (across wet and dry areas; Table 5.2).

Environment-dependent total selection on growth was significant in females under closed canopy and dry treatments and there was significant environment-dependent selection on max cups in males in dry areas. Overall, selection was stronger under dry and closed conditions than wet or open conditions. These results are presented in Chapter 4 and repeated here for comparisons with reaction norms. Under closed canopy, total selection on growth in females was significantly stronger in dry than in wet areas, and selection favored larger females in the dry than in the wet areas (Figure 5.1). However, females (and males) tended to grow larger in the closed-wet than in the closed-dry treatment (Table 4.1). While female genotypes were plastic for growth across wet and dry closed canopy areas, their plasticity was not in the direction favored by selection. Selection on females for higher growth differed across open and closed canopy treatments in dry areas. Females tended to grow larger in the open-dry than in the closed-dry treatment and selection favored larger females in the open-dry treatment. So, across canopy treatments in dry areas, the pattern of plasticity of growth in females was consistent with selection (Figure 5.1). Although selection on max cups differed across light environments in dry areas for males, there was no significant environment-dependent selection on max cups detected in females.

For males, selection favored higher growth in the wet than in the dry closed canopy areas and males tended to be larger in the wet than dry areas. The pattern of growth plasticity for males was consistent with the direction favored by selection, but selection across wet and dry closed canopy areas was not significantly different for males (Table 4.5). In dry areas, males tended to be larger under open canopy than closed canopy and selection favored higher growth in the open canopy areas (Figure 5.1). Again, this pattern of growth plasticity is consistent with

selection, but selection on growth in males across open and closed canopy treatments in dry areas was not significantly different. Selection for higher max cup values for males in dry areas was stronger under open canopy than closed canopy, but males tended to produce the same number of max cups in both open-dry and closed-dry treatments. Thus, while selection significantly differed in strength across environments, there was no plasticity of max cup number across open and closed canopy in dry areas detected in males.

Selection on plasticity

Selection on plasticity of traits, outside of the traits themselves, was neutral for most traits across light and moisture environments. Under open canopy, closed canopy and dry conditions, females experienced selection for higher growth values but lower growth plasticity (Table 5.3). Under closed conditions, total selection was significant, but there was no significant direct selection on growth plasticity. Females were also under total selection for higher max cups and lower max cup plasticity in dry conditions. There was no total nor direct selection on growth or growth plasticity in males. Males did experience total selection for lower early cup and max cup plasticity but only across moisture environments within the light treatments. There was no significant selection detected on traits or their plasticity values in males across light environments in either dry or wet conditions (Table 5.3). In all instances where selection on the plasticity of a trait was significantly strong, the selection coefficient for trait plasticity was negative, and the selection coefficient for the trait was positive, indicating selection for higher trait values but less plastic genotypes.

Sex-specific selection on growth plasticity occurred in closed, open and dry conditions (Table 5.4). In each case, selection for lower growth plasticity was stronger on females than males. There were no differences in selection on the plasticity of cup production traits detected in any treatment.

Positive correlations between growth and growth plasticity in females may stifle selection on growth plasticity. Selection for lower growth plasticity in females was consistent across treatments and significant in two of the treatments, but selection for higher growth and a positive correlation between growth and growth plasticity may neutralize selection on low growth plasticity.

Discussion

Growth and cup production were significantly plastic across light and moisture environments for both sexes of *M. inflexa*, and females showed genetic variation in growth plasticity across light environments. Females were under selection for lower plasticity across both light and moisture treatments, whereas males were under selection for lower plasticity only across moisture treatments. Selection on the plasticity of traits differed between the sexes but in all cases, selection favored less plastic genotypes. Patterns of phenotypic response across conditions where selection is environment-dependent suggest growth-plastic females may be more limited in distribution compared to males in which selection was mainly neutral. Both sexes experienced a cost to plasticity in the form of selection for less plastic genotypes but correlations between traits and trait plasticity values may constrain the response of trait plasticity to selection. Thus, there is not a strong case for sex-specific differences in plasticity of traits to explain the distribution of the sexes of *M. inflexa* across moisture and light environments.

Plastic characters in bryophytes

Genotypes of *M. inflexa* were plastic across both light and moisture environments and all combinations of the treatments used in this experiment elicited expression of plasticity in growth and cup production. Liverworts are notorious for their morphological plasticity (Bischler and Boisselier-Dubayle, 1997) and *M. inflexa* was no exception to this expectation. Water availability and temperature were considered the most influential ecological features that influence variation of morphological features in species of *Marchantia*. Species of *Marchantia* produce thalli that are large and thin with a foliaceous appearance when grown in the shade whereas, plants growing in exposed or harsh habitats have smaller, leathery thalli (Bischler, 1984). Natural populations of *M. polymorpha* subject to gamma radiation show higher tolerance to radiation than populations not exposed to radiation, but whether or not these differences are genetic is unknown (Sarosiek and Wozakowska-Natkaniec, 1968 cited in Longton, 1974). Besides morphological plasticity, non-genetic phenotypic variation in enzymes have been identified within liverwort species and individuals (reviewed in Bischler and Boisselier Dubayle, 1997).

Schuster (1966) touts phenotypic plasticity as an important “adaptive” mechanism in liverworts, but studies to support this claim are lacking. Phenotypic plasticity is expected in species that live in highly variable (Via and Lande, 1985; Van Tienderen, 1991) or

heterogeneous environments (reviewed in Scheiner, 1993) . Shaw and Bartow (1992) found extensive morphological plasticity in *Funaria hygrometrica* on different soil types in highly heterogenous habitat, but low genetic variation for morphological traits among locations. Populations of mosses over a larger scale also showed significant plasticity (Longton, 1972; Forman, 1964 cited therein).

My results indicate that there is little genetic basis for differences in the plasticity of traits in *M. inflexa*, with the exception of growth in females. Both sexes were plastic in growth and cup production across combinations of moisture and light environments and there was variation (mostly non-significant) among genotypes in the direction and degree of plasticity across environments. The lack of significant genetic variation in trait plasticity will impede a response to selection on trait plasticity.

Genetic variation for phenotypic plasticity (genotype x environment interaction) was presumed to be pervasive among organisms (Stearns, 1989). However, Shaw and Bartow (1992) showed that in a moss living on extremely different soil types, there was genetic differentiation of some morphological characters among families at different sites, but no evidence of genetic differentiation of plasticity. Unlike the Shaw and Bartow study, I found differences in plasticity and genetic differentiation of growth rate plasticity in females in one set of environments. This may be extremely important in bryophytes where low genetic variation in morphological traits may take a second seat to variation in phenotypic plasticity for population differentiation.

In a recent study (Pigliucci and Kolodynska (2002), significant among-population variation for trait means but little variation for plasticity of traits was found in *Arabidopsis thalaiana*. But, the plant showed patterns of plasticity thought to be adaptive to different light conditions, and the set of character correlations was stable to changes in light availability. Patterns of plasticity and genetic variation in plasticity in *M inflexa* differ from those in *Arabidopsis* in that genetic variation was low, though present for growth plasticity in females, but character integration (correlations among characters) varies with the environment (Chapters 3 and 4).

Sexual dimorphism

Marchantia inflexa genotypes were plastic in growth and cup production across environments, but there were no significant differences between the sexes in degree of trait plasticity in the environments. There was a non-significant trend for females to exhibit more

plasticity in growth across most environments compared to males, and perhaps a study optimized to detect sex differences (this study was designed to detect selection) might find this difference significant. Previous studies of sexual dimorphism in *M. inflexa* revealed that males invest more in cup production than females (McLetchie and Puterbaugh, 2000), but these patterns may differ depending on the environment (Chapter 4). In a greenhouse study using clonal replicates of *M. inflexa* grown under two light environments, and cup and sex structure production as fitness metrics, females were under direct selection for increased growth plasticity and selection for decreased plasticity in onset of asexual reproduction (Fuselier and McLetchie, 2002). Depending on the fitness metric used and the portion of the life cycle examined, measures of selection may vary.

Under the environmental conditions where selection was strongest (closed and dry), selection on the plasticity of traits was detected. Females and males differed in that there was significant selection for genotypes with low growth plasticity in females, but selection was neutral on growth plasticity in males. Similarly, there was significant selection for less "cup-plastic" males, but selection on cup plasticity in females was neutral.

Plasticity and the distribution of the sexes

Can these sex-specific differences in selection on plasticity of growth and cup production influence the distribution of the sexes? Under closed canopy, males tended (though not significantly) to be more growth-plastic than females, whereas in all other treatments, females tended to be more growth-plastic than males (though not significantly so). Under closed canopy, selection favored higher growth under dry conditions compared to wet conditions for females, yet genotypes were generally larger in the wet than the dry areas. Thus, the plasticity in growth for females across wet and dry areas under closed canopy was not adaptive, and selection favored less growth-plastic females across these environments. Most genotypes were plastic across these environments and most showed plasticity in the opposite direction favored by selection. If, in general females are growth plastic and exhibit non-adaptive patterns of plasticity under closed canopy, females may be less prevalent under closed canopy than males. Under closed canopy across wet and dry conditions, selection favored males with slightly higher growth (though there was no significant environment-dependent selection detected) under wet than dry conditions and males were generally larger under wet than dry conditions. Under closed canopy, across moisture regimes, selection was neutral on growth plasticity in males and any growth

plasticity was in a direction that might be considered adaptive. From these data, it seems that males, plastic or not, might be more prevalent than plastic females under closed canopy, and if most females are plastic, there should be a pattern of higher male occurrence under closed canopy. This is consistent with results based solely on selection on traits and patterns of environment-dependent selection presented in Chapter 4.

This prediction is not supported in terms of environmental correlates and sex distribution among light habitats for the sexes of *M. inflexa* (Chapter 2). Adult population sex ratios not different from 1:1 were reported for *M. inflexa* (McLetchie and Puterbaugh, 2000), but sex ratios vary among populations (Chapter 4) and range from entirely female to entirely male. Previously, it was thought that female *M. inflexa* were the more widely ranging sex, but currently, there are more all-male populations known than all-female populations (Chapter 4). Within populations of *M. inflexa,* females and males are often spatially separated among patches, though this separation is not correlated with an environmental gradient (Chapter 2). However, as pointed out in Chapter 2, to accurately assess habitat use by the sexes, researchers must be able to identify the sex of non-sex-expressing plants. Many plants under low light conditions may delay or forego sex-expression and my data indicate that these may be male plants. These plants won't be counted in sex-ratio censuses based on sex-structure production.

Constraints

The outcome of selection on reaction norms depends on whether environment-dependent selection acts on traits, direct selection on plasticity, and correlations between genotype plasticity and mean phenotype (Scheiner, 1993). I've demonstrated that selection is environment-dependent on *M. inflexa* in the context of light and moisture environments (Chapters 4 and 6). Selection was stronger on females for lower growth plasticity in three of four treatment pairs, but selection on growth plasticity in males was neutral across all environments. Conversely, males were under selection for lower cup production plasticity, but selection on cup production plasticity in females was neutral.

Additionally, the correlations between growth rate and growth rate plasticity were significant and pose a constraint to the evolution of plasticity independent of the trait mean. The negative selection coefficient on growth rate plasticity can be interpreted as a cost to plasticity (Donohue, 2000). In a greenhouse experiment, female *M. inflexa* also showed a cost to the plasticity of asexual reproduction onset under high light, and females were under significantly

stronger direct selection than males for lower plasticity in timing to asexual reproduction in high light (Fuselier and McLetchie, 2002). Thus, costs to plasticity were expressed only under one light environment. These costs combined with correlations between trait and trait plasticity may prevent or slow response to selection by plasticity regardless of the genetic variation present in plasticity.

Although I did not detect sexual dimorphism in plasticity of traits in any environment, this experiment was not designed to optimize detection of differences between the sexes, but rather to detect selection. In future studies to adequately assess sexually dimorphic traits, more genotypes of both sexes and fewer replicates can be used to enhance the detection of sex differences. Previous greenhouse studies either detected significant differences between the sexes (McLetchie and Puterbaugh, 2000) or found non-significant trends in the direction predicted based on other studies (Fuselier and McLetchie, 2002). A study with power optimized to detect sex-specific differences in plasticity in *M. inflexa* may find significant differences in the direction of the trends from this study. Additionally, examination of additional traits, populations and species may reveal very different patterns of selection on traits and their plasticity in bryophytes.

Table 5.1. Genotype-by-environment effects.

Results from analyses of variance to detect plasticity of traits across light and moisture environments and genetic differences in plasticity across environments. MS = mean square, SS = Type III sums of squares, growth is growth over 21 days, growth rate mm^2/21days.

		Effect	DF	SS	MS	F	Pr > F	Effect	SS	MS	F	Pr > F
F	cup1	Light trt	1	161.854	161.854	276.76	**<.0001**	Moisture trt	14.56	14.56	23.57	**<.0001**
		Genotype	25	136.618	5.465	9.34	<.0001	Genotype	136.07	5.44	8.81	<.0001
		Genotype*light	25	39.183	1.567	2.68	**<.0001**	Genotype*moist	9.54	0.38	0.62	0.93
	cups/mm^2/day	Light trt	1	321.220	321.220	45.04	**<.0001**	Moisture trt	84.01	84.01	11.72	**0.0006**
		Genotype	25	279.410	11.176	1.57	0.04	Genotype	277.82	11.11	1.55	0.04
		Genotype*light	25	142.929	5.717	0.8	0.74	Genotype*moist	200.23	8.01	1.12	0.31
	cups/mm^2	Light trt	1	0.472	0.472	6.03	**0.0141**	Moisture trt	0.24	0.24	3.04	0.08
		Genotype	25	2.970	0.119	1.52	0.05	Genotype	2.96	0.12	1.51	0.05
		Genotype*light	25	1.713	0.069	0.88	0.64	Genotype*moist	1.67	0.07	0.85	0.67
	max cups	Light trt	1	186.772	186.772	170.67	**<.0001**	Moisture trt	47.83	47.83	42.54	**<.0001**
		Genotype	25	250.736	10.029	9.16	<.0001	Genotype	250.16	10.01	8.9	<.0001
		Genotype*light	25	35.498	1.420	1.3	0.15	Genotype*moist	20.19	0.81	0.72	0.84
	growth	Light trt	1	120049.833	120049.833	73.78	**<.0001**	Moisture trt	26203.76	26203.76	15.86	**<.0001**
		Genotype	25	166794.905	6671.796	4.1	<.0001	Genotype	166179.99	6647.20	4.02	<.0001
		Genotype*light	25	58529.572	2341.183	1.44	0.07	Genotype*moist	17209.78	688.39	0.42	1.00
	growth rate	Light trt	1	257.890	257.890	78.11	**<.0001**	Moisture trt	19.89	19.89	5.92	**0.015**
		Genotype	25	435.251	17.410	5.27	<.0001	Genotype	433.70	17.35	5.16	<.0001
		Genotype*light	25	122.647	4.906	1.49	0.06	Genotype*moist	35.45	1.42	0.42	0.99
M	cup1	Light trt	1	94.673	94.673	150.02	**<.0001**	Moisture trt	5.84	5.84	8.8	**0.003**
		Genotype	25	40.333	1.613	2.56	<.0001	Genotype	40.32	1.61	2.43	<.0001
		Genotype*light	25	21.033	0.841	1.33	0.12	Genotype*moist	9.70	0.39	0.58	0.95
	cups/mm^2/day	Light trt	1	159.877	159.877	135.32	**<.0001**	Moisture trt	25.30	25.30	20.7	**<.0001**
		Genotype	25	86.016	3.441	2.91	<.0001	Genotype	85.98	3.44	2.81	<.0001
		Genotype*light	25	19.740	0.790	0.67	0.89	Genotype*moist	25.63	1.03	0.84	0.69
	cups/mm^2	Light trt	1	77.212	77.212	9.54	**0.002**	Moisture trt	8.15	8.15	1	0.32
		Genotype	25	304.092	12.164	1.5	0.05	Genotype	305.53	12.22	1.51	0.05
		Genotype*light	25	126.766	5.071	0.63	0.92	Genotype*moist	197.41	7.90	0.97	0.50
	max cups	Light trt	1	0.516	0.516	5.23	**0.0223**	Moisture trt	0.60	0.60	6.07	**0.0138**
		Genotype	25	1.245	0.050	0.5	0.98	Genotype	1.22	0.05	0.5	0.98
		Genotype*light	25	1.312	0.052	0.53	0.97	Genotype*moist	2.90	0.12	1.18	0.25
	growth	Light trt	1	73585.539	73585.539	89.46	**<.0001**	Moisture trt	28614.81	28614.81	34.31	**<.0001**
		Genotype	25	104692.755	4187.710	5.09	<.0001	Genotype	104354.46	4174.18	5	<.0001
		Genotype*light	25	10316.026	412.641	0.5	0.98	Genotype*moist	8945.11	357.80	0.43	0.99
	growth rate	Light trt	1	153.434	153.434	102.12	**<.0001**	Moisture trt	35.25	35.25	22.96	**<.0001**
		Genotype	25	250.327	10.013	6.66	<.0001	Genotype	249.58	9.98	6.5	<.0001
		Genotype*light	25	17.851	0.714	0.48	0.99	Genotype*moist	16.23	0.65	0.42	0.99

Table 5.2. Correlations. Correlation coefficients (above) and significance values (below) for correlations between trait and trait plasticity in female and male *Marchantia inflexa*.

		Females	Males
Dry	growth	**0.53**	0.13
		0.004	0.49
	cup 1	0.10	0.21
		0.61	0.27
	max cup	0.24	-0.02
		0.24	0.93
Wet	growth	**0.68**	0.24
		0.0001	0.19
	cup 1	**0.52**	**0.39**
		0.006	0.03
	max cup	**0.62**	**0.5**
		0.001	0.005
Open	growth	**0.41**	0.17
		0.03	0.36
	cup 1	**0.56**	**0.36**
		0.003	0.05
	max cup	**0.47**	**0.5**
		0.01	0.005
Closed	growth	-0.04	0.08
		0.84	0.69
	cup 1	0.25	-0.02
		0.21	0.91
	max cup	0.22	-0.14
		0.26	0.44

Table 5.3. Selection on traits and trait plasticity.
Beta = direct selection coefficient and s = total selection coefficient; values in bold indicate significant selection on the plasticity of a trait.

			Direct selection				Total selection			
Sex	Treatment	Trait	Beta	Error	t	P	s	Error	t	P
Females	Closed	cup 1	0.09	0.035	2.53	0.02	0.07	0.018	4.24	0.0003
		cup 1 plasticity	-0.01	0.016	-0.37	0.72	-0.03	0.017	-1.96	0.06
		Growth	0.07	0.020	3.74	0.001	0.08	0.019	4.04	0.001
		growth plasticity	-0.03	0.015	-1.8	0.09	**-0.03**	**0.016**	**-2.22**	**0.04**
		max cup	-0.05	0.040	-1.16	0.26	0.07	0.019	3.51	0.002
		max cup plasticity	0.01	0.014	0.57	0.57	0.00	0.018	0.13	0.90
	Open	cup 1	0.07	0.055	1.36	0.19	0.07	0.026	2.57	0.02
		cup 1 plasticity	0.00	0.020	0.06	0.95	0.01	0.023	0.63	0.54
		Growth	0.09	0.019	4.56	0.0002	0.09	0.024	3.76	0.001
		growth plasticity	**-0.05**	**0.016**	**-3.41**	**0.003**	**-0.05**	**0.020**	**-2.47**	**0.02**
		max cup	-0.02	0.058	-0.31	0.76	0.07	0.023	3.22	0.004
		max cup plasticity	0.03	0.017	1.92	0.07	0.02	0.020	1.22	0.24
	Dry	cup 1	0.07	0.031	2.22	0.04	0.08	0.022	3.61	0.001
		cup 1 plasticity	0.02	0.014	1.25	0.22	-0.03	0.020	-1.47	0.15
		Growth	0.15	0.024	6.18	<.0001	0.14	0.021	7.01	<.0001
		growth plasticity	**-0.09**	**0.018**	**-5.35**	**<.0001**	**-0.11**	**0.016**	**-6.98**	**<.0001**
		max cup	-0.04	0.031	-1.19	0.25	0.08	0.021	3.92	0.001
		max cup plasticity	-0.02	0.014	-1.45	0.16	**-0.04**	**0.020**	**-2.03**	**0.05**
	Wet	cup 1	0.00	0.047	-0.05	0.96	0.07	0.021	3.48	0.002
		cup 1 plasticity	-0.03	0.024	-1.18	0.25	0.00	0.017	0.05	0.96
		Growth	0.06	0.021	2.67	0.01	0.08	0.025	3.16	0.004
		growth plasticity	-0.02	0.018	-1.09	0.29	-0.02	0.021	-0.91	0.37
		max cup	0.08	0.056	1.5	0.15	0.09	0.020	4.44	0.0002
		max cup plasticity	0.01	0.025	0.42	0.68	-0.01	0.017	-0.85	0.41
Males	Closed	cup 1	0.08	0.058	1.41	0.17	0.06	0.022	2.6	0.01
		cup 1 plasticity	-0.02	0.019	-1.2	0.24	**-0.04**	**0.018**	**-2.32**	**0.03**
		growth	0.04	0.033	1.31	0.20	0.08	0.022	3.6	0.001
		growth plasticity	0.03	0.018	1.66	0.11	0.01	0.018	0.67	0.51
		max cup	-0.04	0.062	-0.65	0.52	0.05	0.021	2.33	0.03
		max cup plasticity	-0.03	0.021	-1.23	0.23	**-0.05**	**0.018**	**-2.5**	**0.02**
	Open	cup 1	0.07	0.033	2.15	0.04	0.11	0.019	5.66	<.0001
		cup 1 plasticity	-0.01	0.016	-0.39	0.70	-0.01	0.015	-0.98	0.33
		growth	0.02	0.020	1.24	0.23	0.08	0.019	4.19	0.0003
		growth plasticity	0.02	0.014	1.76	0.09	0.00	0.016	0.09	0.93
		max cup	0.05	0.038	1.27	0.22	0.13	0.019	6.69	<.0001
		max cup plasticity	-0.02	0.017	-1.28	0.21	**-0.03**	**0.015**	**-2.08**	**0.05**
	Dry	cup 1	0.08	0.041	1.95	0.06	0.09	0.019	4.87	<.0001
		cup 1 plasticity	-0.02	0.021	-0.97	0.34	-0.02	0.015	-1.06	0.30
		growth	0.01	0.028	0.45	0.66	0.06	0.021	3.03	0.01
		growth plasticity	0.01	0.017	0.4	0.70	0.00	0.018	0.18	0.86
		max cup	0.01	0.041	0.29	0.77	0.08	0.017	4.58	<.0001

Table 5.3 continued

	max cup plasticity	0.02	0.020	0.93	0.36	0.00	0.016	0.16	0.88
Wet	cup 1	0.05	0.043	1.2	0.24	0.11	0.025	4.4	0.0002
	cup 1 plasticity	0.02	0.023	0.77	0.45	-0.01	0.019	-0.39	0.70
	growth	0.08	0.026	3.03	0.01	0.10	0.020	5.12	<.0001
	growth plasticity	-0.01	0.018	-0.76	0.45	-0.03	0.018	-1.51	0.14
	max cup	0.00	0.054	0.03	0.97	0.12	0.027	4.33	0.0002
	max cup plasticity	0.00	0.025	0	1.00	-0.01	0.020	-0.42	0.67

Table 5.4. Sex-specific selection on plasticity of traits.
Results from analyses of covariance; a significant sex by trait (or trait plasticity) interaction indicates sex-specific selection. Interaction terms in bold indicate significant sex-specific selection on trait plasticity in *Marchantia inflexa* grown in four environments.

Treatment	Interaction	Type III SS	Mean Square	F Value	Pr > F
Closed	**growth plasticity x sex**	0.0296	0.0296	3.87	0.05
	cup 1 plasticity x sex	0.0018	0.0018	0.22	0.64
	max cup plasticity x sex	0.0304	0.0304	3.62	0.06
Open	**growth plasticity x sex**	0.0329	0.0329	4.16	0.05
	cup 1 plasticity x sex	0.0022	0.0022	0.3	0.59
	max cup plasticity x sex	0.0147	0.0147	2.32	0.13
Dry	**growth plasticity x sex**	0.1026	0.1026	13.26	0.001
	cup 1 plasticity x sex	0.0032	0.0032	0.39	0.54
	max cup plasticity x sex	0.0244	0.0244	2.99	0.09
Wet	growth plasticity x sex	0.0002	0.0002	0.03	0.86
	cup 1 plasticity x sex	0.0003	0.0003	0.05	0.83
	max cup plasticity x sex	0.0043	0.0043	0.63	0.43

Figure 5.1. Reaction norms.

Average growth over 21 days in female and male *Marchantia inflexa* grown in four treatments at a field site. C = closed canopy, O = open canopy.

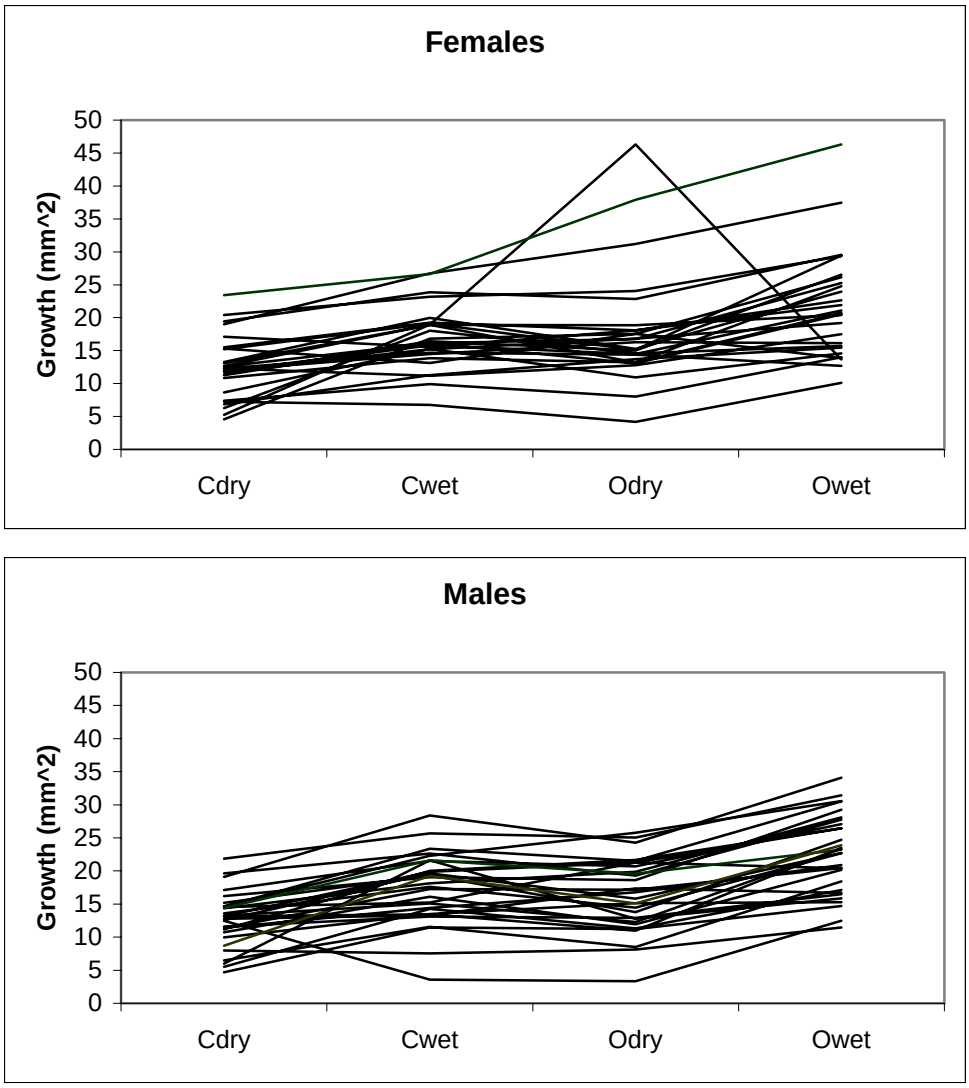
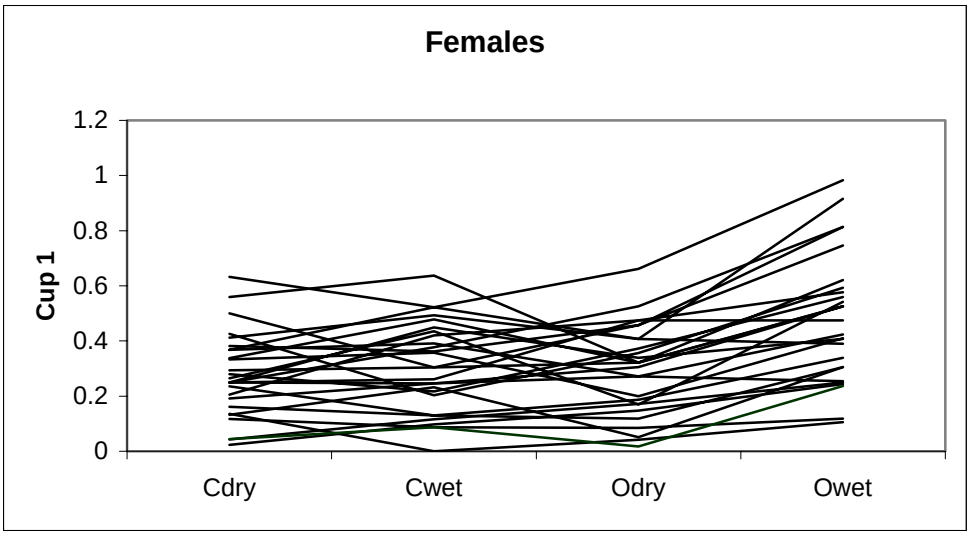


Figure 5.1 continued. Average number of cups produced by female and male *Marchantia inflexa* over 21 days in four field environments.



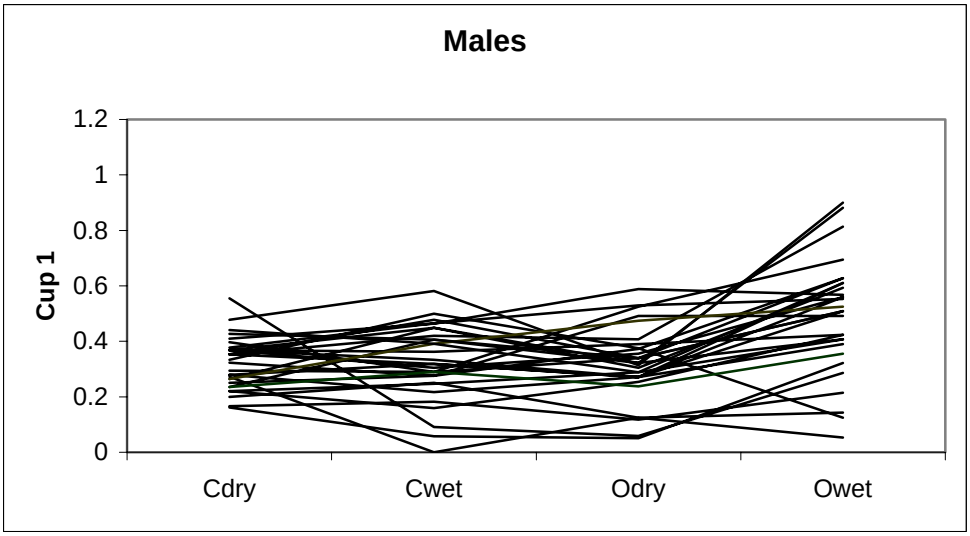
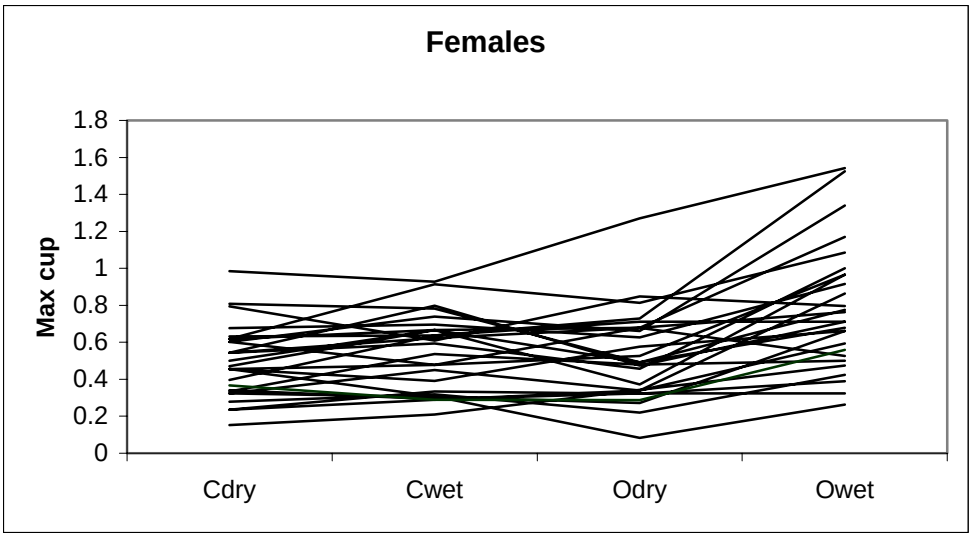


Figure 5.1 continued. Average total cup production over 54 days for female and male *Marchantia inflexa* grown in four field treatments.



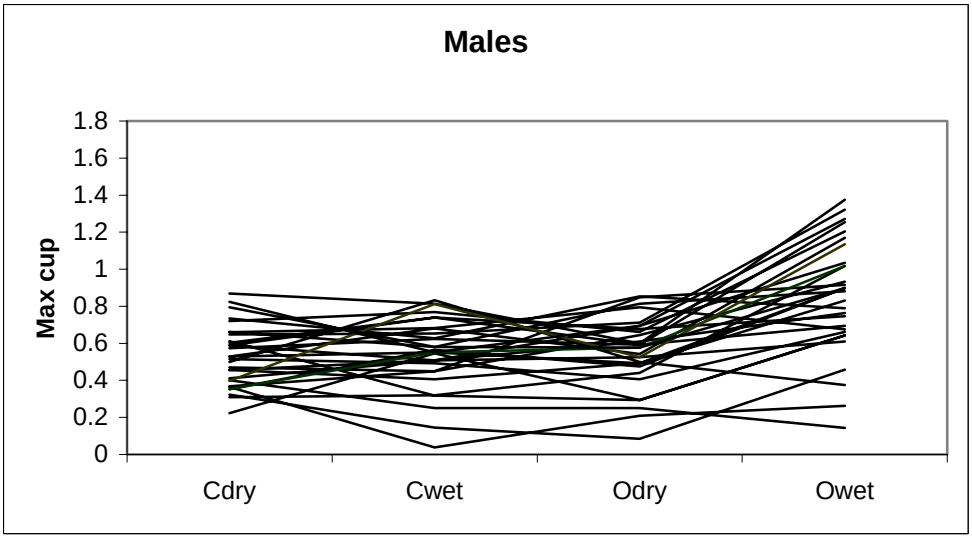
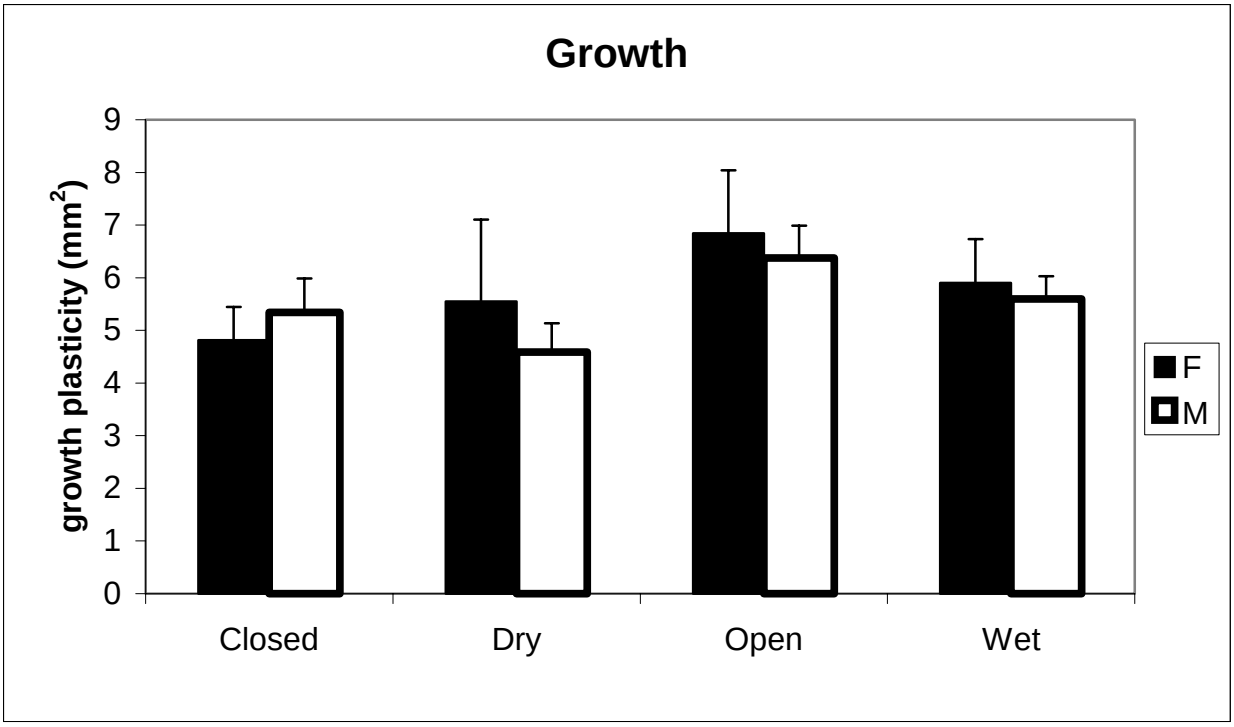


Figure 5.2. Plasticity of traits.
Average plasticity (absolute value of difference between traits in 2 environments) for female and male *Marchantia inflexa* grown in four field treatments.



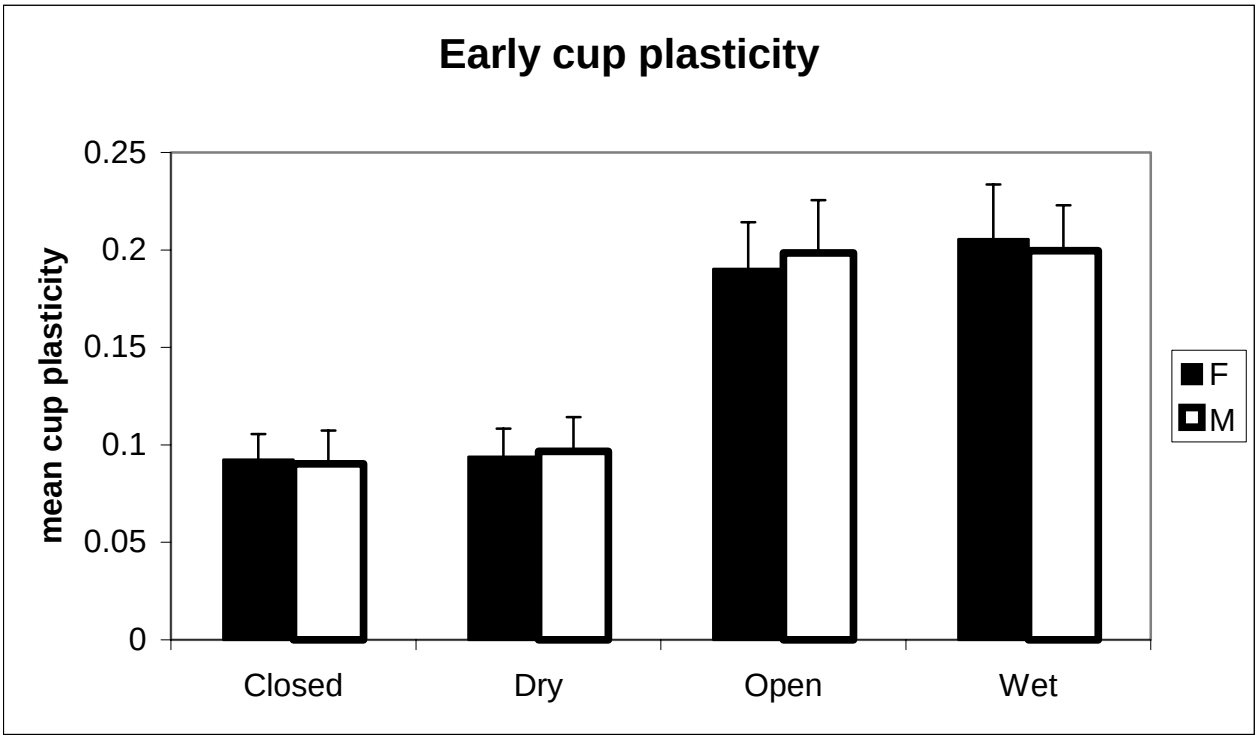


Figure 5.2 continued.

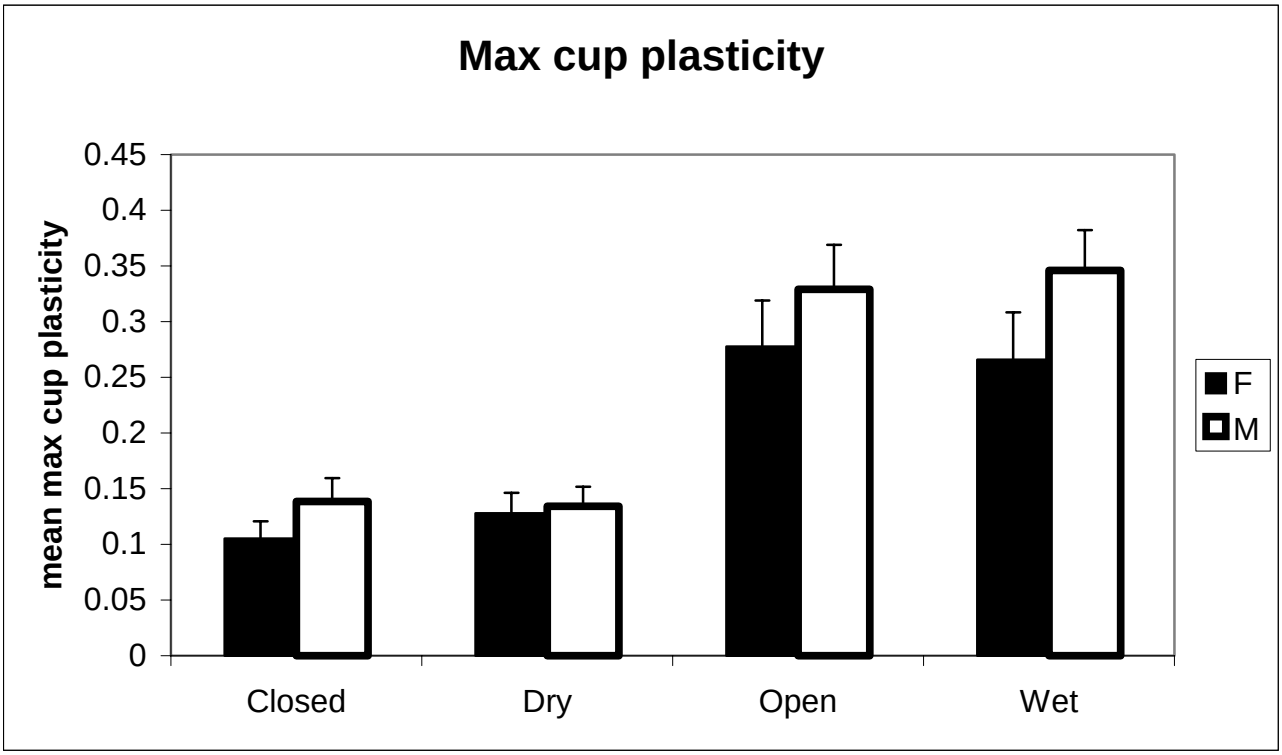
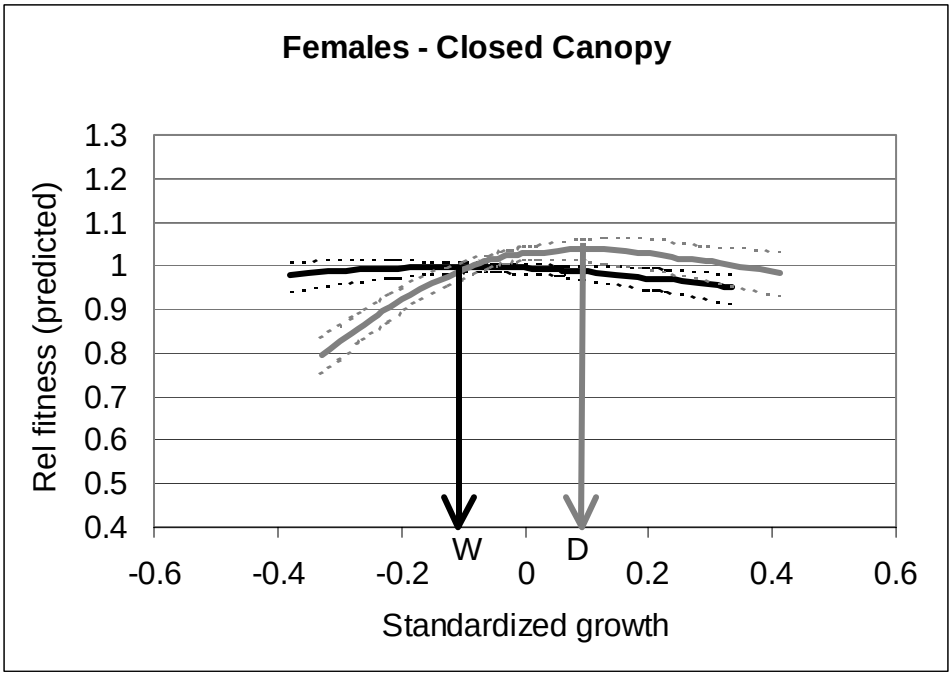


Figure 5.3. Selection and reaction norms.
Reaction norms and fitness functions for traits under conditions where environment-dependent total selection was significant.



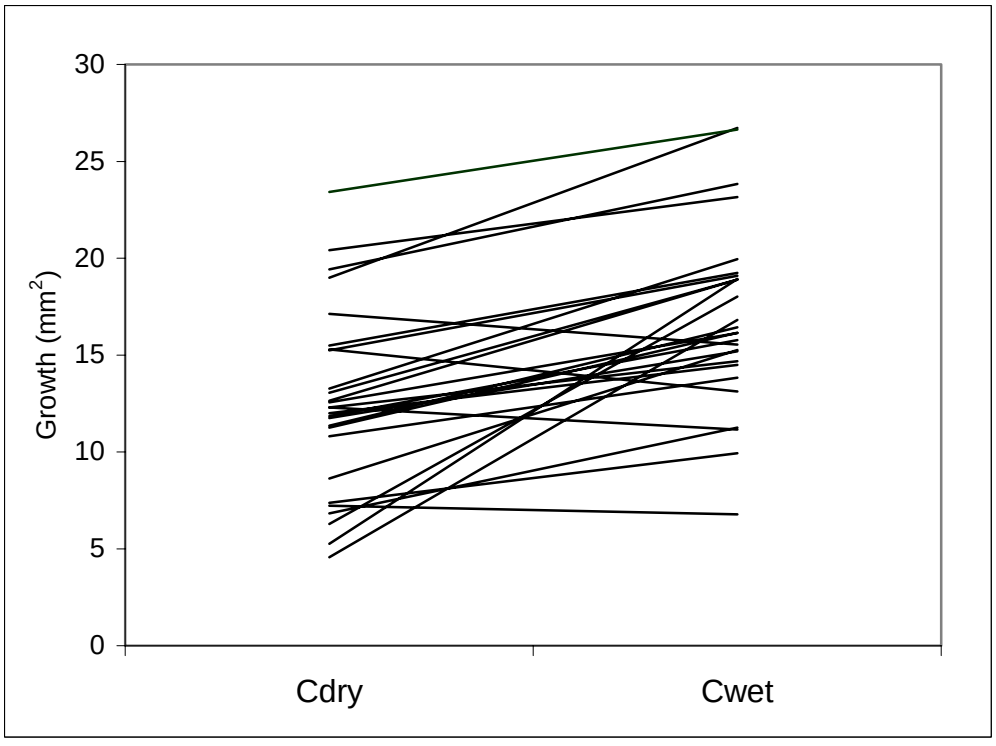
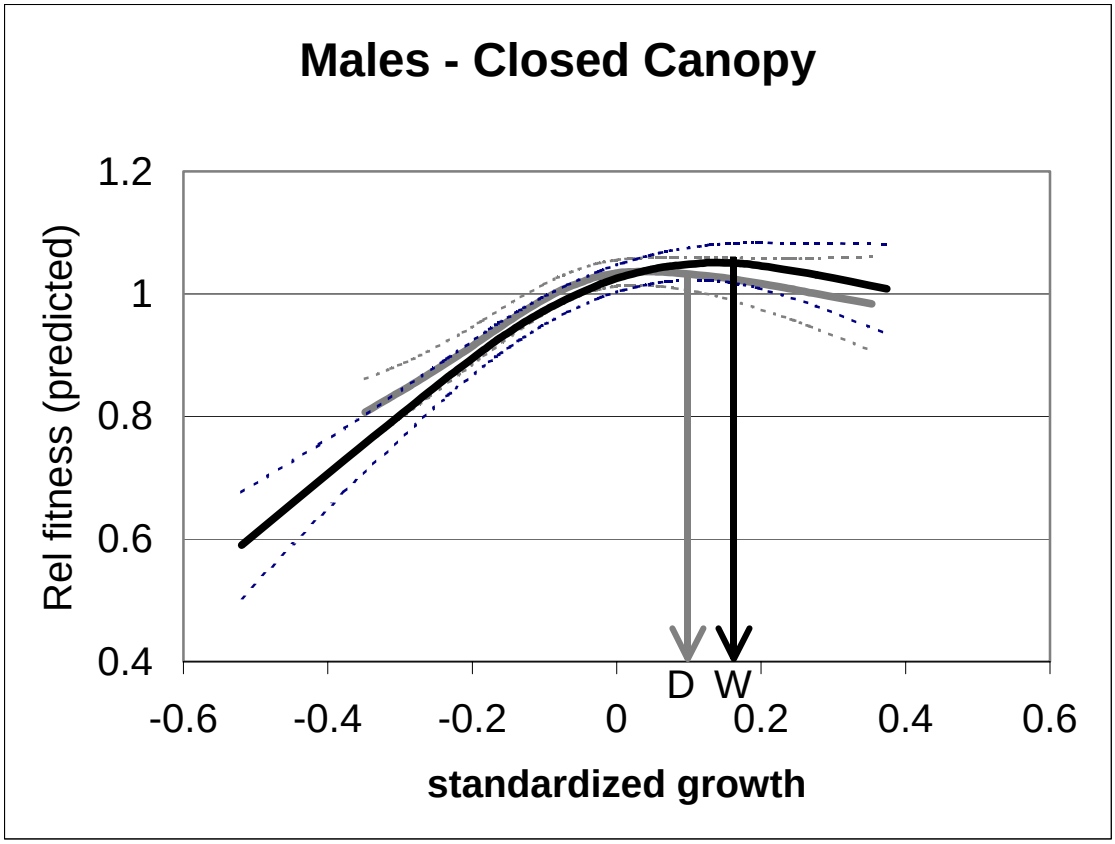


Figure 5.3 continued.



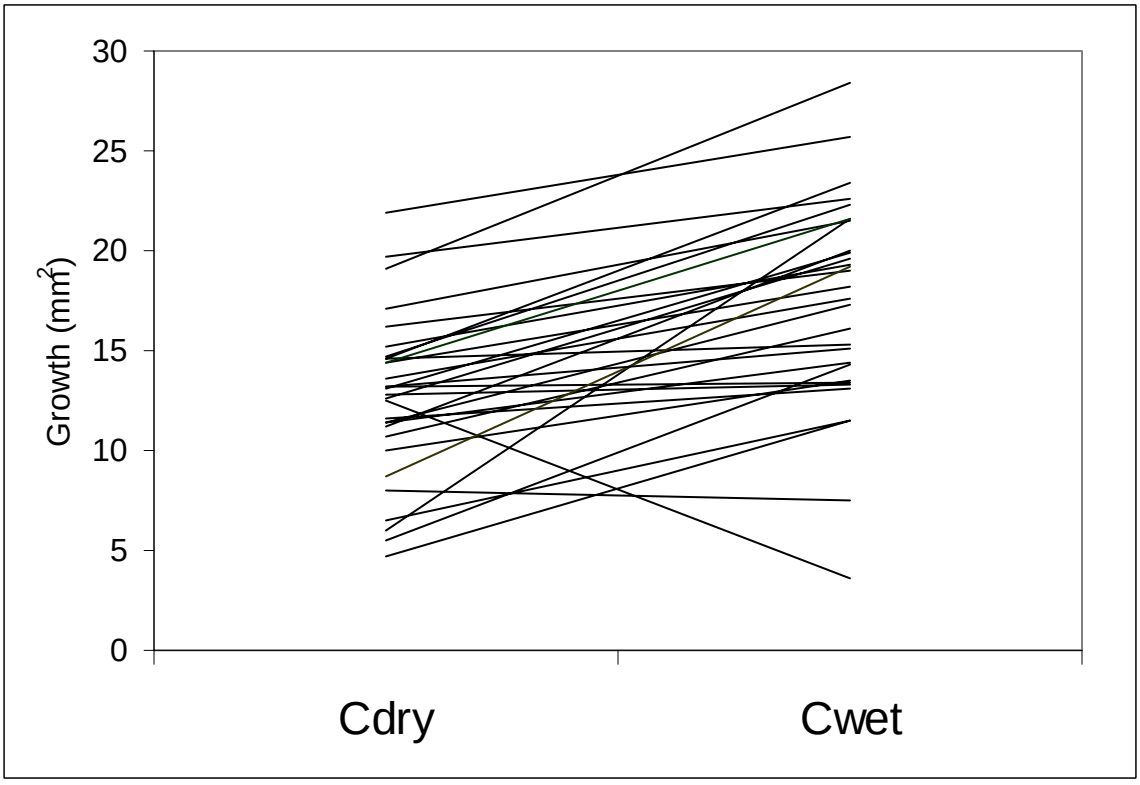
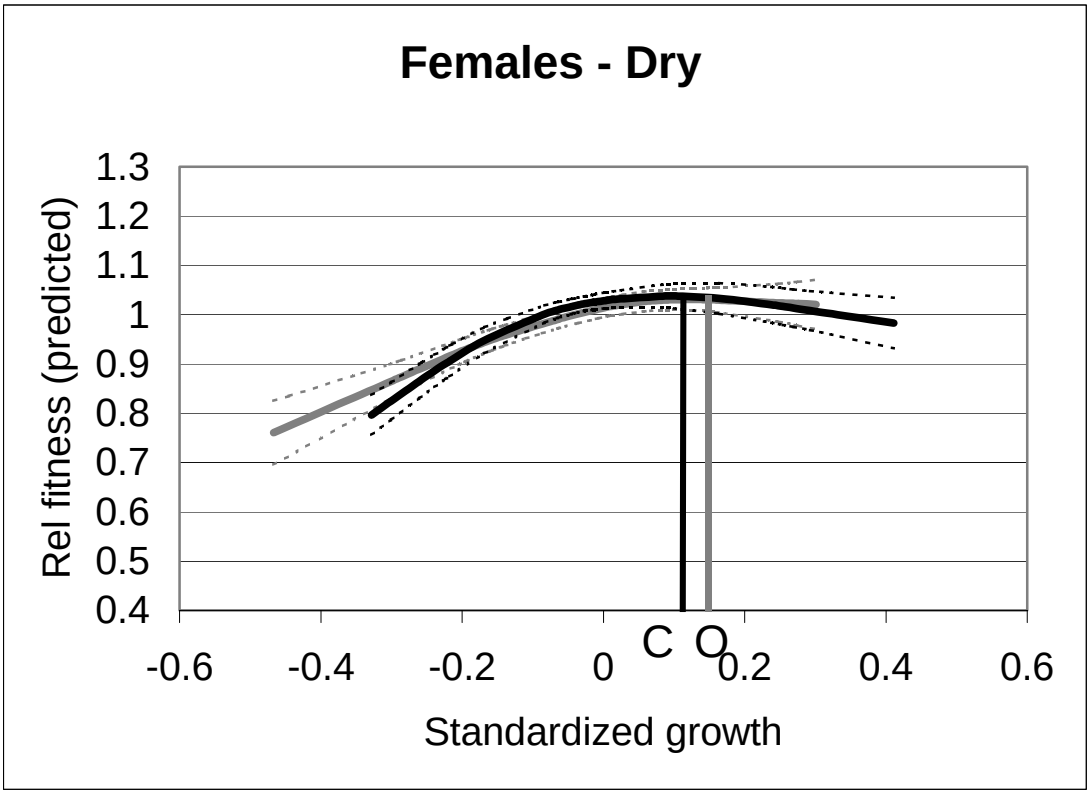


Figure 5.3. continued.



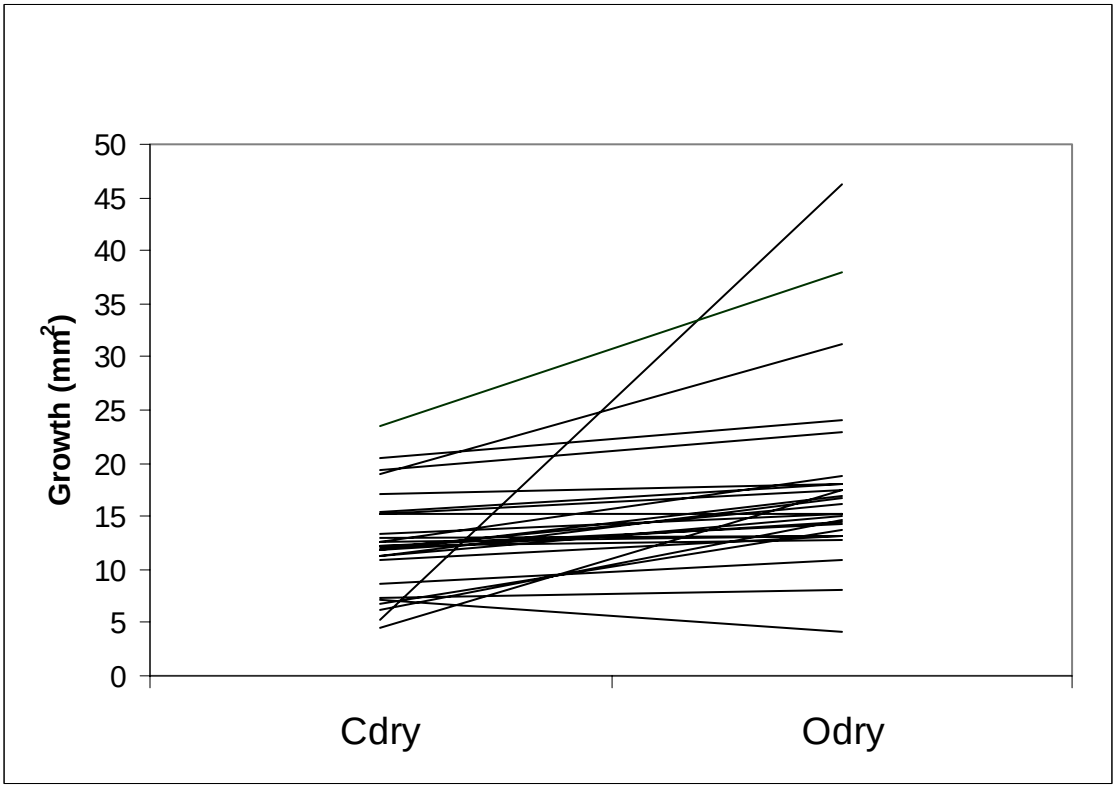
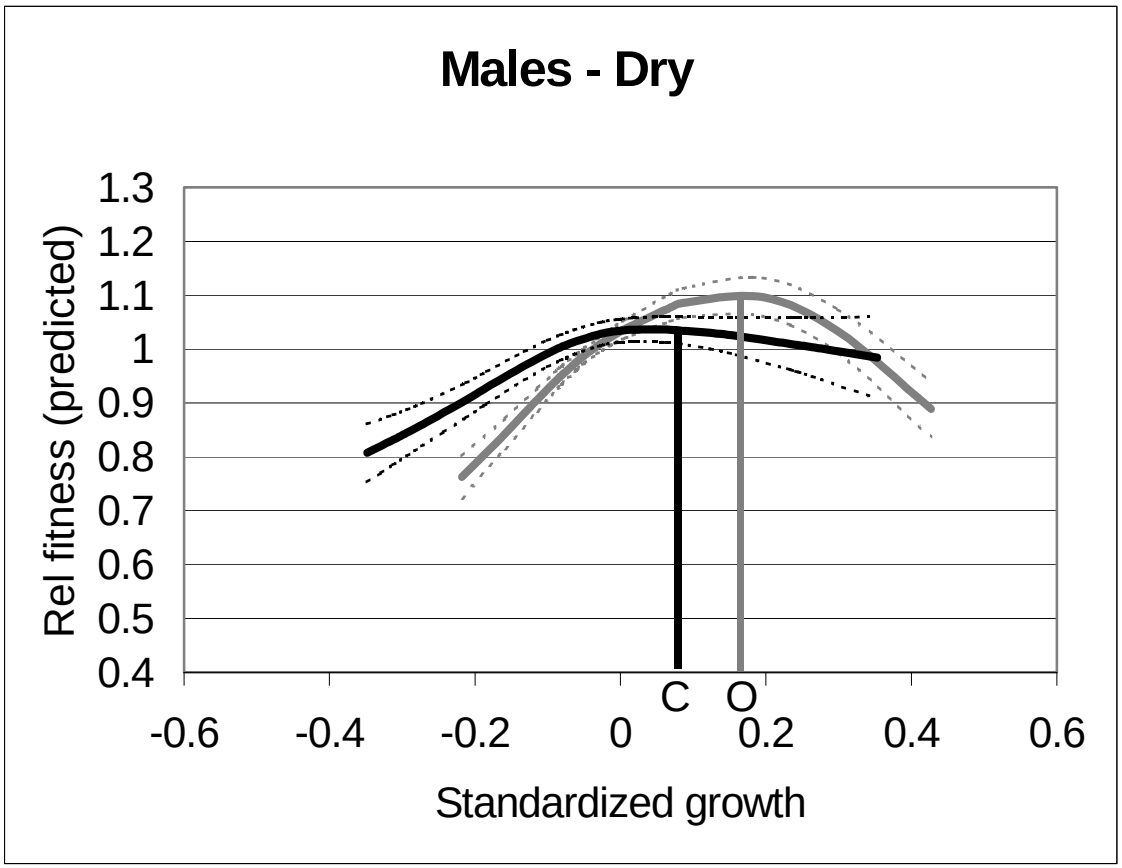


Figure 5.3. continued



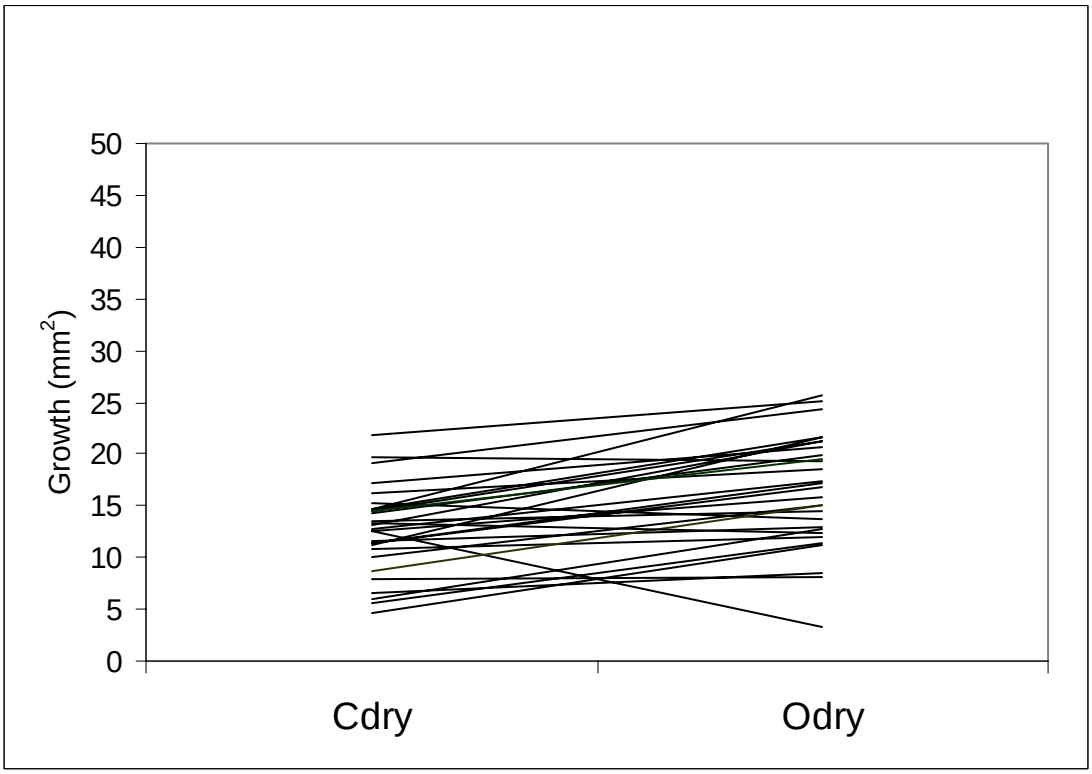
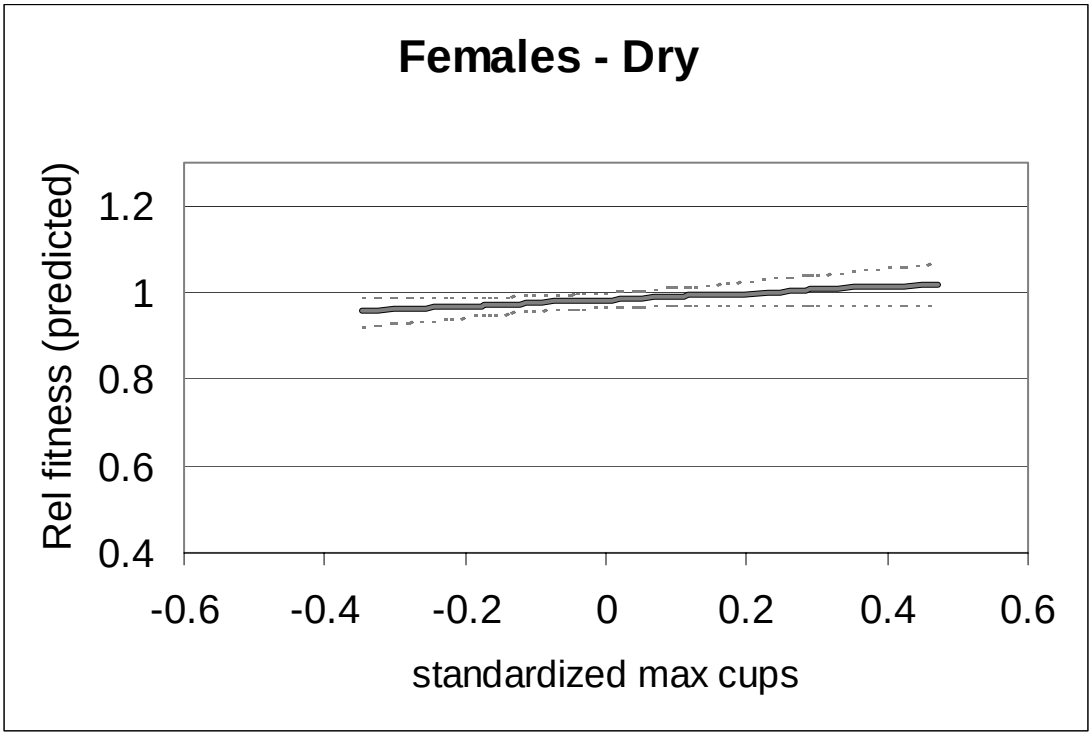
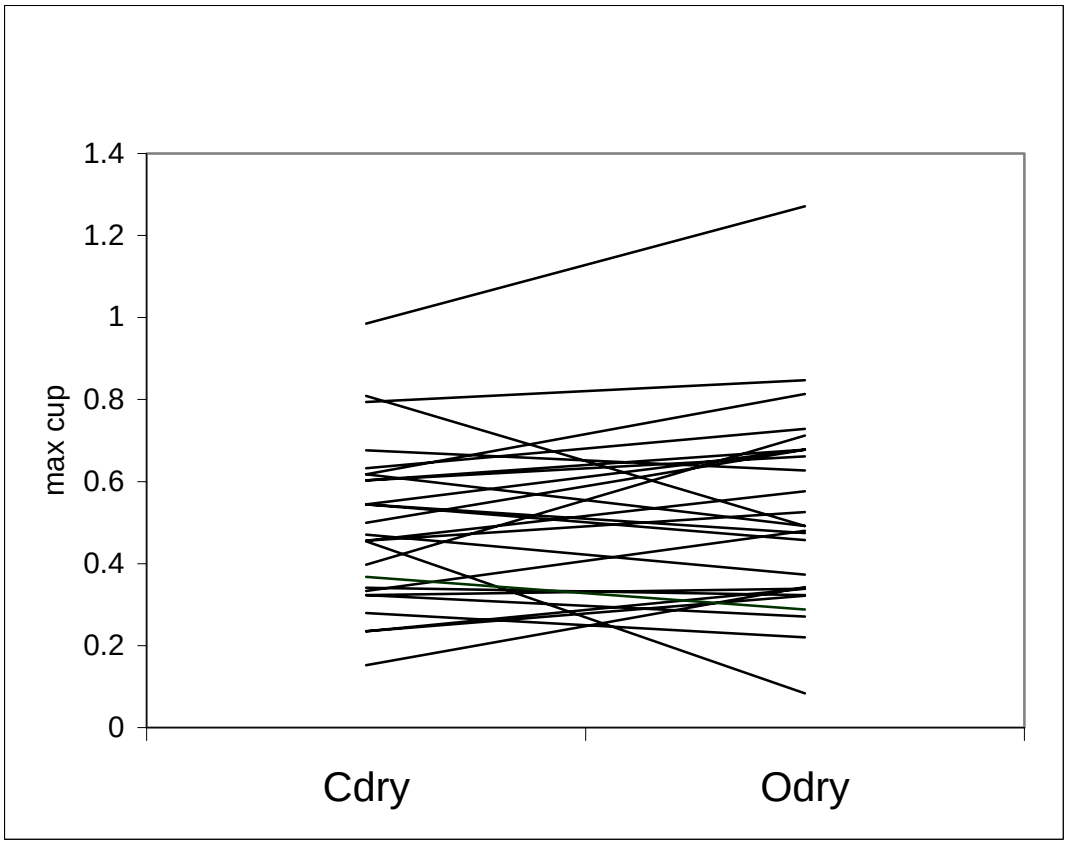


Figure 5.3. continued.





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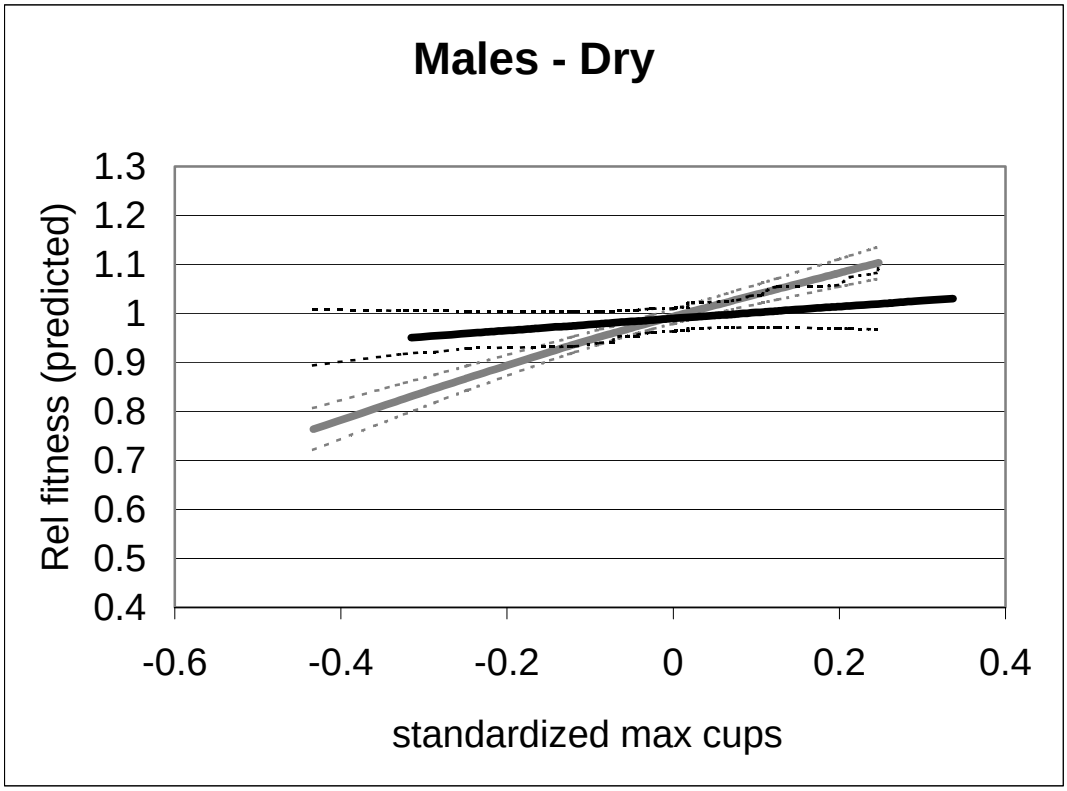
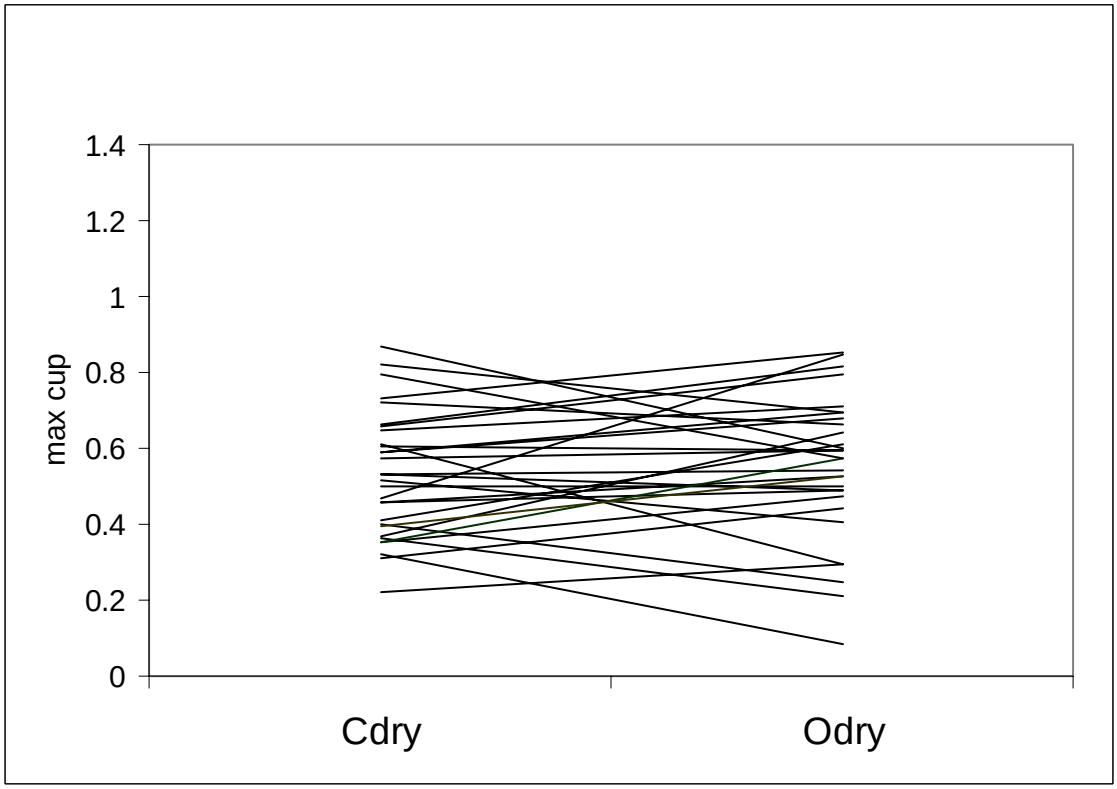




Chapter Six: Maintenance of sexually dimorphic pre-adult traits in *Marchantia inflexa*

Summary

Marchantia inflexa, a dioecious thallose liverwort, is sexually dimorphic in clonal expansion traits. I used selection analyses to measure the magnitude and direction of selection on clonal fitness to uncover possible mechanisms for the maintenance of pre-adult sexually dimorphic characters. I planted replicates of genotypes of female and male *M. inflexa* in two light environments in a greenhouse and measured morphological and phenological characters associated with growth and asexual reproduction. Timing to onset of asexual reproduction and plant size early in development were under sex-specific selection in a low light environment. Additionally, females exhibited a sex-specific cost of plasticity in the timing of their onset of asexual reproduction in high light. Selection on asexual fitness tended to shift traits toward monomorphism rather than sexual dimorphism. Whereas, the expressed phenotype of females was congruent with patterns of selection acting on sexual fitness. I detected negative tradeoffs between asexual and sexual fitness components in females in one light environment. Opposing selective forces acting on asexual and sexual fitness components may explain how sexual dimorphisms persist in the face of selection for monomorphism in the pre-adult phase.

Introduction

In dioecious seed plants, females and males can differ in morphological, physiological, and life history traits (reviewed in Geber et al., 1999). Sexually dimorphic traits may evolve and be maintained through natural selection when sex-specific selection leads to different phenotypic trait optima for the sexes (Putwain and Harper, 1972; Dawson and Geber, 1999). In combination with sex-specific selection, environment-dependent selection may lead to habitat partitioning and spatial segregation of the sexes (Lloyd and Webb, 1977; Kohorn, 1994; Dawson and Geber, 1999; Delph, 1999) which can maintain sexual dimorphisms in vegetative and reproductive characters. Sex-specific costs of plasticity may contribute to the spatial segregation of sexes when selection on traits is environment-dependent and plasticity of a trait, independent of the trait itself (Van Tienderen, 1991), is under sex-specific selection.

The focus of studies of plant sexual dimorphisms has been on differences between mature females and males in traits associated with sexual reproduction. Sexual dimorphism is expected in adult plants in which the sexes exhibit differences in resource allocation as a result of different costs of sexual reproduction (Lloyd and Webb, 1977; Charnov, 1987; Lloyd, 1982; Meagher, 1984; Meagher and Antonovics, 1982; Shine, 1989; Eppley et al., 1998; Delph, 1999). Monomorphism of the sexes is expected in pre-adults because plants exhibit indeterminate growth and nonreproductive individuals, regardless of sex, likely experience similar growth constraints (Lloyd and Webb, 1977). However, pre-adult sexual dimorphisms have been reported in seed plants and bryophytes for characters including, growth rates (Allen, 1919; Godley, 1964; Lloyd, 1973; Shaw and Gaughan, 1993; McLetchie and Puterbaugh, 2000), and asexual reproductive rates (Voth and Hamner, 1940; McLetchie and Puterbaugh, 2000). Sexual dimorphism in vegetative characters associated with pre-adult plants may result from correlated selection on adult sexual reproductive traits such as inflorescence architecture (Kohorn, 1994; Geber, 1995). Measuring selection on sexually dimorphic characters in pre-adults is important because natural selection may act with different magnitudes and in opposing directions among life stages of individuals (Schluter and Smith, 1986; Andersson, 1994; Preziosi and Fairbairn, 2000), and the adaptive significance of adult sexual dimorphisms may be confounded by the action of strong selection on sexually dimorphic traits in pre-adults (Purrington and Schmitt, 1998).

I used *Marchantia inflexa* (Nees et Mont), a dioecious, thallose liverwort, to examine the maintenance of sexually dimorphic pre-adult traits. In *Marchantia,* sex determination is under genetic control of sex chromosomes (Bischler, 1986). *Marchantia inflexa* females and males are sexually dimorphic in growth and asexual reproductive rates (McLetchie and Puterbaugh, 2000). *Marchantia inflexa* ranges from the southern USA to northern Venezuela (Bischler, 1984). Caribbean populations are typically female-biased and include sexually reproducing females and males (McLetchie and Puterbaugh, 2000). Populations in the USA are typically unisexual and maintained solely through asexual reproduction (Schuster, 1992).

Pre-adult *M. inflexa* reproduce asexually via the production of specialized asexual propagules. This makes *M. inflexa* especially suitable for studies of selection on asexual fitness because, unlike most plants, asexual reproduction and growth are distinct processes. I used a measure of asexual reproductive output as a metric for asexual fitness. Fitness in seed plants is

typically some measure of total sexual reproductive output, i.e., number of sexually produced offspring contributed to the next generation. In clonal plants, clonality results in the production of new individuals and the spread of the genotype and thus is considered a measure of fitness (Fagerstrom, 1992; Watson et al., 1997; Shaw and Beer, 1997). My measure of "asexual fitness" is in accord with the measure of gametophyte fitness proposed for mosses (Shaw and Beer, 1997) and with clonal fitness for a meristem-meristem plant cycle (Fagerstrom, 1992). It is not uncommon for bryophyte populations, and even species, to become entirely unisexual (Longton and Schuster, 1983), with the absence of males most common (Longton and Schuster, 1983; Stark et al., 1998). In the case of unisexual bryophyte species, asexual reproductive output is only estimate of fitness. Thus, both asexual and sexual reproduction contribute to an individual's lifetime fitness.

I focused on asexual reproductive output because all bryophytes display some mode of asexual reproduction, all have the potential for clonal expansion (During, 1990; Newton and Mishler, 1994), and for many dioecious species, sexual reproduction is rare (Longton and Schuster, 1983). In dioecious clonal plants, females and males can allocate resources differently to sexual and asexual reproductive processes. Therefore, selection acting separately on asexual and sexual fitness components may have implications for the evolution and maintenance of sexual dimorphisms.

The purpose of this study was to uncover possible mechanisms for the maintenance of sexual dimorphisms in pre-adult traits via selection on asexual fitness. I used phenotypic and genotypic selection analyses (Lande and Arnold, 1983; Rausher, 1992) to estimate the magnitude and direction of selection on traits in female and male *M. inflexa* in two light environments. I asked the following questions: (1) Is sex-specific selection acting on asexual fitness of *M. inflexa* in such a way as to maintain sexual dimorphisms in pre-adult characters? (2) Is selection environment-dependent? (3) Is there sex-specific selection on plasticity of traits, independent of the traits themselves, that is consistent with the geographical distribution of the sexes? I hypothesized that selection on asexual fitness would be sex-specific in direction or magnitude because *M. inflexa* exhibits sexual dimorphic pre-adult traits. Given what appears to be a wider geographic distribution of females relative to males and the prevalence of female-biased populations (Bischler, 1984; but see McLetchie and Puterbaugh (2000) where sex ratio did not differ from 1:1), I predicted that selection on sexually dimorphic traits would be environment-

dependent. Further, I hypothesized that the more widely distributed sex, females, would be more plastic than males and that males might experience an environment-dependent cost of plasticity consistent with their limited geographical distribution. Although my focus was on asexual reproduction, I also included a limited analysis of selection on sexual fitness.

Materials and methods

To measure sex-specific and environment dependent selection on pre-adult characters in *M. inflexa*, I grew replicates of female and male genotypes under two different light environments in a greenhouse. Stock plants used in this experiment were collected in June 1999 along Quare River in the Hollis Reservoir watershed on the island of Trinidad, The Republic of Trinidad and Tobago. I collected 40 – 43 vegetative tips from patches where only females or only males were expressing sex. The collected tips were presumed to be female are male respectively. Each tip was collected from a different, randomly chosen patch along 2 - 3 km of stream length, and all patches were separated from each other by water. This collection method increased my likelihood of collecting individuals that were not members of the same genet. Field-collected thallus tips were individually transplanted into pots and maintained in a greenhouse on a capillary watering system as described below.

Alan Whittemore (New York Botanical Garden, Bronx, New York, USA) verified identification of the species and voucher specimens were deposited at the Missouri Botanical Garden (St. Louis, Missouri, USA, specimen nos. MO92113 and MO92115) and the National Herbarium of the Republic of Trinidad and Tobago (St. Augustine, Trinidad specimen no. TRIN34616, D. N. McLetchie, collector).

I planted gemmae, from randomly chosen female and male stock plants, into plastic pots (5.9 cm diameter and 2.7 cm deep) in Feburary 2000. I used a total of 16 female and 14 male genotypes because one of the presumed males was actually a female. Several gemmae per pot were planted on steam-autoclaved soil (collected from the North Farm, University of Kentucky, Lexington, Kentucky, USA) and these were thinned to 1 plant/pot 10 d after planting. I had a total of 420 pots, consisting of seven replicates per genotype across two shade treatments. Pots had individual lids fitted with either 55% or 73% shade cloth, to provide treatments of high light or low light, respectively. Pots were placed on a single greenhouse table with locations randomized. Pots were placed directly on a capillary mat, one edge of which was submerged in a water trough, and filled with deionized water.

The planting day was considered day 0. I checked plants every 2 d beginning on day 16 to record date that the mericell first split into two mericell regions (referred to as split), date of onset of asexual reproduction (referred to as cup onset), and date of first sex structure production (sex expression). Number of cups was counted on day 120 and number of sex structures counted on day 150. Photographs of plants were taken every 2 wk beginning on day 49 using a Nikon Coolpix 950 digital camera. Total green plant area was measured in squared millimeters on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the internet at http://rsb.info.nih.gov/nih-image/).

Shade treatments were chosen based on McLetchie and Puterbaugh (2000) and unpublished preliminary trials in which plants were grown successfully from gemmae through sexual maturation in 0%, 55%, 63% and 73% shade (McLetchie, unpublished data). The low-light environment was chosen because, although levels of sex expression changed in relation to canopy closure in the field (McLetchie, unpublished data), under extremely low light plants have severely retarded growth rates (Fuselier, unpublished data).

Greenhouse temperatures ranged from 22 ^{o}C at night to 25 ^{o}C during the daytime, day-length ranged from 11 h to 13.5 h throughout the year. In Trinidad, plants experience day-lengths of 12 to 13 h, temperatures from 24 ^{o}C to 26 ^{o}C, on average, through the year (Muller, 1982). To compare greenhouse light conditions to field light conditions I used hemispherical photographs, taken at low sun angles (to avoid scattering of light), though a 180^{o} fish-eye lens (FC-E8) on a tripod-mounted digital camera (Nikon 950, Nikon Corporation, Tokyo, Japan). Images were analyzed using Scanopy (Reagent Instruments Inc, Quebec, Canada) to estimate the amount of photosynthetically active photon flux density (PPFD, mol/m^2/day) reaching a site. I used only direct PPFD and assumed no cloud cover. I used two images from field sites and a single greenhouse image. Photographs from the field were taken as part of an unrelated field experiment (McLetchie, unpublished data), and were chosen to represent the highest and lowest light levels among 20 microsites with *M. inflexa*. In the field, PPFD ranged from 19.03 to 5.23 mol/m^2/day. In the greenhouse, PPFD was 12.25 mol/m^2/day under 55% shade and 7.34 mol/m^2/day under 73% shade. Therefore, the light environments in the greenhouse were within the range of those experienced by plants at field sites.

Clonal fitness

The number of cups on day 120 was used as a metric for asexual fitness. This is an appropriate measure of asexual fitness because the number of cups on a plant is positively related to the number of gemmae produced (McLetchie and Puterbaugh, 2000) and numbers of gemmae represent asexual reproductive output. The cup count on day 120 was late enough that plants in both shade treatments were well into cup production by this time.

Selection analyses

I used age at first split, age at onset of asexual reproduction, and area at day 49 in phenotypic and genotypic selection analyses to examine directional and nonlinear selection on female and male phenotypes (Lande and Arnold, 1983). Previously, area, number of cups, and number of mericell splits were described as dimorphic in *M. inflexa* (McLetchie and Puterbaugh, 2000). Timing to first mericell split and plant area at day 49 represent differences in mericell production and early growth. Timing of cup onset is an important characteristic of asexual reproduction because gemmae production and dispersal are likely important to patch colonization.

Selection analyses take into account not only direct selection but also indirect selection that can obscure the influence of direct selection on a trait. Partial regression coefficients of standardized traits regressed onto relative fitness provide measures of direct selection, selection gradients (Beta), after controlling for correlations with other characters. The coefficient from a regression of a single trait on relative fitness provides a measure of total selection, the selection differential (s), which includes effects of indirect selection via correlated characters (Lande and Arnold, 1983). Inclusion of quadratic terms in addition to standardized trait values permits the examination of patterns of direct (gamma) and total (g) disruptive and stabilizing selection. Additionally, a significant interaction between two traits indicates the action of correlative selection and indicates that the relationship between two traits is under selection. A selection differential statistically different from zero indicates that a trait has not reached its equilibrium value (Lande, 1980; Lande and Arnold, 1983).

Trait values were standardized by sex and shade treatment by subtracting the mean and dividing by the standard deviation of the measure. Fitness values were made relative within each sex and treatment by dividing by mean fitness in each sex-shade group. Data were not normally distributed, and residuals from preliminary analyses were likewise non-normal. Transformations

did not improve normality. I used jackknife regression, a resampling method that is unbiased by nonnormal data, via the program Freestat (Mitchell-Olds, 1989) to generate more accurate measures of standard error and more reliable probability values for selection analyses. Correlations among standardized variables were examined using PROC CORR in SAS (1990) but because the data were non-normal, probability values were considered approximate.

Phenotypic selection analyses included all plants (each gemmule) as "individuals," whereas in genotypic selection analyses, I used the genotype mean measure for the traits in each sex-shade group. Unlike phenotypic selection analyses, genotypic selection analysis reduces the likelihood that results are biased by environmentally induced correlations that impact fitness (Rausher, 1992). I present two analyses from the same experimental data because of the limitations of both phenotypic and genotypic selection analyses. My phenotypic selection analyses suffer the limitations of possible environmentally induced correlations and correlations due to genetic relatedness. My genotypic selection analyses are the stronger and more conservative method but suffers the limitation of reduced power. I did not use genotypic selection data in nonlinear regression because of reduced power to detect differences. I interpret my results in light of both analyses and stress those results that are significant in phenotypic selection analyses and, whether significant or not, are of the same direction and magnitude in the genotypic selection anaylses. Selection gradients and differentials significant in the phenotypic selection analyses alone, and with contradictory results in the genotypic selection analysis, cannot be definitively interpreted.

Sex-specific and environment-dependent selection

I used analyses of covariance (ANCOVA) within each shade treatment with relative fitness as the dependent variable, sex as a main effect, and the standardized plant traits as covariates to examine if selection was sex-specific. A significant sex-by-trait interaction indicated sex-specific selection (Donohue et al., 2001). To determine if selection was environment-dependent, I used similar ANCOVAs within each sex with shade as a main effect; a significant interaction between shade and trait indicated environment-dependent selection. Selection gradients were considered significantly different if for two gradients the mean ± 1 SE (generated from jackknife regression) did not overlap. ANCOVAs were conducted using SAS (SAS, 1990).

Plasticity

I used plasticity of a trait and the mean genotype trait value in genotypic selection analyses to examine selection on plasticity of traits independent of the traits themselves (Donohue et al., 2001). I calculated plasticity for each genotype as the difference between values of the trait in the two shade treatments such that maximum plasticity would have a positive value. Relative fitness was calculated as the number of cups divided by the mean number of cups for the genotype across the two shade environments. I conducted selection analyses for each sex-shade combination. I used the same variables in an ANCOVA where relative fitness was the dependent variable, sex was the main effect, and standardized plasticity and trait values were covariates. An interaction between sex and trait plasticity indicated sex-specific selection on the plasticity of that trait independent of the trait itself.

Sexual fitness

I conducted additional phenotypic selection analyses with female plants that expressed sex in 55% shade and had sex structures on day 150 to examine the magnitude and direction of selection on sexual fitness and possible tradeoffs between asexual and sexual reproductive processes. Number of sex structures present on day 150 was used as a metric for sexual fitness because this should be directly and positively related to spore production. My measure of sexual fitness is potentially a representation of the number of sexually produced progeny contributed by a plant to the next generation. However, this metric for sexual fitness may be flawed because it is unknown how closely the number of sex structures translates into spore progeny. Split, area, cup onset, and timing to sex expression were standardized and regressed onto relative sexual fitness. I conducted a second analysis to examine the relationship between trait plasticity and sexual fitness by regressing the traits and their plasticities (excluding onset to sex expression) onto sexual fitness. Numbers of plants that expressed sex in 73% shade and number of males that expressed sex in 55% shade were too few to analyze. Finally, I conducted a second phenotypic selection analysis using standardized values of split, area, cup onset, and timing to sex expression regressed on relative asexual fitness. This permitted a comparison of the magnitude and direction of selection acting on sexual and asexual fitness components.

Results

Plants were followed through day 157, and at that point, there were no differences in mortality between females (15%) and males (14%). Females and males tended to produce cups

earlier and grow larger in 55% compared to 73% shade (Table 6. 1). Females ($t = -1.26$, $P < 0.11$) and males ($t = -1.94$, $P < 0.05$) that expressed sex in 55% shade tended to be larger than genotype replicates in the same treatment that didn't express sex ($N = 15$ pairs). Although some of these traits were not significantly different between the sexes, they follow the pattern of sexual dimorphism described by McLetchie and Puterbaugh (2000) for plants in 55% shade.

Genotype mean trait values for time to split and area were negatively correlated for females in 55% shade and for males in both treatments. Area was also negatively correlated with cup onset in females in 55% shade (Table 6. 2). There were no significant correlations between traits for females in 73% shade.

Selection analyses

In 73% shade, selection favored females and males that produced cups earlier and males with larger area at day 49 (Table 6. 3). Females in 73% shade also showed significant disruptive selection for cup onset (indicated by a positive gamma). This suggested that selection favored females that produced cups earlier or later but females with intermediate values of cup onset had reduced fitness. There was significant direct but no significant total selection for larger area in males in 73% shade. In 55% shade, direct selection for earlier cup production was nearly significant for females in phenotypic selection and significant in genotypic selection analyses. Males showed disruptive selection for area (Table 6. 3) in 55% shade. Correlative selection terms included in nonlinear models (split × cup, cup × area, and area × split) were not significant in phenotypic selection analyses and thus not presented here. Significance in the phenotypic and nonsignificance in the genotypic selection analyses were likely indicative of low power to detect genotypic selection. On the other hand, significance in the genotypic and nonsignificance in phenotypic analysis is an indication of environmentally induced correlations in the phenotypic analysis (Rausher, 1992). My results are interpreted in the light of both analyses in that they were either significant in both or significant in the phenotypic selection analysis and of similar direction and magnitude in the genotypic selection analysis.

Significant direct selection combined with nonsignificant total selection (as in the case of area of males in low light) results when there is direct selection on the trait, but this is masked by selection acting in opposing directions on correlated variables. Area was negatively correlated with time to split for males in both shade treatments (Table 6. 2). Direct positive selection on

area combined with a negative correlation between area and split produce nonsignificant selection differentials via contrasting selection pressures.

Cup onset for females in 73% shade was not significantly correlated with other variables. However, cup onset in females in 55% shade was negatively correlated with area (Table 6. 2). There may have been some correlation (albeit not significant) that influenced total selection for cup onset in 55% shade such that the differential was nonsignificant. There may also have been correlated variables that influenced total selection but were not included in the models.

Sex-specific selection

Sex-specific phenotypic selection for larger area and earlier cup onset was detected for plants in low light (Table 6. 4). Under 73% shade, males experienced stronger direct and total selection for larger area compared to females, and direct selection for earlier cup onset was stronger for females than males (Table 6. 4). When genotypic means were used in this analysis, these differences were not significant. Detection of differences using genotype mean phenotypes was confounded by small sample size in genotypic selection analyses. The direction and relative magnitude of selection on area and cup onset for females and males were similar in the genotypic and phenotypic analyses (Table 6. 3). No significant differences in magnitude or direction of selection between the sexes were detected for plants in 55% shade (Table 6. 4).

Environment-dependent selection

Females in 73% shade were under stronger direct and total selection for earlier cup onset compared to females in 55% shade (Table 6. 4) in both phenotypic and genotypic analyses. Total selection on males for early cup onset was stronger in 73% shade than in 55% shade. Genotypic selection analysis indicated that direct selection for earlier cup onset was stronger on males in 73% shade than on those in 55% shade. Total directional selection on males in 55% shade favored smaller size, whereas in 73% shade, total selection favored larger size (Table 6. 4). Because no significant difference between shade treatments for direct selection on area in males was detected, there was a change in the trait but it resulted from indirect selection acting on correlated characters.

Plasticity

Females tended to exhibit greater plasticity of cup onset (0 = 56.97) compared to males (0 = 39.64) but these differences were not significant in a t -test (Figure 6.1). In genotypic selection analysis, females exhibited a cost of plasticity in onset of asexual reproduction in 55% shade.

There was no significant direct selection detected for other trait plasticities in either sex for either treatment (Table 6. 5). When trait and trait plasticities were compared in an ANCOVA, results showed that females were under significantly greater direct selection than males for lower plasticity in timing to asexual reproduction in 55% shade ($F = 7.02$, $P = 0.02$; Table 6. 5 and Figure 6. 1). Additionally, the magnitude of selection on plasticity in first mericell split differed between the sexes ($F = 5.33$, $P = 0.03$). Males with a plastic response for split in 55% shade were not favored by selection, whereas plastic split response was favored in females in 55% shade. However, neither of these selection differentials were significant in selection analyses. Probability values from the ANCOVA were used for this analysis because residuals were normally distributed. Sex-specific differences might have resulted as a statistical artifact of comparing a highly variable population (females) with a less variable population (males), but because the magnitude of direct selection for females was very high, the results likely indicate an actual pattern.

Sexual fitness

Females in 55% shade that expressed sex early were favored by direct selection (Table 6. 6) in a phenotypic selection analysis. Results of a genotypic selection analysis were of similar direction and magnitude but no significant selection was detected (analysis not shown). A negative tradeoff between sexual and asexual fitness components was detected (Figure 6. 2). Females exhibited direct selection for later sex expression ($Beta = 0.496 \pm 0.13$, $t = 2.866$) when timing to sex expression was included in the multiple regression for asexual fitness (split, cup onset, area and time to sex expression regressed onto number of cups as a metric of fitness). Phenotypic correlations indicated that females that expressed sex earlier had fewer cups (low asexual fitness) but had more sex structures (high sexual fitness; Table 6. 7, Figure 6. 2).

I conducted a genotypic selection analysis using split, cup onset, area, and their plasticities regressed onto sexual fitness to examine the influence of sexual fitness on trait plasticity. Unlike the analysis with asexual fitness, plasticity in timing of cup onset was not significant but there was significant direct selection for higher plasticity in area (Table 6. 8) detected for sex-expressing females in 55% shade.

Discussion

Sex-specific selection on pre-adult traits was detected in *Marchantia inflexa*, but selection on asexual fitness favored monomorphism of the sexes. Selection on sexual fitness,

rather than asexual fitness, may maintain the sexual dimorphisms observed in female *M. inflexa*. Selection for early cup onset, although sex-specific in magnitude in low light, was in the same direction for both sexes. Because males have earlier onset of asexual reproduction than females do and selection on both sexes favored earlier onset, stronger selection on females drives the sexes to be more similar in timing of onset to asexual reproduction. Also, in 73% shade, males tended to be larger than females and direct selection favored larger males, but there was no total selection for larger area in males because of a constraining correlation of size and time to mericell split. Finally, females are known to have a wider geographic distribution than males and were expected to be the more plastic of the sexes. Females tended to be more plastic than males in onset to asexual reproduction but, in terms of asexual fitness, females incurred a cost for this plasticity in 55% shade. Thus, selection favored females that were more similar to males in cup onset plasticity in one environment. These trends toward monomorphism of the sexes via selection on asexual fitness may be reversed when the same traits are examined in the context of sexual fitness. My results indicated a trade-off between asexual and sexual fitness for females in high light. Selection on sexual fitness favored females with earlier onset of sex expression, but significant selection was not detected on other characters, indicating that these characters may be at their equilibrium values (Lande, 1980; Lande and Arnold, 1983).

Selection on pre-adult traits

Differences in trait means between the sexes detected in this study were consistent with McLetchie and Puterbaugh (2000) and indicate that sexual dimorphisms do occur early in the life cycle in traits associated with asexual reproduction. This is significant because most studies of sexual dimorphism have been restricted to comparisons of adult life stages (Geber, 1999). Age of onset of sexual dimorphism and the degree of correlation between selection on juvenile and adult traits are unknown for most dimorphic dioecious plants. Lloyd and Webb (1977) suggested that sexual dimorphisms should not occur prior to sexual maturity. In female-biased populations of *Rumex acetosa*, sexual dimorphisms in phenology were not evident until shortly before sexual reproduction (Korpelainen, 1993). Sexual dimorphisms in resource allocation in *Silene latifolia* were not evident until plants began investing in sexual reproduction (Delph and Meagher, 1995). My results corroborate these studies in that I found selection on asexual fitness favored monomorphism of the sexes in pre-adult characteristics. However, sexual dimorphisms were evident in plants prior to sexual reproduction. Pre-adult sexually dimorphic traits have been

detected in angiosperms and bryophytes for characters such as germination ability (Newton, 1972; Conn and Blum, 1981; Cameron and Wyatt, 1990; McLetchie, 1992; Carrol and Mulcahy, 1993; Purrington, 1993; Shaw and Gaughan, 1993; Taylor, 1994; Shaw and Beer, 1999; McLetchie, 2001), embryo competitive ability (Conn and Blum, 1981), and regeneration rates (Longton and Greene, 1979). My results suggest that the expression of pre-adult sexual dimorphisms in the face of opposing selection pressure may result from correlations with traits associated with sexual fitness in adults (Geber, 1999).

Sex-specific selection

Selection should favor different phenotypic optima in the two sexes if natural selection maintained sexual dimorphism via sex-specific selection (Geber, 1995; Kohorn, 1994). At an equilibrium level of sexual dimorphism, selection differentials for traits in the two sexes are not significantly different from zero and each sex rests at its respective trait optimum (Geber, 1999). Sex-specific selection has been implicated in the evolution of sexual size dimorphism in cases in which size is heritable and differences between the sexes evolve as a product of differential selection pressures and low genetic correlations between the sexes (Lande, 1980; Slatkin, 1984). High genetic correlations of traits between the sexes will slow the evolution of dimorphisms even under sex-specific selection regimes and will extend the period of time that suboptimal phenotypes are expressed in a population (Lande, 1980; Meagher, 1984). I detected sex-specific selection in pre-adult traits in *M. inflexa*, but the direction of selection in relation to asexual fitness did not drive the sexes toward different phenotypic optima. Although disruptive selection acted on cup onset in females in low light and in males on area in high light, given the strength and magnitude of directional selection on phenotypes, these patterns do not implicate different phenotypic optima for the sexes. Knowledge regarding the heritability of the traits considered in this study is lacking but must be elucidated to assess the impacts of genetic correlations in these traits between the sexes.

Environment-dependent selection

Environment-dependent selection and adaptive significance of light environments to sexually dimorphic clonal expansion traits may maintain sexual dimorphisms via spatial segregation of the sexes. In seed plants, light environment influences internode elongation and flowering response among other traits, and there is evidence that photomorphogenic shade avoidance responses are adaptive (Schmitt et al, 1995; Dudley and Schmitt, 1996). In

bryophytes, characteristics of the light environment are important in timing and speed of gametangial induction (Benson-Evans, 1964; reviewed in Longton, 1990), production of gemmae (Lockwood, 1975), and thallus growth (Voth and Hamner, 1940).

A number of studies on angiosperm sexual dimorphism document how adaptations that increase mating success influence responses to sexual selection such that vegetative sexual dimorphisms are correlated with environmental characteristics (Dawson and Geber, 1999). For example, males of wind-pollinated taxa may specialize on drier habitats to increase chances of pollen dispersal (Dawson and Bliss, 1989), whereas females may have higher reproductive success in protected areas. Differences in morphology related to habitat differences may be maintained as a result of the greater stresses imposed by reproduction (usually on the female) in the environment in which it occurs (Wallace and Rundel, 1979). If females and males are better adapted to different environments, spatial segregation of the sexes and biased sex ratios along environmental gradients may result (Dawson and Bliss, 1989; Dawson and Ehleringer, 1993). Also, if the resource cost of reproduction is higher for one sex, that sex is expected to be under selection to increase resource uptake (Dawson and Geber, 1999). Thus, biased sex ratios correlated with habitat characters may result because one sex specializes on high quality habitats and drops out of suboptimal habitats.

I detected environment-dependent selection in *M. inflexa* in relation to asexual fitness, but there were no patterns that suggested that one sex consistently outperformed the other sex in either light environment. The magnitude of selection on females for early onset of asexual reproduction differed across environments such that selection was stronger under low light than under high light. Male *M. inflexa* experienced greater total selection for larger area and early cup onset in low light compared to high light. Relative to plants in high light, males in low light expressed maladaptive cup onset and size phenotypes and females expressed a maladaptive cup onset phenotype. However, females tended to be slightly smaller than males in low light, a trend favored by selection. In nature, the frequency with which the sexes experience different light environments and the importance of additional environmental variables such as temperature and photoperiod will influence response to environment-dependent selection and may contribute to the spatial distribution of the sexes.

Plasticity

Costs of plasticity may arise in organisms through a physiological cost of being plastic in an environment (Van Tienderen, 1991). When maintenance of trait plasticity is costly, nonplastic genotypes will be favored over plastic genotypes with the same trait mean. Sex-specific selection on plasticity and sex-specific costs of plasticity may influence the geographic distribution and contribute to spatial segregation of the sexes and skewed sex ratios. Because female *M. inflexa* are more widely distributed than males and populations are typically female-biased (Bischler, 1984; but see McLetchie and Puterbaugh (2000) where 1:1 sex ratios were found), I predicted that females were the more plastic of the sexes. Sex-specific selection for increased plasticity in females relative to males in traits associated with clonal expansion would provide evidence for an influence of plasticity on the spatial distribution of the sexes. I detected a cost of plasticity in onset of asexual reproduction in females that is incongruent with the wide distribution of females relative to males. When plasticity of traits was examined in the context of sexual fitness, females were under direct selection to increase plasticity in size. This is in accordance with my original hypothesis that females should display more plasticity, as evidenced by their wider geographic distribution, and that selection should act to increase plasticity in females.

Adult sex ratios of bryophyte are frequently skewed from a 1:1 ratio at the local patch and population levels. In liverworts and mosses, the ratios are most commonly female-biased (Longton and Schuster, 1983; Bowker et al., 2000 and references therein) and in some cases, entirely female (Longton and Schuster, 1983). In the southern USA, the northernmost reach of *M. inflexa's* range, some populations of are entirely female or entirely male (Schuster, 1992). If, as my results indicate, females are more plastic than males, females might thrive in environments where males fail. This pattern is in accordance with the distribution of sex ratios in the species, and suggests a need for further research on adaptive plasticity and environmental correlates of sex ratios in *M. inflexa* populations.

Sexual fitness

Differences in resource allocation as a result of different costs of sexual reproduction between the sexes can lead to sexual dimorphism (Lloyd and Webb, 1977; Charnov, 1982; Lloyd, 1982; Meagher and Antonovics, 1982; Meagher, 1984; Shine, 1989; Eppley et al., 1998; Delph, 1999). Physiological condition and future reproduction compete with current

reproduction to produce negative phenotypic correlations and trade-offs among traits (Stearns, 1992). A negative correlation between sexual and asexual reproductive output is predicted by life-history theory, but clear-cut empirical demonstrations of this tradeoff are few (Cheplick, 1995; but see Sutherland and Vickery, 1988; Reekie, 1991; Westley, 1993). I detected a trade-off between asexual and sexual fitness for female *M. inflexa* in high light. In high light, sexual fitness was highest for females that produced sex structures earlier. However, females that produced sex structures early also produced cups later. Late onset of cup production resulted in lower asexual fitness.

These opposing selective forces will interact to determine the pattern of selection experienced over an individual's lifetime (Schluter and Smith, 1986; Schluter et al., 1991). In the context of sexual fitness, for females in high light, selection differentials for split, area, and asexual reproductive onset were not statistically different from zero, indicating that these traits were at their equilibrium. Selection on asexual fitness favored monomorphism of the sexes, but this was incongruent with patterns of phenotypic expression observed in nature. Thus, it appears that selection on sexual fitness rather than asexual fitness, exerts more influence on phenotypic evolution of sexually dimorphic characters in females.

A greater relative influence of sexual fitness for females is expected because most sexually dimorphic traits in plants are believed to be a consequence of higher costs of reproduction incurred by females relative to males (Lloyd and Webb, 1977). Higher costs of sexual reproduction lead to larger trade-offs with other traits for females (Putwain and Harper, 1972; Lloyd and Webb, 1977; Meagher and Antonovics, 1982). To compensate for these trade-offs, females may allocate more energy than males to leaf tissue early in life so they will have resources to allocate to sexual reproduction later (Delph, 1990; Delph, et al., 1993). Unfortunately, my data for male allocation to sexual reproduction was limited, and I were unable to compare the sexes in terms of selection on both sexual and asexual fitness components. My findings do however, underline the importance of investigating selection on components of fitness in different life stages of plants to reveal possible mechanisms for the maintenance of sexual dimophisms in dioecious species.

Table 6.1. Trait values for the sexes.
Mean character values for all individual females and males in two shade treatments. Area is size of plant 49 d after planting; timing to mericel split, cup onset, and sex onset are given as number of days after planting. Sample size is in parentheses.

Characters	Females	Males
73% shade		
Mericell split	39.0 (109)	38.0 (98)
Area (mm^2)	21.3 (109)	24.6 (98)
Cup onset	122.6 (106)	100.6 (90)
Number of cups	3.8 (106)	6.0 (90)
Sex onset	171.0 (4)	-
Number sex structures	1 (4)	0
55% shade		
Mericell split	32.5 (112)	32.5 (99)
Area (mm^2)	65.0 (112)	64.5 (99)
Cup onset	62.1 (102)	56.9 (94)
Number of cups	9.7 (102)	11.6 (94)
Sex onset	132.9 (40)	128.7 (8)
Number sex structures	5.0 (40)	1.6 (8)

Table 6.2. Among-trait correlations.
Pearson correlation coefficients between genotype mean characters in females and males in two shade treatments. 55% shade is above the diagonal and 73% shade, below. Split refers time to first mericell split, cup refers to timing to first cupule production, and area refers to size of plant 49 d after planting. Probability values are considered approximate due to nonnormality of data. ** P = 0.02, * P =< 0.05

	Character		
	Split	Cup	Area
*Females (*N = *16*)			
Split	-	0.25	-0.57**
Cup	0.17	-	-0.58*
Area	-0.24	-0.06	-
Males (*N* = 14)			
Split	-	0.17	-0.56*
Cup	0.19	-	0.28
Area	-0.75**	-0.45	-

Table 6.3. Results of selection analyses.
Selection gradients (Beta and Gamma) and differentials (s and g) from phenotypic selection analyses for directional (Beta and s) and nonlinear selection (gamma and g) on plants in two shade treatments. Gradients and differentials from genotypic selection analyses are given in parentheses for those terms significant in either analysis or in subsequent analyses. * $P < 0.05$, ** $P < 0.02$, ‡ $P < 0.06$

	Direct selection		Total selection	
Character	Beta ± SE directional	Gamma ± SE nonlinear	s ± SE directional	g± SE nonlinear
Females 73% shade				
Split	0.053 ± 0.114	-0.004 ± 0.143	-0.150 ± 0.081	-0.094 ± 0.039
Area	-0.082 ± 0.157	0.124 ± 0.142	0.149 ± 0.141	0.012 ± 0.035
Cup onset	-0.820 ± 0.104** (-0.976 ± 0.319**)	0.631 ± 0.224**	-0.782 ± 0.087** (-0.995 ± 0.311)	-0.523 ± 0.088
Males 73% shade				
Split	0.207 ± 0.095	-0.018 ± 0.052	-0.280 ± 0.100	-0.074 ± 0.026
Area	0.640 ± 0.168* (0.349 ± 0.172)	0.226 ± 0.202	0.631± 0.136	0.401 ± 0.118
Cup onset	-0.379 ± 0.073** (-0.611 ± 0.132**)	0.170 ± 0.114	-0.525 ± 0.083* (-0.816 ± 0.222)	-0.245 ± 0.040
Females 55% shade				
Split	0.018 ± 0.133	0.074 ± 0.094	-0.114 ± 0.378	0.008 ± 0.033
Area	0.076 ± 0.109	-0.054 ± 0.108	0.221 ± 0.304	-0.005 ± 0.054
Cup onset	-0.215 ± 0.092‡ (-0.270 ± 0.090*)	0.052 ± 0.045	-0.265 ± 0.090	-0.028 ± 0.026
Males 55% shade				
Split	-0.114 ± 0.067	0.030 ± 0.050	-0.065 ± 0.062	0.008 ± 0.011
Area	-0.199 ± 0.294	0.285 ± 0.108*	-0.135 ± 0.273	0.268 ± 0.076
Cup onset	-0.141 ± 0.078 (0.131± 0.431)	-0.092 ± 0.056	-0.115 ± 0.084	-0.117 ± 0.060

Table 6.4. Sex-specific and environment-dependent selection.

Analysis of covariance F ratios for interaction terms based on phenotypic and genotypic selection gradients that represent direct selection (Beta) and differentials that represent total selection (s) for female and male plants in two light treatments. *P < 0.05, **P < 0.001

	Sex-specific selection					Environment-dependent selection			
Interaction	55% shade		73% shade		Interaction	Females		Males	
	Beta	s	Beta	s		Beta	s	Beta	s
	Phenotypic selection					Phenotypic selection			
Split × sex	0.05	0.64	0.59	0.54	Split × shade	0.03	0.08	1.79	0.19
Area × sex	0.66	1.74	7.17*	8.07*	Area × shade	0.69	0.04	3.31	3.90*
Cup × sex	2.16	0.00	13.49*	2.62	Cup × shade	13.57**	13.17**	2.92	3.99*
	Genotypic selection					Genotypic selection			
Split × sex	0.31	0.53	0.02	0.08	Split × shade	0.59	0.42	0.10	0.37
Area × sex	0.52	0.33	0.73	1.09	Area × shade	0.10	0.07	0.13	0.63
Cup × sex	0.24	0.17	0.92	0.28	Cup × shade	4.14*	5.84*	4.19*	3.52

Table 6.5. Genotypic selection analysis results.
Selection gradients from genotypic selection analysis of traits and the plasticity of the traits included in a multivariate regression on asexual fitness for plants in two shade treatments. **P < 0.02

Trait plasticity	Females		Males	
	55% shade	73% shade	55% shade	73% shade
Split plasticity	0.643	-0.570	-0.442	0.048
Area plasticity	-0.063	0.425	-0.341	0.352
Cup onset plasticity	-1.136**	-0.494	0.127	-0.946

Table 6.6. Selection on sex-expressing females.
Selection gradients (Beta) and differentials (s) from the phenotypic analysis of traits regressed on sexual fitness for female plants in 55% shade that expressed sex (*N* = 31). Split refers to time to first mericell split, cup refers to timing to first cupule production, area refers to size of plant 49 d after planting, and sex onset refers to timing of first sex structure production. * P < 0.02

Trait	Beta	s
Split	0.041	0.052
Area	-0.135	-0.216
Cup onset	0.133	0.249
Sex onset	-0.915*	0.243

Table 6.7. Trait correlations and sexual fitness.
Correlations for traits used in analyses of sexual fitness for females in 55% shade. *N* = 31, * P < 0.02, ** P < 0.001

Trait	Area	Onset of asexual reproduction	Number of cups	Onset to sexual reproduction	Number of sex structures
Split	-0.53**	0.55**	-0.37*	0.11	0.05
Area		-0.44*	0.26	0.0006	-0.20
Cup onset			-0.49	-0.19	0.23
Number of cups				0.38*	-0.23
Sex onset					-0.56**

Table 6.8. Selection plasticity based on sexual fitness.
Selection gradients (Beta) from genotypic selection analysis based on sexual fitness for females that expressed sex in 55% shade. * P < 0.05

Trait	Beta
Split plasticity	-0.595
Area plasticity	1.170*****
Cup onset plasticity	-0.162

Figure 6.1. Plasticity in onset of asexual reproduction.
Plasticity of onset of asexual reproduction in female and male *Marchantia inflexa* genotypes in two shade treatments given as genotype mean number of days to first cupule produced.

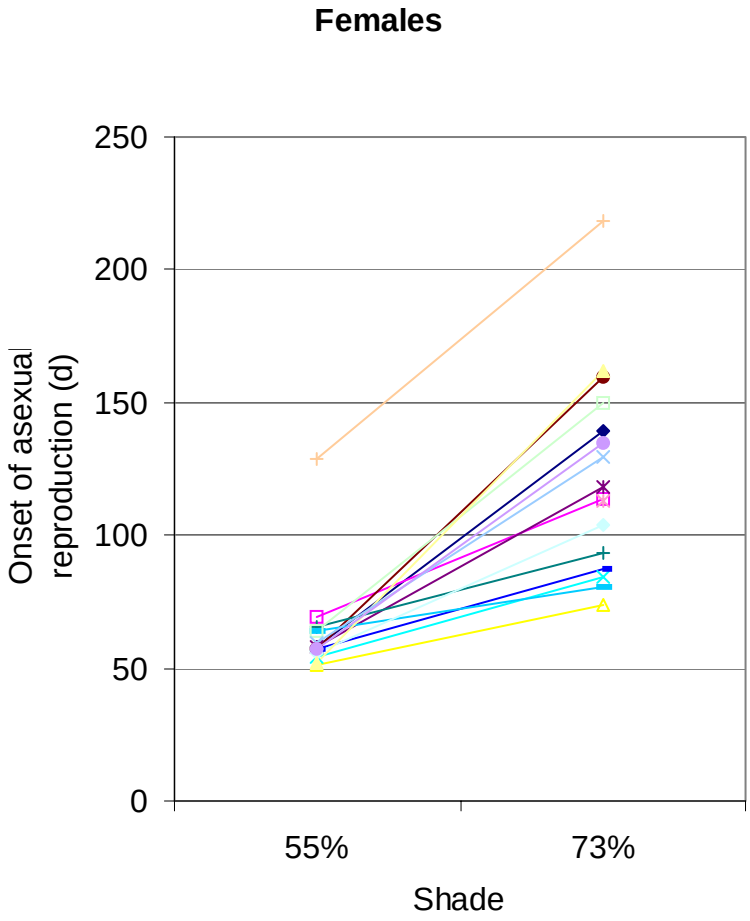


Figure 6.1 continued.

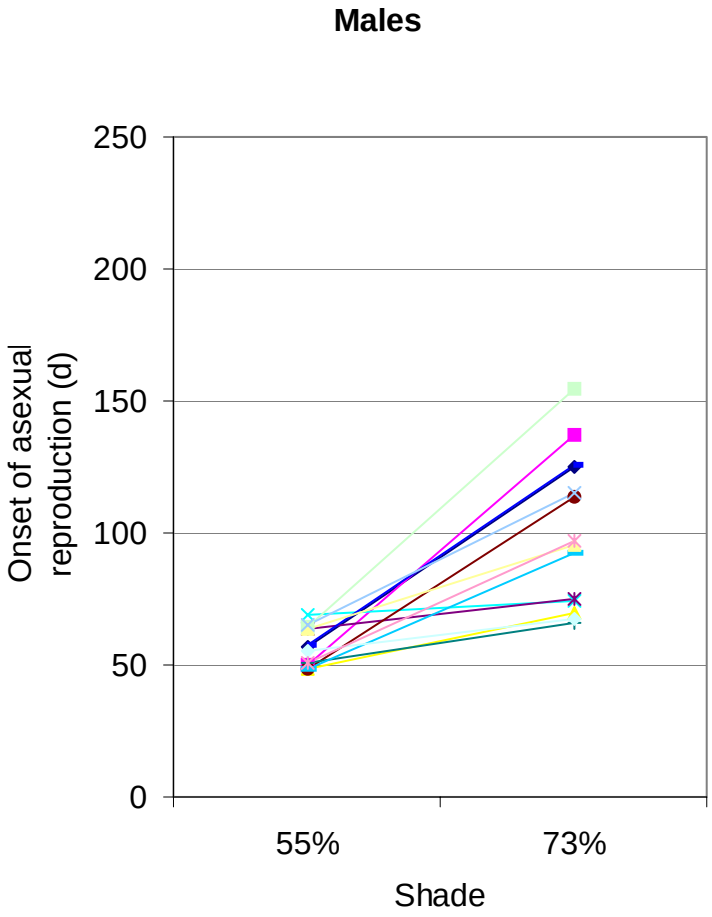
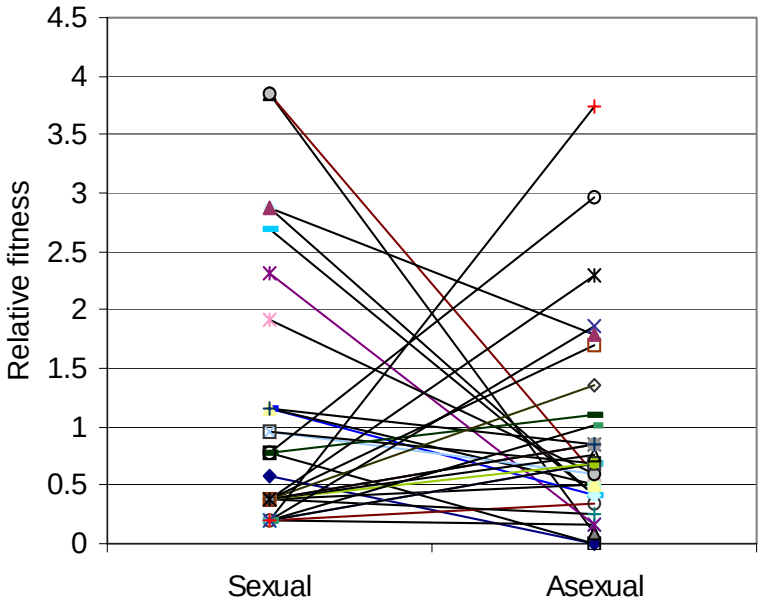


Figure 6.2. Trade-offs of asexual and sexual fitness.
Relative sexual and asexual fitness for *Marchantia. inflexa* sex-expressing females grown in 55% shade that were used in the phenotypic selection analyses.



Chapter Seven: Dissertation Summary & Insights for Further Research

My dissertation research provides an in-depth investigation of sexual dimorphism in *Marchantia inflexa* with the aim of understanding the role of natural selection in maintaining sexual dimorphism in dioecious liverworts. There is a high incidence of dioecy in liverworts relative to angiosperms, and many liverworts are sexually dimorphic. I found that sexually dimorphic characters in *Marchantia inflexa* may be maintained via a combination of sex-specific and environment-dependent selection and, in a heterogeneous habitat, selection may promote the coexistence of both sexes in a population. Factors in nature appeared to promote sexual reproduction; the sexes broadly overlapped in habitat use and sporophytes occurred in both-sex populations. Phenotypes of females were consistent with selection on sexual fitness rather than selection on asexual fitness, suggesting an importance of sex. However, differences among populations and between population types (single-sex and both-sex) indicate that investment in sexual reproduction and the degree of sexual dimorphism varies in a species.

The prevalence of sexual size dimorphism (females larger than males), more male investment in asexual reproduction, and indications of male desiccation tolerance point to potential ecological and evolutionary importance of sexual dimorphism in liverworts. In particular, sexually dimorphic characters may influence sex ratios in bryophyte populations, and biased sex ratios can lead to a lack of successful sexual reproduction. Because sexual reproduction is often tied to "evolutionary potential", sexually dimorphic life history strategies in liverworts may have far-reaching effects. I investigated whether sexually dimorphic characters resulted from habitat specialization of the sexes or sex-specific natural selection, and whether sexual dimorphism and sex ratios varied among populations of *Marchantia inflexa*.

It was not surprising that the sexes of *Marchantia inflexa* overlapped in distribution within populations because the sexes must be in very close proximity for successful sexual reproduction. It was surprising to find that there was no definitive trend for females and males to use different light environments. However, my results were influenced by plant phenology. Future experiments should follow population phenology throughout the entire year, censusing the sexes periodically. Given that sexes are only identifiable when they have sex structures, there are limitations to this technique in that habitat use by non-expressing plants is not included.

A sex marker that permits identification of non-expressing plants is needed to fully explore habitat use by the sexes and to obtain reliable sex ratio data.

Among population variation in sexually dimorphic traits, as evidenced in a common garden experiment, indicated that populations were genetically differentiated, and the degree to which populations were sexually dimorphic varied. Why sexual dimorphism varied among populations is an interesting question for further investigation. There may be local ecological/environmental factors in populations that promote sexual dimorphism, and because liverwort populations are likely subjected to genetic drift, these differences may be quite important in population differentiation. Differences in degrees of sexual dimorphism may be related to the degree to which trade-offs among growth, asexual and sexual reproduction are influenced by local environmental factors.

An interesting pattern to emerge from my study of among-population differences was the similarity between the Oklahoma and Florida male populations. Two growth phenotypes were evident, and plants from single-sex populations tended to have one phenotype while plants from both-sex populations, the other. These patterns call for population genetic studies of the species to elucidate the evolutionary history of the group. I know from unpublished, preliminary data that the plants are not genetically distinct in ITS sequence, as would be expected if they were different species. Thus, the phenotypic differences are likely not a case of cryptic speciation or hybridization with a commonly occurring congener, *M. paleacea*.

Another question begging further investigation is, why more male than female populations? Further genetic studies and expanded sampling may reveal that single-sex populations actually harbor both sexes, or that there are more female populations than I've found. It is not unlikely that single-sex populations did originate from one clone that found refugia during the Pleistocene. If so, what is the connection between growth phenotypes in the single-sex populations and survival of post-Pleistocene expansion? Studies of the selective environment in these populations combined with phylogenetic reconstructions of the population's origins may shed light onto the evolution and expression of alternative growth phenotypes.

My studies of selection provided insight into the maintenance of sexual dimorphism in liverworts. Sex-specific selection in nature does play a role in the maintenance of sexually dimorphic life history characters and, in combination with environment-specific selection, may

promote genetic variation and the persistence of both sexes in a population. Rather than differences in the direction of selection on the sexes, primarily selection is sex-specific in strength and type; with males experiencing stronger selection than females in most cases. That selection acted on the sexes differently in different environments is intriguing. There were trends for larger males to have higher fitness and for selection to favor larger males in shaded areas whereas, in open areas, females grew larger and larger females experienced higher fitness. If this pattern translates into a space occupation advantage for males over females in shady areas, habitat heterogeneity within populations may be crucial for the maintenance of both sexes in a population.

Bryophytes are notoriously plastic and phenotypic plasticity may outweigh genetic differentiation in local fitness and population, phenotypic, differentiation. If bryophytes lack the genetic variation to make local adaptation possible, as some assume (i.e., the old adage that bryophytes are an evolutionary dead end), phenotypic plasticity may make up for the lack of genetic variation in terms of plant colonization and survival ability. However, despite sex-specific selection on the plasticity of traits, there were significant constraints to the evolution and expression of plasticity. Differences between the sexes in plasticity of traits or selection on plasticity did not adequately explain the distribution of the sexes.

Sexual reproduction may have the greatest influence on promoting sexual dimorphism. I found that sexual and asexual fitness trade-off within individuals, but that the individual phenotype is in accord with selection on sexual rather than asexual fitness (for females). This indicates that sexual reproduction may be very influential in liverworts; more so, than previously thought. It is sometimes assumed that because these plants are clonal, and may even generate genetic variation via somatic mutation, that sexual reproduction may not be as “important” to bryophytes as it is in seed plants. There is clearly a need to further assess the importance of sexual reproduction and asexual methods of generating genetic variation in liverwort populations.

The influence of sex in populations may also mean that sexual dimorphism is a result of sexual selection, in terms of numbers of successful matings that a genotype incurs. In dioecious thallose liverworts that produce asexual propagules, males may use asexual propagules to “move” within populations for access to mates. Better for males to invest in asexual reproduction and then sex, than ever to invest much in growth. Males may find refuges within

populations, such as low light areas, where they have a growth advantage, and produce asexual propagules that move to female-dominant patches. While near a female, they grow for a short period of time, produce gametangia and fertilize females before being overgrown by females. Many males produce cupules on their sex structures toward the end of the sex season – this would help them to escape overgrowth in a female-dominated patch. Additional investigations into gametangial initiation, gemmae dispersal, and growth in occupied patches is needed to corroborate these hypotheses.

My studies of sex-specific natural selection in nature and on sexual versus asexual fitness are unique to the bryophyte literature. Additionally, because the study organism was clonal, I was able to investigate genotypic natural selection to detect both sex-specific and environment-dependent selection. These experiments contribute to the literature on the maintenance of sexual dimorphism because they are among the first attempts to provide empirical evidence for theories regarding the maintenance of sexually dimorphic characters via selection. In relation to detecting environment-dependent selection, I investigated sexual dimorphism in plasticity of characters and constraints to the evolution of sexual dimorphism in trait plasticity. This is especially applicable to bryophytes because they are notoriously plastic but there are no investigations on selection on plasticity in bryophytes. Overall, my research resulted in a complex picture of the maintenance of sexual dimorphism and population differentiation shed light onto the evolutionary mechanisms promoting genetic divergence within and among populations and the persistence of both sexes in liverwort populations

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Wolfe, L. M., AND A. Schmida. 1997. The ecology of sex expression in a gynodioecious Israeli desert shrub (*Ochradenus baccatus)*. *Ecology* 78: 101-110.
Worley, A. C., AND L. D. Harder. 1996. Size-dependent resource allocation and costs of reproduction in *Pinguicula vulgaris* (Lentibulariaceae). *Journal of Ecology* 84: 195-206.
Wyatt, R. 1977. Spatial pattern and gamete dispersal distances in *Atrichum angustatum*, a dioicous moss. *The Bryologist* 80: 284-291.
_____. 1994. Population genetics of bryophytes in relation to their reproductive biology. *Journal of the Hattori Botanical Laboratory* 76: 147-157.

Vita

Linda Fuselier

Born: New Orleans, LA, USA 19 Oct 1965

EDUCATION

M.S., Environmental Biology. Emporia State University, Emporia, KS. 1993. Thesis title: Habitat restoration and seasonal habitat use by Neosho madtoms (*Noturus placidus*), and seasonal variation of fish assemblages in the Cottonwood River, Kansas. Major professor: Dr. David R. Edds

B.A., Biology, with Secondary Teacher Certification. Southwestern College, Winfield, KS. 1987. (Cum laude)

TEACHING EXPERIENCE

Advanced Ecology Lab Teaching Assistant, University of Kentucky. (2003)

Lecturer, University of Kentucky. (2002)

Undergraduate Research mentor, University of Kentucky, (2001-2002)

Undergraduate Research mentor, Academy of Natural Sciences in Philadelphia. (1999)

Ecology Recitation Teaching Assistant, University of Kentucky. (2000)

Adjunct Professor, Murray State College, Tishomingo, OK (1993-1994)

Naturalist, Kansas Department of Wildlife and Parks. (1992)

General Biology Lab Instructor, Emporia State University and University of Kansas (1991-1992, 1994)

High School Biology Teacher, Unified School District #430, South Brown County, Kansas. (1988-1991)

Substitute Teacher, Kansas school districts. (1987)

RESEARCH EXPERIENCE

Dissertation research, University of Kentucky (1999-2003)

Fellowship Research through University of Kansas and National Security Education Program fellowship (1997)

Fisheries Scientist II, Academy of Natural Sciences in Philadelphia, Patrick Center for Environmental Research. (Jan 1998 – Jul 1999)

Stream Fisheries Biologist, Kansas Department of Wildlife and Parks (Jan 1995 - Oct 1996)
Master's thesis research, Emporia State University (1991-1993)

Field Technician, Nature Conservancy (1993)

Research Assistant, University of Oklahoma Biological Station (1993)
Graduate Research Assistant Emporia State University (1991)

Field Technician, Flint Hills National Wildlife Refuge (1990)

Research Technician, Voyageur's National Park (1989)

Undergraduate Research Assistant, Iowa State University dairy farm (1983-1985)

PUBLICATIONS

Fuselier, L. and D. N. McLetchie 2002. Maintenance of sexually dimorphic pre-adult traits in *Marchantia inflexa* (Marchantiacae). American Journal of Botany 89:592-601.

Fuselier, L.C. 2001. Impacts of *Oreochromis mossambicus* (Perciformes: Cichlidae) upon habitat segregation among Cyprinodontids (Cyprinodontiformes) of a species flock in Mexico. Revista de Biologia Tropical 49: 647-656

W.C. Hession, T.E. Johnson, D.F. Charles, D.D. Hart, R.J. Horwitz, D.A. Kreeger, J.E. Pizzuto, D.J. Velinsky, J.D. Newbold, C. Cianfrani, T. Clason, A.M. Compton, N. Coulter, L. Fuselier, B.D. Marshall, J. Reed. 1999. Ecological benefits of riparian reforestation in urban watersheds: study design and preliminary results. Environmental Monitoring and Assessment. 63: 211-222

Heckert, Megan, Linda Fuselier and R.J. Horwitz. 1999. Habitat use by *Fundulus heteroclitus* and *F.diaphanus* and effects of species co-occurrence. J. Penn. Acad. Sci. 73:22-26
Fuselier, L. and D. R. Edds, 1997. Seasonal variation in pool and riffle fish assemblages in a mitigated reach of a midwestern USA stream. Southwestern Naturalist 41:229-306.

Fuselier, L. and D. R. Edds, 1994. An artificial riffle as fish habitat restoration for a threatened madtom. North American Journal of Fisheries Management 15:499-503.

Fuselier, L. and D. R. Edds, 1994. Seasonal variation in habitat use by the Neosho madtom (Teleostei: Ictaluridae: *Noturus placidus*). Southwestern Naturalist 39:217-223.

Fuselier, L. and D. R. Edds, 1994. Habitat partitioning among three species of map turtles, genus *Graptemys* (Testudines, Emydidae). Journal of Herpetology 28:154-158.

Fuselier, L. and D. R. Edds, 1992. *Phoxinus erythrogaster* Cypriniformes: Cyprinidae) range extension in Kansas. Kansas Academy of Science 96:227-228.

Fuselier, L. and C. Mammoliti. 1996. County Records for Fishes in the Neosho River Basin in Kansas. Transactions of the Kansas Academy of Sciences 99:157-160.

Wilkinson, Christopher and L. Fuselier. 1997. Neosho madtoms (*Noturus placidus*) in the South fork of the Cottonwood River: Implications for Management of the species. Transactions of the Kansas Academy of Sciences 100:162-165.

TECHNICAL REPORTS

Fuselier, L. 1998. Fisheries and aquatic habitat section on benefits of tidal restoration in a Delaware marsh *in* Status and ecological benefits associated with the restoration of the Augustine Creek Impoundment, CH2MHILL report, 19 pp.

Fuselier, L. 1998. Fish section *in* ANSP 1997 Savannah River biological surveys for Westinghouse Savannah River Company. Rept. No. 98-10F. Acad. Nat. Sci. Phila.

Fuselier, L., 1998. Fish section *in* ANSP Aquatic field studies in the Congaree River near Columbia, South Carolina, 1997. Rept. No. 98-4F. Acad. Nat. Sci. Phila. 144 pp.

Fuselier, L., 1998. Fish section *in* ANSP. Biological and chemical studies of the Guadalupe River, 1996-1997. Rept. No. 98-9D. Acad. Nat. Sci. Phila. 208 pp.

Fuselier, Linda and David Edds. 1993. Neosho Madtom movements and seasonal habitat use, Final Report to Kansas Department of Wildlife and Parks, November, Pratt, Kansas.

Fuselier, Linda and David Edds. 1993. Neosho Madtom Colonization of Artificial Riffle Habitat, final Report to Kansas Department of Wildlife and Parks, November, Pratt, Kansas.

Fuselier, Linda. 1996. Neosho River Basin Monitoring Program. Report submitted to Kansas Department of Wildlife and Parks, December, Pratt, Kansas.

GRANTS RECEIVED

Dissertation Year Fellowship, 2003-2004, $16,000
Sigma Delta Epsilon, Graduate Women in Science research award, 2002, $900
Kentucky Academy of Science, Botany Fund Award, 2002, $1100
Dissertation Enhancement Award, 2001, $2000
Torrey Botanical Society 2001, $1000
Ribble research grant 2000, $500
2001, $500
2002, $800
Kuehne Award 2000, $400
2001, $200,
Norcross Wildlife Foundation 1999, $10,000
Heritage Conservancy demonstration grant for restoration of tidal wetland 1998, $2500
National Security Education Program graduate fellowship 1997, $6400
Tinker Foundation Grant for study in Latin America 1997, $800
1996, $650
Explorer's Club Grant for scientific exploration and travel 1997, $1150
Panorama Grant, University of Kansas Natural History Museum 1996, $551
Kansas Department of Wildlife and Parks 1991-1992, $1995
Kansas Department of Wildlife and Parks, 1992-1993, $13,750
US Fish and Wildlife Service 1991, $2438
1992, $1977
US Fish and Wildlife Service, Wildlife Extension Agreement 1992, $4310

SCHOLARSHIPS AND AWARDS

Botanical Society of America, honorable mention for student paper (2001, 2002)
Graduate Student Development Award (2000)
Ribble Fellowship for research funding (2000, 2001, 2002)
Ida Hyde Scholarship for Women in Science (1996)
Best Paper Presentation, Kansas Chapter of American Fisheries Society (1993, 1996)
Outstanding Thesis in Biological Sciences, (1993)
Phi Kappa Phi Honor Society membership (1992)

Skinner Award, American Fisheries Society (1992)
Boylan Outstanding Graduate Scholar Award (1992)
Outstanding Fisheries Student, Kansas Chapter American Fisheries Society (1992)
Newbold Graduate Fund, Zavos Graduate Fellowship in Biology, (1991)
Academic Achievement Assistantship, (1991)
Presidential Scholarship, Southwestern College (1986-1987)
Academic Achievement Scholarship, Iowa State University (1983)

PAPERS PRESENTED

Fuselier, L and D. N. McLetchie. Habitat use by the sexes of a dioecious liverwort. Botanical Society of America, 2003.

Crowley, P., H. Davis, A. Ensminger, L. Fuselier, J. K. Jackson, K. N. Sudler, and D. N. McLetchie. Simple and complex models of overgrowth competition. International Clonal Workshop, 2003

Fuselier, L. Growth and reproduction of *Marchantia inflexa* from single and both-sex populations. American Bryological and Lichenological Society, 2002.

Fuselier L. and N. McLetchie. Maintenance of sexually dimorphic pre-adult traits in a thallose liverwort. Botanical Society of America, 2001

Fuselier, L., A. Echelle, R. A. Van den Busch, C. Rodriguez, and M. L. Smith. Phylogeography of *Cyprinodon* on Hispaniola. American Society of Ichthyologists and Herpetologists (ASIH), 2001

Fuselier, L. Effects of introduced fishes upon a species flock in Laguna Chichancanab, Yucatan. ASIH, 1997

Fuselier, L. Changes in Fish Community Structure and Distribution in the Neosho River Basin in Kansas. ASIH,1996

Fuselier, L. Changes in Fish Community Structure and Distribution in the Neosho River Basin in Kansas. Kansas Academy of Science, 1997

Fuselier, L. Biotic integrity of Neosho River Basin tributary streams in Kansas. Kansas AFS, 1996

Fuselier, L. Habitat, behavior and morphology; segregation of *Etheostoma spectabile* and *E. radiosum* in an Oklahoma stream. Southwestern Association of Naturalists,1995

Fuselier, L. and D. Edds. An artificial riffle as fish habitat restoration. Midwest Fish and Wildlife Conference, 1995

Fuselier, L. and D. Edds. Seasonal variation in pool and riffle fish assemblages in a mitigated stream reach of the Cottonwood River. Southwestern Association of Naturalists, 1994

Fuselier, L. and D. Edds. An artificial riffle as mitigation of habitat loss for a threatened madtom, *Noturus placidus*. Kansas AFS, 1993

Fuselier, L. and D. Edds. Habitat partitioning among three species of map turtles, genus *Graptemys*. Southwestern Association of Naturalists,1993

Fuselier, L. and D. Edds. Seasonal and Spatial variation in fish assemblages in the Cottonwood River, Kansas. Oklahoma Academy of Sciences, Great Plains Limnology Conference,1993

Fuselier L. and D. Edds. Niche overlap comparisons among three species of map turtles in Kansas. Kansas Academy of Sciences, Southwestern Association of Naturalists, & Kansas Herpetological Society, 1992

INVITED LECTURES AND SYMPOSIA

American Society of Ichthyologists and Herpetologists, pupfish evolution symposium, 2001.
Botanical Society of America & American Bryological & Lichenological Society, symposium on evolutionary constraints, 2001.
Introductory Ecology lecture at UK, 2000.
University of Kentucky, ecology seminar 1999.

Kansas Wildlife Society Conclave, 1996.
Environmental Assessment and Ecology courses at KU, 1995-1996.

UNIVERSITY AND COMMUNITY SERVICE

Search committee for Dean of Libraries at University of Kentucky, 2002
Electronic theses and dissertations committee, University of Kentucky, 2002
GEM-SET – Girls E-mentoring in science, engineering and technology, 2001-2002
Women in Natural Sciences group University of Kentucky. 1999-2001
English as a second language tutor, Literacy of America, volunteer (1998)
Women's Programming Board, Emporia State University, 1991-1993;
Homebound students – tutored homebound, pregnant teens 1988-1990

PROFESSIONAL MEMBERSHIPS

Botanical Society of America
American Bryological and Lichenological Society
Sigma Delta Epsilon (women in science)
National Science Teachers Association

EDUCATION-RELATED TRAVEL

Trinidad and Tobago; field studies of thallose liverwort, 2001
Dominican Republic; collecting and field experiments, 1999
Yucatán, México; research at Mexican research institute, 1997
México, faculty sponsor on ecology field trips, 1993, 1994
San Salvador Biological Station, Bahamas, coral reef and tropical ecology course, 1993
Peru, Amazon basin, tropical field ecology, Pittsburg State University, 1992