Chapter

BRYOPHYTES: SURVIVAL IN A DRY WORLD THROUGH TOLERANCE AND AVOIDANCE

Dale H. Vitt, Barbara Crandall-Stotler, and Andrew Wood*
Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901, USA

ABSTRACT

Drought provides a major stress in the lives of many plants, especially those with limited abilities to retain water for long periods of time for physiological processes. Mosses, liverworts, and hornworts are among plants that are poikilohydric, yet many species within these three lineages occupy drought-prone habitats and in fact can occur in some of the harshest conditions on the planet. Their success in establishing and occupying these habitats is largely due to their physiological tolerance to desiccation, whereby individuals survive complete loss of free water. Additionally, some species have a number of morphological adaptations or life history features that enhance their abilities to withstand dehydration. We argue here that many members of both moss and liverwort lineages are desiccation tolerant, allowing them to survive in periodically dry habitats, and that in many of the moss lineages this physiological desiccation tolerance is remarkably high. However, in other lineages with less tolerance to desiccation, morphological or life history adaptations are present that also allow survival under periodic drought. These avoidance strategies are somewhat different in mosses than in liverworts and hornworts.

INTRODUCTION

Drought is an abiotic environmental situation that occurs when potential evapotranspiration is greater than incoming precipitation and is often associated with the loss of water from tissues and cells causing dehydration stress. Plants can avoid water deficit stress either through the evolution of a complex set of morphological (succulent growth forms) and physiological features (*e.g.,* C_4 and CAM photosynthetic pathways and anatomy) or by temporally escaping drought through shortened life cycles. Another strategy for avoiding water deficit stress is

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∗ Corresponding author Email: dvitt@siu.edu

physiological tolerance wherein plants have evolved the physiological abilities to survive cellular desiccation.

Mosses, with over 12,700 species (Magill, 2010), and liverworts, with about 7,500 species (von Konrat et al., 2010) form the second and fourth largest groups of land plants, respectively (ferns are third), and in the past have been considered (along with hornworts) to comprise a Division of plants called Bryophyta. Although many textbooks still incorrectly define bryophytes as non-vascular green plants, these three groups of plants are at best distantly related. These three groups form a grade of organization best defined by similarities in life cycle and ecological roles - wherein the sporophyte generation remains attached and is partially or wholly dependent on the photosynthetic gametophyte generation. These groups differ fundamentally in both gametophyte and sporophyte development and have few, if any, shared derived features (Crandall-Stotler, 1974; 1984). In mosses and most liverworts, the gametophyte has stems and leaves, and most species of mosses have at least some form of internal water conducting tissues, but these are usually poorly developed. In contrast, the gametophytes of all hornworts are fairly simple thalli.

Mosses and leafy liverworts have other features in common that apparently have evolved independently. Among these features are: 1) leaves only a single cell in thickness; 2) leaves that never have a petiole and are attached to the stem along a wide insertion; and 3) a lack of ability to retain water (poikilohydric) and cell turgor for an extended length of time (but a remarkable physiological ability to tolerate desiccation). This tolerance to desiccation can be illustrated by comparing an African violet to a moss, each growing on a window sill and left without water. The African violet remains photosynthetically active for an extended period of time – perhaps a week or two, avoiding the lack of water by retarding water loss by a thick impervious cuticle and by active water movement through well-developed water-conducting tissues from underground roots to photosynthetically active, aboveground stems and complex leaves that lose water only through transpiration from stomates. After some period of weeks the cells lose turgor and the plant shuts down physiologically, but when rewetted does not revive and subsequently dies. The moss, in contrast, also begins this trial photosynthetically active, and after a few hours dries out and becomes quiescent, but when rewetted becomes active once again. These two different strategies for survival, one of avoiding desiccation through various morphological traits and the second of tolerating desiccation by physiologically shutting down cell functions, allow vascular plants and bryophytes to occupy and function in very different habitats and ecological situations. Although most mosses and liverworts are desiccation tolerant to some degree, they also avoid drought through a number of morphological avoidance mechanisms.

DESICCATION TOLERANCE—A PRIMER

Strategies of Water Economy

As exemplified by our African violet and moss examples, land plants have evolved two major strategies for water economy: homoiohydry and poikilohydry. Homoiohydry is defined as "striving to maintain a high water potential under water limiting conditions" and poikilohydry as "the inability to control water loss to the environment" (Wood, 2005).

Homoiohydry is the dominant land plant strategy, and homoiohydric plants (such as the African violet) have evolved a variety of physiological and anatomical features that maintain a high internal water potential (Bewley, 1979; Nobel, 1983). Poikilohydry, on the other hand, is a defining feature of mosses, liverworts, and hornworts, and poikilohydric plants are unable to control water loss to the environment with the result that cellular water content fluctuates in concert with external water availability. Land plants are constantly losing water to the surrounding environment because the surrounding air is extremely "dry" relative to the plant. Some of the adaptations required for invasion of the land by aquatic plants would have required several adaptations including: 1) tolerance of vegetative tissues to desiccation; 2) reproductive and fertilization strategies in non-aqueous environments; 3) gas exchange across a liquid-air interface; and 4) enhanced ion and metabolite transport.

The vast majority of plants cannot survive equilibrium with dry air and will die upon complete drying; however, a number of plant species can survive complete drying (i.e., desiccation). Desiccation is the complete loss of "free" water from an organism that corresponds to <0.1 g H_2O g^{-1} dry mass (10% water content or less) (Alpert, 2005; Gaff, 1971). These "desiccation-tolerant" plants equilibrate with dry air (50% RH and 20° C) and resume normal metabolic function upon rehydration (Bewley, 1979; Proctor et al., 2007). Wood (2005) proposed that the successful land-invading plant would have been compact, poikilohydric, and desiccation tolerant. In addition, the first successful land-invading plant would have been able to tolerate high irradiation and temperature fluctuations.

Desiccation tolerance, or "drying without dying," is one of the most amazing phenomena in biology and has been the topic of a number of reviews (see Alpert, 2005; Bewley, 1979; Oliver et al., 2005; Proctor et al., 2007; Wood, 2007) and books (Black & Pritchard, 2002; Jenks & Wood, 2007). Over the past 30 years of research, several general concepts have emerged: 1) desiccation tolerance is observed in each of the three domains of life (Archaea, Bacteria, and Eukarya); 2) desiccation tolerance is often associated with tolerance to other abiotic stresses; 3) in many plants and animals, desiccation tolerance is limited to specialized structures such as cysts, tubers, spores, or seeds; 4) desiccation tolerant reproductive structures are found within the mosses, liverworts, pteridophytes, gymnosperms, and angiosperms; 5) in many plants and animals, vegetative tissues (*i.e.*, non-specialized structures) are desiccation tolerant; and 6) vegetative desiccation tolerance is a common feature of mosses and liverworts.

DISTRIBUTION OF DESICCATION TOLERANCE

Desiccation tolerance within vegetative tissues is widely distributed in land plants. Approximately 320 species of vascular plants (less than 0.15% of the total) possess vegetative desiccation tolerance (Porembski & Barthlott, 2000). They reside within nine pteridophyte families (Adiantaceae, Aspleniaceae, Davalliaceae, Grammitidaceae, Hymenophyllaceae, Isoëtaceae, Polypodiaceae, Schizaeaceae, and Selaginellaceae) and ten angiosperm families (Acanthaceae, Cactaceae, Cyperaceae, Gesneriaceae, Labiatae, Liliaceae, Myrothamnaceae, Poaceae, Scrophulariaceae, and Velloziaceae). Interestingly, vegetative desiccation tolerance is undocumented in the gymnosperms. Of the approximately 21,000 species of mosses, liverworts, and hornworts, the majority are postulated to be desiccation tolerant and able to survive brief desiccation of modest intensity (Proctor & Pence, 2002; Proctor et al., 2007;

Wood, 2007). Two hundred and ten bryophyte species (approximately 1% of the total) have been experimentally determined to be desiccation tolerant (Wood, 2007) and have been identified within four of the eight classes of mosses (Andreaeopsida, Bryopsida, Polytrichopsida, and Tetraphidopsida), two of the three classes of liverworts (Marchantiopsida and Jungermanniopsida), and one of the two classes of hornworts (Anthocerotopsida).

Experimental Determination of Desiccation Tolerance

The ability of vegetative tissues to revive from the air-dried state is a common characteristic of mosses (Proctor et al., 2007); however, the speed and intensity of the drying event is critical. Oliver & Bewley (1997) proposed that "fully desiccation tolerant" species can survive extremely rapid water loss, and that "modified desiccation tolerant" species can only survive slow water loss. The vast majority of desiccation tolerant bryophytes are "fully" tolerant while the vast majority of desiccation tolerant vascular plants are "modified" tolerant. Stable drying environments over a wide range of relative humidity (RH) can be created within an enclosed space using known solutions. Wood (2007) proposed five categories of desiccation tolerant bryophytes. Category 1 is the majority of documented desiccation tolerant species and they can survive equilibrium with extremely dry air (*i.e.*, 0–30% RH or less than -162 MPa). Category 2 species can survive equilibrium with moderately dry air (i.e., 70–80% RH or -30 to -48 MPa). Category 3 species can survive equilibrium with 70–80% RH, die at 0–30% RH, but will survive severe water deficit stress if the gametophores are ''hardened'' by either slow drying or exposure to a mild desiccation pre-treatment (*i.e.*, 96% RH for 24 h). Category 4 can survive 0-30% RH and Category 5 can survive 70-80% RH only if the gametophores are hardened.

The 'Austin Protocol' has been proposed as a simple and standardized procedure for determining vegetative desiccation tolerance (Wood, 2007). This protocol evaluates the modulated chlorophyll fluorescence in plant tissues equilibrated at two relative humidity set points (*i.e.*, 67% RH and 23% RH). Mosses and liverworts that recover from equilibration at 67% RH are classified as "modified" desiccation tolerant and those that do not recover are classified as desiccation sensitive. Species able to recover from equilibration at 23% RH are classified as "fully" desiccation tolerant. Based upon this system of classification, essentially every desiccation tolerant species in the Andreaeales, Bryales, Dicranales, Grimmiales, Hookeriales, Hypnales, Orthotrichales, Polytrichales, and Pottiales are either Category 1, 2, or 3. This means that (with few notable exceptions) all desiccation tolerant mosses can survive equilibration with 23% RH (either with or without pretreatment) and are fully desiccation tolerant. Similar results are seen in the liverworts. Every documented desiccation tolerant species in the Marchantiopsida and most of the species in the Jungermanniopsida can survive equilibration with 23% RH (either with or without pretreatment).

MOSSES – A PRIMER

Mosses, Division Bryophyta, seemingly appear in the fossil record relatively late, with the earliest confirmed fossils from the Upper Carboniferous (Krassilov & Schuster, 1984). By

Cretaceous time, many of the modern families and genera were present, and by early Tertiary, even some modern species seem to have fossil occurrences.

Moss Habitats

Mosses occur in all of the major biomes of the world. They dominate the ground layer in the boreal forest and are foundational species of northern peatlands. They are among the most abundant epiphytes in higher elevation tropical rainforests and with liverworts and algae form soil crusts in prairies and deserts. In alpine and montane areas they may form complete cover on boulders and dominate many cliff faces, ledges, and rock falls. In temperate and oceanic regions, they form conspicuous carpets on the forest floor and are epiphytic on tree trunks. Their canopies have a rich internal structure and are the homes for numerous insects, rotifers, tardigrades, and other invertebrates. Only a few species are aquatic and few have any ability to tolerate salt. If one were to place moss species onto Grime's triangle (Grime, 1988) of ecological strategies, they would largely fall either as ruderals or as stress tolerators, and almost none would be classified as competitors (Grime et al., 1990). Mosses occupy many relatively harsh habitats that periodically are subject to drought, including habitats that are present in early succession, where desiccation is the key limiting factor for most species.

Moss Phylogeny

Currently there are a number of robust molecular-based reconstructions of moss phylogeny at the family rank and above (Cox et al., 2010). Morphologically, the major lineages of mosses are defined by features of the peristome (Vitt, 1984). The phylogeny as presently constructed shows eight major lineages (Figure 1) along with several more isolated ones with only a few taxa. Lineage 1 (Sphagnales) - contains the genus *Sphagnum*, with many unique sporophytic characters and gametophores having complex leaves of dead hyaline cells and living green cells. There is some evidence that although *Sphagnum* as a genus may have evolved quite early compared to other moss lineages, its diversification occurred only in the Miocene (Shaw et al., 2010). Much of the diversity of *Sphagnum* is in tropical and temperate regions where it occurs overhanging on cliff faces and forming mats on moist soil, but a small number of species have evolved decomposition-resistant gametophores and are a major component of peat and foundation species of northern peatlands. Lineage 2 (Andreaeales) - contains the rock mosses with only two genera that are highly desiccation tolerant and occur only on periodically dry rock surfaces in arctic and alpine areas of the world. It is in this early lineage that desiccation tolerance is clearly evident. Lineage 3 (Polytrichales) - contains the structurally complex Polytrichaceous mosses, with well-developed water and food conducting systems, complex multi-layered leaves with cuticle and photosynthetic lamellae (resembling the palisade layer of vascular plant leaves) and underground rhizoidal mats.
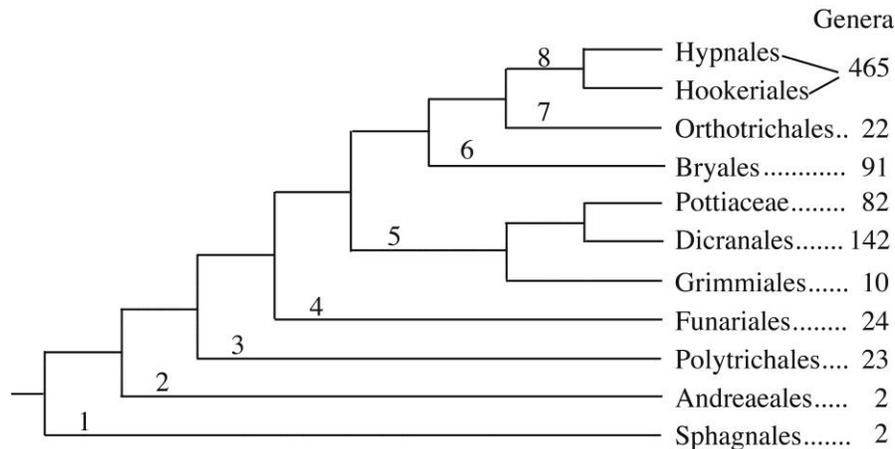
Figure 1. The major lineages (as Orders) of the mosses (Division Bryophyta) summarized from Cox et al. (2010). Numbers above the branches refer to the lineage numbers discussed in the text; 1 = mosses with nematodontous peristomes; 4 = mosses with diplolepideous opposite peristomes; 5 = mosses with haplolepideous peristomes; 6-8 = mosses with diplolepideous alternate peristomes (after Vitt, 1984). Numbers to right are approximate number of genera based largely on Cox et al. (2010).

The species occur on soil, occasionally on organic matter over rocks, and include the largest of the mosses (*Dawsonia*), reaching 1m in height.

Lineage 4 (Funariales) -contains genera related to *Funaria*, a lineage morphologically defined by peristome features (arthrodontous, diplolepideous with opposite endostome and exostome) (Edwards, 1984). The species occur on moist soil and organic matter, and have large, thin-walled cells, a strong costa, and are acrocarpous (producing perichaetia terminal on stems). Epiphytic and rock substrates are not colonized by species in this lineage and tolerance to desiccation is limited.

Lineage 5 (Grimmiales, Dicranales, Pottiaceae) - contains the Dicranoids, a large assemblage of genera all with a unique peristome (arthrodontous, haplolepideous), acrocarpous placement of archegonia, and mostly sympodial growth (erect and with few branches). This is the first lineage that contains species able to occupy an epiphytic habitat, although most species occur on mineral soil or on organic soil over rocks.

Lineage 6 (The Bryales) – This lineage has acrocarpous plants with a diplolepideous, ciliate peristome with endostome segments positioned alternate to exostome teeth (Vitt et al., 1998). The species are specialized to habitats of moist shaded soils, stream sides, and disturbed soils. Although a few species are epiphytes, these are rare.

Lineage 7 (Orthotrichales) – This is a lineage with peristome features similar to the Hypnales and Bryales, but with no endostomial cilia. These genera have species highly specialized for epiphytic habitats, especially in tropical forest canopies.

Lineage 8 (Hypnales, Hookerales) – Defined by arthrodontous, diplolepideous, ciliate, and alternate peristomes, these are pleurocarpous (with lateral placement of archegonia), have complex branched gametophytes, many with differentiated stems and branches, and occupy a wide range of habitats. In particular they seemingly are highly adapted for epiphytism and competitive forest floor habitats. One specialized lineage within the group, the Hookeriales, has apparently lost much of their desiccation tolerance and occupy shaded, mesic habitats that rarely if ever experience drought.

MOSSES – AVOIDANCE ENHANCEMENTS TO DESICCATION TOLERANCE

The evolution of increased physiological tolerance to desiccation is paramount to mosses being able to occupy drier and drier habitats.
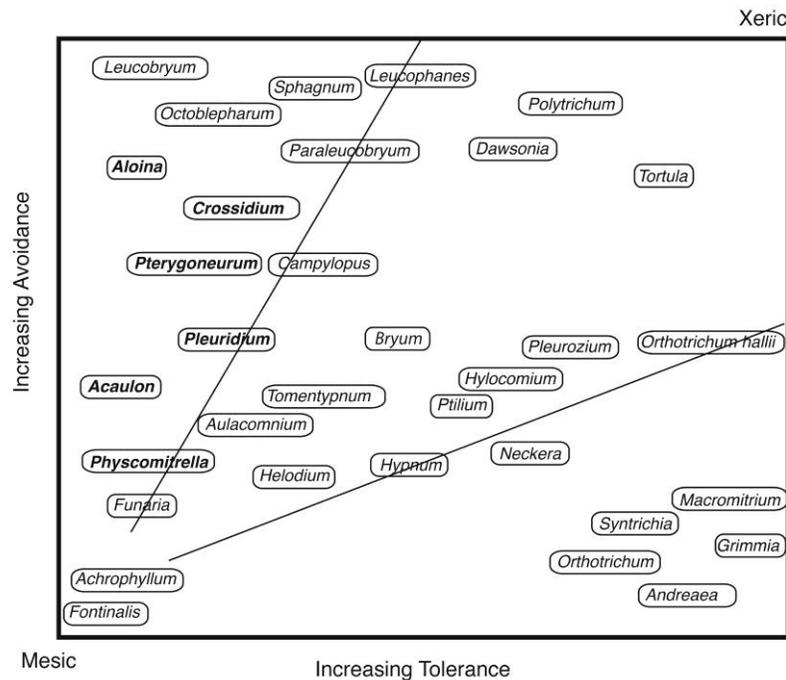

Figure 2. Conceptual model showing the distribution of select moss genera along a dryness gradient (mesic to xeric). Oblique lines delineate major strategies from largely avoidance (upper left) to largely tolerance (lower right). Boldface names are genera with escape strategies.

Additionally, mosses have enhanced their physiological tolerance with a suite of morphological or life history features. These morphological adaptations increase the ability of some species of mosses to occupy even more desiccation prone habitats. In most cases, specific avoidance features have evolved convergently in two or more lineages. In agreement with Oliver et al. (2000) we argue here that physiological tolerance to desiccation was to some extent present in the ancient moss lineage from the beginning. Adaptation to drought advanced either through increasing physiological desiccation tolerance or through morphological and life history changes leading to the ability to avoid desiccation. Conceptually, many moss lineages have increased their physiological tolerance to drought, but also have developed morphological avoidance mechanisms that enhance habitat availability (Figure 2). Surprisingly, few mosses are truly aquatic and, as expected, those that are have little tolerance to drought, but even the aquatic *Fontinalis* (of streams and pools) can be induced if slowly dehydrated (Cruz de Carvahlo et al., 2011; 2012; 2014). A few species occur in deeply shaded, wet habitats such as seeps, beneath overhanging vegetation, and on exposed tree roots. These species also have little or no drought tolerance and occur sporadically through the moss lineages. Especially

noteworthy are species in the Hookeriales that are characterized by flattened leaf arrangements and large thin-walled leaf cells (*Achrophyllum, Distichophyllum,* and *Hookeria*).

## Protection of Photosynthetically Active Leaf Cells

At least three distinct lineages have leaves in which enlarged, dead, porose hyaline cells (often termed leucocysts) partially or completely encase smaller, photosynthetically active (green) cells (often termed chlorocysts). In *Sphagnum*, the leaves are one cell layer thick with large hyaline cells that alternate with, and partially or completely surround, the smaller green cells (Figure 3).

The green cells are a variety of shapes (in transverse section), ranging from circular, trapezoidal, to triangular. Most species have green cells exposed on one side of the leaf, which may facilitate gas exchange as the surface is exposed to air rather than water of the adjacent hyaline cells.

In *Leucobryum*, small green cells are sandwiched between 2-3 (and up to 7) layers of dead porose hyaline cells (Figure 4). Cardot (1900) demonstrated that the structure of this multi-layered leaf is derived from modification of costal cells. In *Leucobryum* and related genera (*Cladopodanthus*, *Ochrobryum*, *Schistomitrion*, and *Terrestria*) the hyaline cells lie opposite to one another, with the green cells diamond-shaped. Like *Leucobryum* and related genera, *Paraleucobryum* has peristomial affinities with genera having a 'Dicranoid' peristome (long, bifid peristome teeth with vertical lines of ornamentation); however, the most recent molecular phylogeny of mosses (Cox et al., 2010) places *Paraleucobryum*, along with *Dicranum*, in a lineage separate from that of *Leucobryum* (which is sister to *Campylopus*, a genus with some species having a costa with some hyaline/green cell development as well as a distinct leaf lamina). *Paraleucobryum* has a very similar leaf structure to that of *Leucobryum* with green cells sandwiched between a dorsal and ventral layer of hyaline cells; however, in *Paraleucobryum*, the green cells are large, of similar size to the hyaline cells, and there are some green cells in the outer dorsal layer of cells; like *Leucobryum* the hyaline cells are opposite to one another and alternate to the diamond-shaped green cells (Figure 5).

In *Leucobryum* and *Paraleucobryum*, the multi-layered part of the leaf, consisting of a layer of green cells enveloped by two or more layers of hyaline cells, is always associated with a narrow, unistratose leaf lamina and appears to have been derived from costal elements. Compared to these genera is the situation in *Leucophanes* (Leucophanaceae, but closely related to the Calymperaceae) where small diamond-shaped green cells are sandwiched between a dorsal and ventral layer of large hyaline cells; however, in this case there is a well-developed costa with stereids (fibers) present as well (Figure 6) - thus here the multi-layered leaf is seemingly derived from leaf laminal origins. A number of genera, apparently related to the Calymperaceae (with short, single, stubby, papillose peristome teeth), have a superficially comparable leaf structure; among these are *Exodictyon*, *Exostratum*, *Arthrocormus*, and *Octoblepharum*.

These genera have 3-7 sided green cells sandwiched between multiple (up to 8-9) layers of hyaline cells and no costa or unistratose lamina (Figure 7). *Exodictyon* also has green cells on both outer leaf surfaces in addition to a central layer. *Octoblepharum albidum* is a very common tropical moss that occurs at the bases of palm trunks in coconut plantations, an extremely dry and exposed habitat.
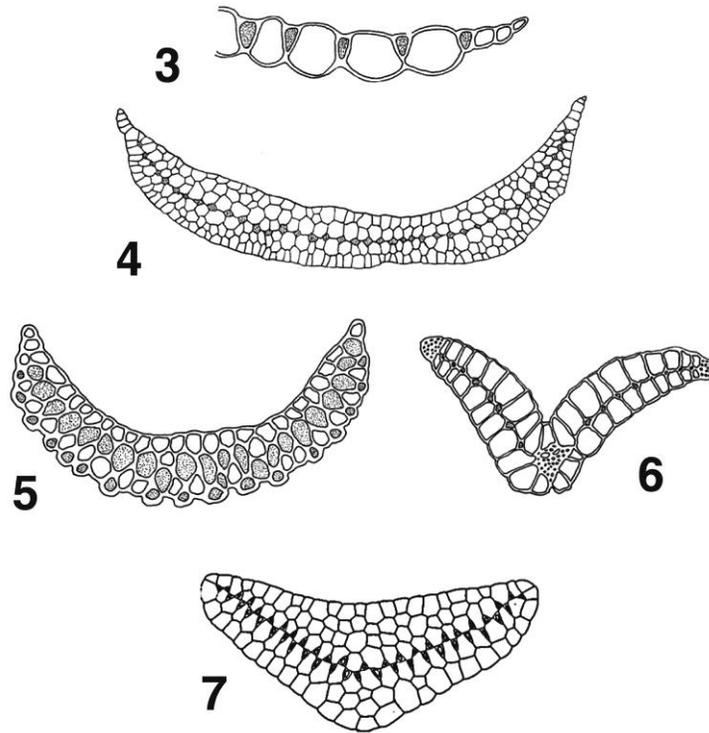
Figures 3-7. Transverse sections through mid-portions of leaves from genera with dead hyaline cells protecting living green cells (stippled). 3. *Sphagnum*; 4. *Leucobryum*; 5. *Paraleucobryum*; 6. *Leucophanes*; 7. *Octoblepharum*.

In addition to these genera with multistratose leaves, a large number of mostly tropical moss genera have single-layered leaves with photosynthetically active green cells in the distal portion and dead porose hyaline cells (in one layer) in the proximal portion (*e.g*., *Syrrhopodon*, *Leucophanella*, and *Mitthyridium*). It appears that porose hyaline cells, in many cases surrounding active living green cells, have appeared in a number of distinct lineages, serving to extend the activity of a reduced number of photosynthetically active cells for a longer period of time under drought conditions.

Complex Canopies

Mosses, like vascular plants, have variable canopies of leaves, branches, and stems, all of these important in controlling water balance and gas exchange; especially important are surface roughness and stem density and position (Rice & Schneider, 2004; Rice et al., 2001). Mosses found in the earlier moss lineages are acrocarpic (perichaetia terminal on main stems) and have sympodial branching (thus after a fertilization event growth of the gametophore stops with a new stem arising from beneath the terminal perichaetium). This situation severely limits branching frequency, and hence complex canopy development is often not well-developed in these species (but see LaFarge-England, 1996 for a detailed discussion). Pleurocarpy (perichaetia formed from lateral buds) is a foundational feature of the crown lineage of mosses (the Hypnales) and it is in pleurocarpous mosses that monopodial branching (branching from

lateral buds) along with lateral perichaetia that complex branching patterns can provide protection from evaporative stress. Additionally, complex canopy development allows for the expansion of colonies in both horizontal and vertical directions providing a competitive advantage. If one envisions a boreal forest floor, dominated by *Hylocomium splendens* (pleurocarpic sympodial), *Pleurozium schreberi* (pleurocarpic monopodial), and *Ptilium crista-castrensis* (pleurocarpic monopodial), the daily routine is for the moss canopy to be moist and functioning in early morning owing to dew formation; however, by mid-day on a sunny day the moss canopy is evaporating moisture and the mosses rapidly become non-functional. The amount of time on a daily basis that the moss is hydrated and photosynthetically active is strongly correlated to the growth rate and to carbon fixation. Thus there is a strong advantage to the presence of three-dimensional complex canopies composed of richly branched plants where growth cannot only be vertical from the apical bud of the primary stem, but also continue for two or more years from lateral branches (Benscoter & Vitt, 2007).

Endohydric Water Conduction

Genera within the Polytrichales extend their tolerance to drought by having highly complex gametophores. More so than any other mosses these species have a well-developed cuticle, a complex leaf laminal structure and dense vertical lamellae on the adaxial surface of the leaf that increases photosynthetic ability, a differentiated underground rhizoidal mass, and an internal conducting system of dead water-conducting cells (hydroids) and living nutrient/food conducting cells (leptoids) that connect leaves to stems through leaf traces (unlike other mosses that may have a costa and stem conducting elements, but these do not connect through the stem cortex). When active, these species have leaves that are straight and bent 90 degrees to the stem, but as they desiccate, they twist and curl against the stems and on rewetting unfurl and are alive and well - thus they are clearly desiccation tolerant, but they extend their active period by a series of mechanisms that effectively move water from below ground to the above ground structures. Species in this lineage are among the largest of mosses, attaining nearly 3-10 decimeters in height. Smaller species (1-10 mm in height) are often not only reduced in stature, but have less well-developed conducting systems, fewer lamellae, and are more desiccation tolerant - these genera (*Atrichum*, *Psilopilum*) are among the most derived in the family lineage (Cox et al., 2010; Hyvonen et al., 2004).

Ectohydric Water Conduction

Many mosses occur emergent from wetland substrates. They are largely dependent on wicking water upward from a permanent supply, yet they occur well above the water level (Busby et al., 1978). Vitt & Glime (1984) called these mosses 'emergents.' Few if any of these species have efficient internal water conducting systems, and they rely on a set of external stem features that conduct water upward owing to evaporative pressure. For example, *Aulacomnium palustre*, some species of *Philonotis*, *Paludella squarrosa*, and *Tomentypnum* species have a dense mat of rhizoids that completely covers the stems. Some mosses have branched or filamentous, green, stem outgrowths called paraphyllia that in some species form a tomentum over the stems (*e.g*., *Helodium* spp., *Cratoneuron commutatum*, and some *Thuidium* species).

Species of *Sphagnum* have complex branching structure of hanging and spreading branches that provide numerous, small spaces for capillary movement.

Shortened Functional Life Cycle and Enhanced Photosynthetic Apparatus

One life history strategy widely used by mosses is to avoid drought by shortening the functional life cycle, thereby temporally escaping periods of drought. Annuals and species with even shorter, ephemeral life styles largely avoid periods of drought by completing the gametophytic life cycle phase (or by having short-lived gametophores) during a brief, moist growing season and weathering the dry season as a diaspore (or in some cases as protonema or in a desiccated state). The problem with this strategy is that photosynthetic activity is severely limited by length of the wet period. One strategy to remedy this problem is to increase the structural components in which photosynthesis takes place. Several mechanisms are seemingly present in mosses, including leaves with the adaxial costal surface having: 1) photosynthetic pads; 2) filaments; 3) lamellae; or 4) adaxial leaf laminal surface having convex cell walls. Shortened life cycles not only include modification to the gametophyte generation (Vitt, 1981), but also to the sporophyte generation. Such modifications are reduction in seta length resulting in capsules immersed in the vegetative leaves, capsule axis compressed to a globose shape, loss of peristome function, and lack of an operculum (cleistocarpy) that results in spores being deposited in the same place as the parent plant. Escape also occurs by shortening the complete life cycle to an ephemeral status (life cycle completed in a few weeks only) and is present in a number of moss families. Examples are species of *Physcomitrium*, *Physcomitrella patens*, *Aphanorregma* and some species of *Entosthodon* in the Funariaceae, and *Discelium nudum* and *Gigaspermum* in closely related families of the lineage. The Dicranoid lineage, species with tiny, bulbiform plants and cleistocarpous, immersed capsules, include *Pleuridium*, *Eccremidium*, *Pseudephemerum*, and *Cleistocarpidium* in the Ditrichaceae and *Bruchia* in the Bruchiaceae. The classic genus, *Ephemerum*, with tiny (less than one mm) high gametophores arising from a persistent protonema has in the past been placed near Funariaceae, but recent molecular data place these species in the Pottiaceae, in the Dicranoid lineage. This last family contains well over 15 genera with shortened life cycles and these genera are found in at least 2-3 separate lineages (Zander, 1993). Examples include *Acaulon* and the distantly related species of *Astomum* (currently placed with species in *Weissia*) and *Aschisma*.

Although these species with shortened life histories are evident in at least three ordinal lineages, only species in one has structural photosynthetic enhancements. Within the Pottiaceae, *Crossidium* has a costal pad of photosynthetic filaments (Figures 8-9), *Aloina* has not only filaments but the leaf laminae are inrolled over the filaments providing a pseudo multi-layered leaf (Figure 10), *Pterygoneurum* species have a costal pad with wavy lamellae (Figure 11), *Pseudocrossidium* in one lineage and *Stegonia*, some *Tortula* species (Figure 12) and *Microbryum* in another have costal pads with enlarged thin-walled photosynthetic cells. Finally *Plaubelia*, *Hyophila*, and *Timmiella* (Figure 13) have laminal cells with highly convex ventral walls - these apparently acting as lenses concentrating sunlight to the chloroplasts. Most of these genera with shortened life cycles differ from their close relatives in greatly reduced or lack of papillae on leaf cells and sporophytes that have lost any functional spore dispersal abilities. In most of these species, peristomes are lacking and have no organized capsule dehiscence (Vitt, 1981).
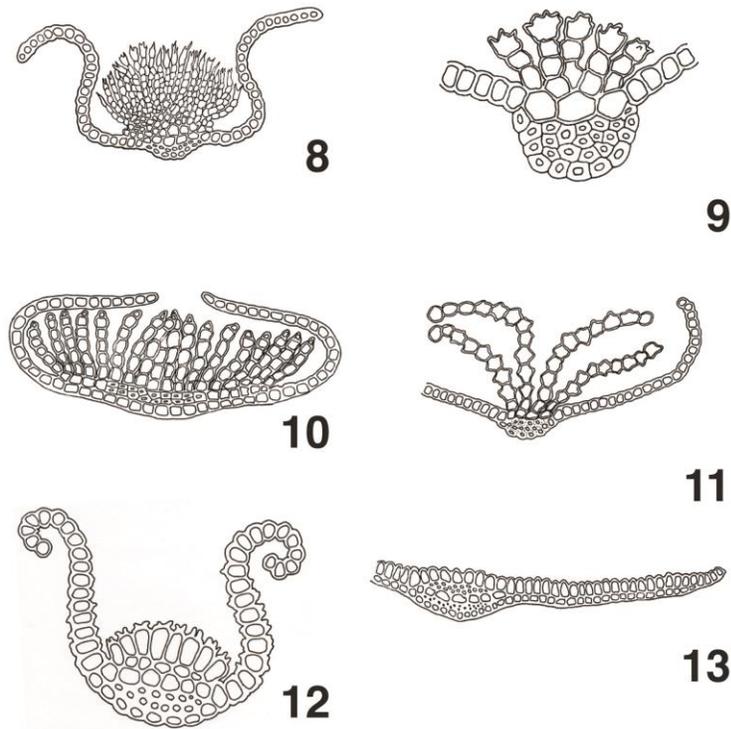
Figures 8-13. Transverse sections through the upper portion of leaves from species with an enhanced photosynthetic apparatus. 8. *Crossidium squamiferum*; 9. *Crossidium aberrans*; 10. *Aloina pilifera*; 11. *Pterygoneurum ovatum*; 12. *Tortula atrovirens (= Desmatodon convolutus)*; 13. *Timmiella anomala*.

Multi-Stratose Leaves

Scattered among genera inhabiting a variety of xeric habitats are a number of species that have photosynthetic leaf cells in 2-3 layers (or strata). These species occur in a number of independent lineages and usually as isolated species. For example, *Orthotrichum hallii* and *O. bolanderi*, both species growing on dry rocks, have bistratose leaves and are in separate lineages within the family (Vitt, 1971). *Syntrichia caninervis*, a desert soil species, has bistratose leaves, as does *Didymodon bistratosa*, a species of rock ledges. *Macromitrium*, a genus wherein many species occur within tropical forest canopies and on tree branches, has a number of species with bistratose leaves (*e.g.*, *M. tongense*). Species of *Grimmia* that occur exclusively on xeric, exposed rock faces have many species with bistratose leaves. Although there are no data on actual tolerances, the correlation between habitat and bistratose leaves suggests that this could be a method of extending the length of time that cells can be active by reducing the ratio of evaporative surface to cell volume.

Falcate-Secund Leaves

Occurring throughout the lineages of mosses are species that have all leaves curved to one side of the stem forming semi-circles. Most of these species have the leaves pointed downward towards their substrate – such as vertical tree trunks, horizontal log surfaces, or the forest floor (*e.g.*, *Hypnum*). As mosses desiccate, often on a daily basis, the retention of the last remaining water in an area of photosynthetic activity is of utmost importance as this allows a longer period of positive carbon gain. Falcate-secund leaves characteristically are also curved laterally and form a hollow cavity in the upper inside portion of the leaf, and it is here that water is retained longest.

Leaf Cell Structural Modifications

Almost all moss species are poikilohydric (lack of ability to retain water). They possess only a thin cuticle and although many have conducting elements in the stem and leaves these do not connect through the cortex of the stem; thus efficient internal water movement is lacking in most species. As a result of these inadequacies in being able to move or retain water for any length of time, mosses rely on being able to quickly absorb water from atmospheric sources, enable photosynthesis to rapidly initiate, and continue at a high rate (equal to that of vascular plants: Martin & Adamson, 2001) while the moss plants are hydrated. However, if the unistratose leaf cells are immersed in a water film, the exchange of gases is severely inhibited and if species are growing in sunny habitats leaf temperatures can become high. As a result, not only do moss leaves absorb and move water quickly, they must also provide cell surfaces exposed to the atmosphere and therefore dry out quickly in order to reduce cell temperatures after a wetting event (Proctor, 2009). Additionally, mosses are active at any temperature above 0°C when moist and carry out effective photosynthesis throughout the year, even under the melting snow pack in northern areas.

Mosses that occur in harsh environments such as rock faces, dry land soil crusts, and tree trunks and branches all appear to have a high level of desiccation tolerance, but also have a set of morphological attributes that are associated with these severe habitats. These species have thick cell walls; thus a higher amount of carbon is allocated to structural components than species of mesic habitats. They mostly have papillae - cell wall protuberances in a variety of forms, from mere finger-like bumps to branched, forked, or c- to u-shaped structures. The papillae may serve a variety of purposes. They may create capillary channels that effectively move water along the leaf surface allowing rewetting of the leaf to occur more quickly (Proctor, 1979). They are usually hollow and create an increased cell surface to volume ratio allowing water to be absorbed and lost more quickly, and they create an elevated surface for enhanced gas exchange. Species having papillae often have cells in the upper portion of leaves differentiated from those below - the upper ones photosynthetic with abundant chloroplasts and papillae; while the lower ones lack chloroplasts and are non-papillose, larger, thinner-walled, and clear -– perhaps with greater water retention abilities. In some cases these lower cells have openings (pores) in their walls (Proctor, 1979). Many species occurring in xeric habitats have leaves that are wide-spreading (exposing the adaxial surface to the atmosphere) when moist, but upon drying become crisped and twisted around the stems. This leaf movement is rapid, often occurring within minutes of wetting or drying (*e.g.*, *Syntrichia ruralis*). Although perhaps

related more to temperature than moisture, many xeric species have leaves ending in clear, long or short hair points, these apparently providing a protective layer from harsh, bright sunlight and/or reducing surface temperatures. Since any conducting tissues present in the costa (midrib) of the leaves do not connect to those in the stems, each leaf appears to act as an independent entity, photosynthesizing in its upper portion, transferring the photosynthate to the lower clear cells and storing it there, with no interactions with nearby leaves.

This is fundamentally different from the highly interactive situation in vascular plant leaves wherein turgor is maintained by a strong interconnected water connecting system and carbon is readily translocated. Whereas in vascular plants water maintenance is from the bottom-up throughout the entire plant, in these xeric mosses, water is obtained and transferred only locally from the top-down. It may be that the costa in these xeric species, which is almost always well-developed, serves as a strengthening organ rather than one that provides for water and/or photosynthate movement. When desiccation tolerant moss species dry out, the cell membrane of the individual cells shrinks and contracts away from the rigid, thick cell wall. The cell sap becomes concentrated in small spherical vesicles within each cell surrounded by an intact cell membrane, which is separated from the cell wall. This separation of cell membrane from cell wall can be seen not only in the xeric species of mosses (*e.g.*, *Orthotrichum*, *Ulota*, and *Grimmia*), but also in more mesic species (*e.g.*, *Mnium*, *Bryum*, *Fabronia*, and *Dicranum*).

Resource Allocation

Turetsky et al. (2008) have shown that, in *Sphagnum*, species living on hummocks allocate more of their carbon to structural components compared to species living in wetter lawns and carpets - these latter species allocating more carbon to soluble cell sap components. Thus lawn species have higher primary production in habitats that do not require highly structured canopies, but decompose more rapidly, while drier hummock habitats require more structural organization that leads to increased drought avoidance, but less production and less decomposition. This appears to be true for mosses in general.

The ability of species to tolerate desiccation not only is dependent on a species' physiological tolerance, but also on morphological enhancements. These enhancements require a trade-off in where carbon is allocated. Increased cell wall thickness, cell ornamentation, external structure for water movement, and water-storage cells all require allocation of carbon to non-photosynthetic processes; however, these increase the length of time a cell can be active. Living in drought-prone habitats is not without costs. Sexual allocation, sporophyte abortion, and vegetative propagation are all influenced by dehydration (Stark et al., 1998; 2000). In many cases, these reproduction traits and ecological strategies are strongly influenced by the rate of drying as well as variation in the number of drying events (Stark et al., 2013). Likewise periodic climatic changes can influence the survival abilities of species occurring in desert habitats (Reed et al., 2012).

LIVERWORTS – A PRIMER

Division Marchantiophyta appear much earlier in the fossil record than mosses, with *Metzgeriothallus sharonae* and *Pallaviciniites devonicus*, simple thalloid fossils that are morphologically referable to the Pelliidae, known from the Devonian. Molecular clock estimates of divergence times, which are constrained by fossil placeholders, suggest that the backbone lineages of the division were all established by the end of the Carboniferous and that most of the modern family lineages had diverged by the mid-Cretaceous (Cooper et al., 2012). Despite many lineages having a long evolutionary history, much of the extant species level diversity of liverworts appears to have arisen in the Cenozoic, particularly in the last 80 Myr (Heinrichs et al., 2007; Wilson et al., 2007).

Liverwort Habitats

Liverworts occur on all continents, and inhabit a wide variety of micro-niches from the Arctic to the Antarctic. While abundant in the temperate forests of the northern hemisphere, they are most diversified in the southern hemisphere, especially in the temperate rain forests of the Austral region and the montane, tropical cloud forests of the Andean chain. They often grow intermixed with mosses, along stream banks, on rock walls and bluff tops, on shaded soil on the forest floor or over decomposing logs, as epiphytes on forest trees, and even on or intermixed with *Sphagnum* in peatlands. In the tropical rain and cloud forests of the southern hemisphere, species of the Lejeuneaceae and Metzgeriidae form dense communities on the exposed, upper surface of leaves in the forest canopy, and other taxa from the speciose Lepidoziaceae, Plagiochilaceae, and Lophocoleaceae form thick mats on branches and tree trunks. Despite abundant moisture in these forest communities, such epiphyllous and epiphytic liverworts undergo repeated cycles of rapid drying and re-wetting (Proctor et al., 2007). Although many liverworts grow in moist, protected habitats, many other genera (*e.g*., *Riccia*, *Fossombronia*, and *Exormotheca*) have species that tolerate harsh, seasonally dry habitats, including the deserts of southwestern Africa; northern, central, and western Australia; and the Mediterranean climates of Europe and North America. In fact, in *Riccia* the range of habitat tolerances is extremely broad, ranging from the aquatic habitat of floating *R. fluitans* to the extremely dry, exposed bluff top habitat of *R. dictyospora*.

Field bryologists have long been aware of the vast diversity of moisture, light, and temperature regimes occupied by liverworts and have postulated that many liverworts possess some level of drought tolerance; *i.e*., they can tolerate some degree of drying and metabolic slow-down without dying (Campbell, 1904; Clausen, 1952; 1964). Like mosses, they are poikilohydric plants, in which cellular water potential is basically the same as their surroundings. Their ability to tolerate intermittent, as well as extended dry periods, involves two components: 1) reducing water loss to maintain metabolically suitable cell water content through physiological or structural adaptations; and 2) being physiologically desiccation tolerant, *i.e*., being able to recover cell function after nearly complete loss of free cell water (Alpert & Oliver, 2002; Proctor et al., 2007). As in mosses, how well a species can adapt to desiccation limits to a great extent the habitat in which it grows.

Liverwort Phylogeny

Liverwort gametophores are of three different body types, referred to as simple thalloid, complex thalloid, and leafy morphologies. Although traditional classification schemes suggested that these body forms represented natural groups, phylogenetic reconstructions based on nucleotide sequence data have consistently resolved five backbone lineages, with the most speciose lineage, the leafy liverworts, further divided into two major sub-groups (Forrest et al., 2006; Shaw et al., 2011). Thus, six major lineages are currently recognized (Figure 14).

Lineage 1 (Haplomitriopsida) - This lineage is the first divergence within liverworts, and as such, is also the first lineage of extant embryophytes. This is a small lineage, consisting of three genera in two subclasses, *Haplomitrium* (Haplomitriidae), *Treubia*, and *Apotreubia* (Treubiidae). All species of these genera grow on soil in constantly moist environments and are not tolerant to drought (Wood, 2007).

The gametophytes of *Haplomitrium* consist of erect, radially symmetric leafy shoots that arise from a branched, hyaline, subterranean stolon system, while *Treubia* and *Apotreubia* consist of little-branched, prostrate, lobed thalli. Both stolons and leafy shoots of *Haplomitrium* contain a small, central strand of thin-walled, hydrolyzed cells that are presumed to serve in water conduction and/or storage. In contrast to the hydroids of mosses, these cells bear numerous small wall perforations on both the terminal and lateral walls. All three taxa produce copious secretions of slime that coat the stolons in *Haplomitrium* and ventral midrib surface in *Treubia* and *Apotreubia*. These secretions, which are released through the epidermis, contain abundant insoluble carbohydrates and substantial amounts of water that likely protect the plants during brief periods of drying (Duckett et al., 2006). Since the secretions are more abundant at the growing tips of the stolons and thalli, when plants are exposed to drying, these tips may remain hydrated. Tests of tolerance in *Treubia lacunosa*, using the Austin Protocol (Wood, 2007) show, in fact, about 15% cell survival in shoot apices at 67% humidity level, but no survival or recovery at 23% RH (Crandall-Stotler & Wood, unpublished data).
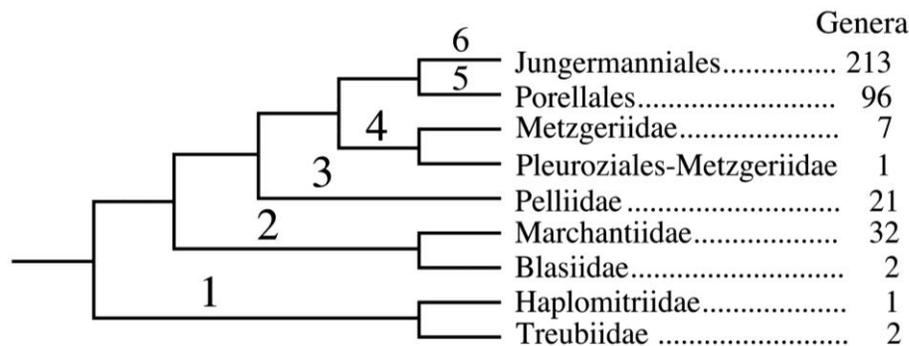

Figure 14. Phylogenetic reconstruction of the backbone lineages of liverworts (Division Marchantiophyta), based on the analyses of Forrest et al. (2006). Numbers above the branches refer to the lineage numbers discussed in the text; lineage 1 = Haplomitriopsida; 2 = Marchantiopsida; 3–6 = Jungermanniopsida. Estimates of generic diversity in each lineage are based on Crandall-Stotler et al. (2009).

Lineage 2 (Marchantiopsida) - This lineage consists of all complex thalloid genera (Marchantiidae) and two simple thalloids, *Blasia* and *Cavicularia* (Blasiidae). The

gametophytes of most members of the complex thalloid clade possess multistratose thalli, with air chambers and air pores, two or more rows of large ventral scales and both pegged and smooth rhizoids. Thalli in *Blasia* and *Cavicularia*, in contrast, are differentiated into a midrib and unistratose wing, are without air chambers or pegged rhizoids, and are unique among liverworts in bearing two rows of *Nostoc*-containing auricles along the ventral side of the midrib. To date, there is no evidence of drought tolerance in the Blasiidae, but several derived genera of complex thalloid liverworts (the Marchantiidae) have been confirmed to be drought and/or desiccation tolerant (Bischler, 1998; Wood, 2007). Among these are *Riccia*, *Corsinia*, *Cronisia*, *Exormotheca*, *Targionia*, and *Monocarpus*; all taxa with fairly small, thick thalli and short life cycles, as well as the much larger plants of the Aytoniaceae, such as *Mannia*, *Asterella*, and *Reboulia*. When one of these plants dries, the sides and apex of the thallus enroll over the dorsal surface, so that the thicker central midrib of the thallus is completely covered by the darkly pigmented ventral scales and rhizoids. This process, of course, greatly decreases the surface area exposed for evaporation and provides protection to the meristematic cells of the apex. When seasonal rains return, the thallus quickly unrolls and growth is renewed, especially at the thallus apex. This scenario, perhaps coupled with intracellular mechanisms of desiccation tolerance, can allow for very long-term dormancy as exemplified by the report that a few specimens of *Riccia macrocarpa* from Tunisia were able to renew apical growth after being stored more than 23 years in dry conditions (Breuil-Sée, 1993). It is interesting that there are no drought tolerant taxa in the Marchantiaceae, which is the first diverging lineage of the true complex thalloids (Bischler, 1998).

Lineage 3 (Pelliidae) - Most of the diversity of simple thalloid hepatics is found in this lineage, including such familiar taxa as *Pellia*, *Fossombronia*, *Moerckia*, *Pallavicinia*, and *Symphyogyna*. Most genera in the lineage grow in constantly moist habitats and are drought intolerant, although they are able to withstand repeated cycles of brief drying and re-wetting. In contrast, *Fossombronia*, the most speciose genus in the lineage, typically inhabits harsh, seasonally dry habitats that require some mechanism for drought tolerance. Notable among these is a short, annual life cycle, production of large, desiccation resistant spores, and formation of swollen, tuberous apices or pendent, subterranean tubers that allow for perennation during the dry season. *Pallavicinia* and *Symphyogyna* as well as other genera of the Palliviciniales are the only group of liverworts outside of *Haplomitrium* to have water-holding conducting strands in the thallus midrib. As in *Haplomitrium*, these cells bear numerous pits on their lateral walls, but unlike *Haplomitrium*, their walls are thick and fibrous. It is probable that these cells serve as an internal water reservoir that releases water to the photosynthetic wing cells during short periods of drying, as occurs even in rain forest habitats, where many of them grow. When ample water again returns, these dead, hollow cells rapidly re-fill as water diffuses in from the surrounding thallus cells. In genera in which the winged thalli arise from subterranean rhizomes, *e.g*., *Jensenia*, *Hymenophyton*, and dendroid species of *Pallavicinia* and *Symphyogyna*, there is a fairly continuous influx of water from the central strand of the rhizome system into the midrib strand cells, so water is always available, even when ambient air humidity levels fall.

Lineage 4 (Metzgeriidae) - This lineage comprises the two most derived families of simple thalloid liverworts, the Metzgeriaceae and Aneuraceae, as well as the leafy liverwort *Pleurozia*. Most species of *Pleurozia* and *Metzgeria* are epiphytes, with some species of *Metzgeria* occurring as epiphylls, while the Aneuraceae are found on moist soil, rocks, and rotting logs in both temperate and tropical forests. Several species of *Riccardia* also occur in the peatlands of

the Andean parámos. The phylogenetic link between *Pleurozia* and the Metzgeriales is puzzling, but well supported by numerous studies. This strange genus bears two rows of leaves, each of which bears a complex dorsal water-sac, postulated to serve as an insect trap.

Although morphologically very different from the small, simple thalli of *Metzgeria*, *Pleurozia* grows via a 2-sided, or lens-shaped, apical cell like all other members of the lineage. Clausen (1964) has confirmed that *Pleurozia purpurea* and three species of *Metzgeria* are drought tolerant, although only a few cells of *Metzgeria* survived treatment at 15% RH levels. The Aneuraceae appears to be drought intolerant although only a few species and none of those from the páramo have been tested (Clausen, 1952; Crandall-Stotler, unpublished data).

Lineage 5 (Jungermanniidae, Porellales) - The "true" leafy liverworts are the last backbone divergence of the division. Their dichotomy into two morphologically well-defined sister groups, the Porellales (lineage 5) and the Jungermanniales (lineage 6) is estimated to have occurred in the early Carboniferous.

The greatest diversity of the Porellales is found in the rain and cloud forests of the tropics, where they occur as epiphytes. All members of the order have incubous leaf insertions, unequally bilobed leaves, with the small ventral lobe often forming an inflated water sac, and precocious, endosporic spore germination. Despite growing in a moist to wet macrohabitat, most members of the Porellales are very desiccation tolerant; *Porella* and *Frullania*, in fact, rival the tolerance of extremely tolerant mosses like *Syntrichia* and *Grimmia* (Crandall-Stotler, unpublished data; Marschall & Proctor, 1999).

Lineage 6 (Jungermanniidae, Jungermanniales) - The Jungermanniales are the largest and most diverse lineage of the leafy liverworts, both morphologically and ecologically. Although there are some epiphytic taxa in the lineage, soil, rock, and fallen logs are more common substrates. Complicate unequally bilobed leaves occur in a few taxa (*e.g.*, *Nowellia*, *Scapania*, and *Schistochila*), but the smaller lobe is dorsal, rather than ventral as in the Porellales. Leaf insertions may be transverse, incubous, or succubous, with the latter being the most common expression in the order. Spore sizes vary and germination is almost always exosporic. Drought tolerance is of scattered occurrence, and is only moderately expressed in many of the taxa in which it has been confirmed (Clausen, 1952; 1964). Genera able to tolerate 12 hours at 15% RH, *i.e.*, highly tolerant taxa, include *Ptilidium*, *Herbertus*, *Mastigophora*, *Jamesoniella*, *Cephaloziella*, *Isopaches*, *Southbya*, and *Gymnomitrion*.

These genera are distributed across the phylogeny of the Jungermanniales, *Ptilidium* being a very early divergence and *Gymnomitrion* in one of the crown groups; however, fewer than 8% of the genera in this lineage have been studied, generally with a single species tested per genus. Increased sampling that includes genera, like *Stephaniella* and *Gongylanthus*, from exposed rocks and dry soils of high elevations, and tropical elements of the most speciose families, including the Lepidoziaceae, Lophocoleaceae, and Plagiochilaceae, are needed to evaluate the distribution and significance of desiccation tolerance in this large, polymorphic lineage.

LIVERWORTS - AVOIDANCE ENHANCEMENTS TO DESICCATION TOLERANCE

Tolerance to extreme drying has been less well studied in liverworts than in mosses, and almost nothing is known about physiological mechanisms at the cell level that allow desiccation tolerant taxa to recover cell function after prolonged drying in controlled conditions. It has been shown that abscisic acid (ABA) enhances recovery of desiccated thalli in normally tolerant *Exormotheca holstii* (Hellewege et al., 1994) and induces desiccation tolerance in the otherwise intolerant *Riccia fluitans* (Pence et al., 2005). Pressel et al. (2009) have shown that the cytological changes that occur during desiccation and recovery in *Southbya nigrella* are comparable to changes also seen in mosses during these processes, including an increase in soluble carbohydrates in the cytosol. Taken together, these results suggest that liverworts likely possess a cell mechanism similar to that of mosses, but this has never been confirmed. Since liverworts are poikilohydric, like mosses, it is not surprising that they possess many of the same structural and life history adaptations to enhance the efficiency of water retention and thereby increase the chances of surviving drought by avoiding desiccation (Figure 15).
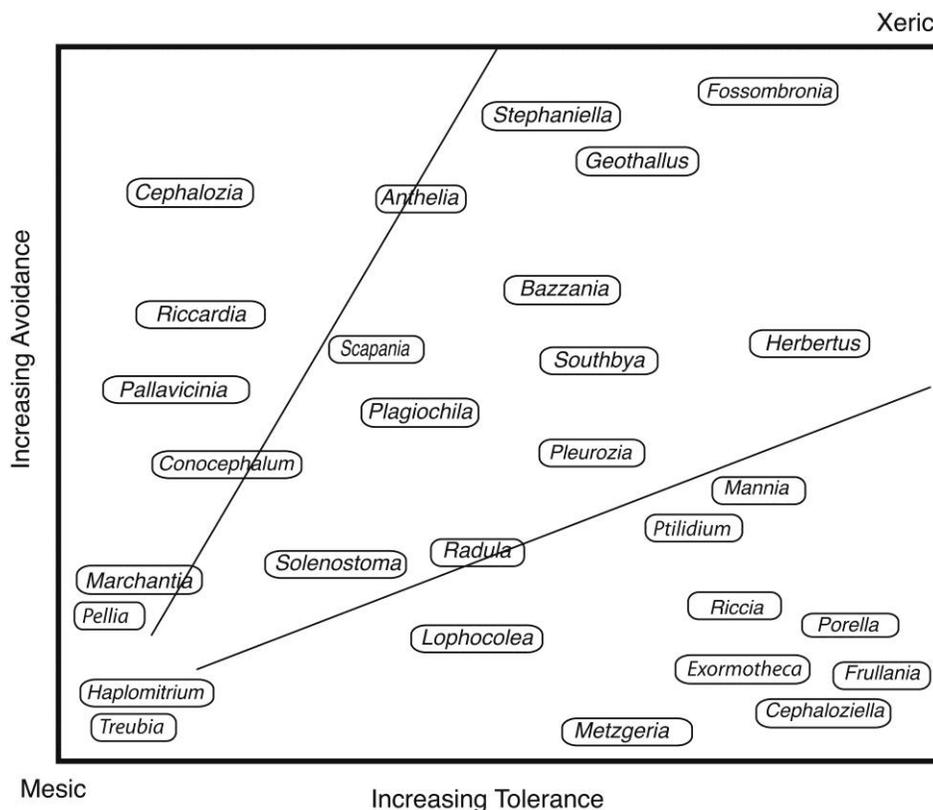

Figure 15. Conceptional model, showing the distribution of select liverwort genera along a dryness gradient. Oblique lines delineate major strategies from largely avoidance (upper left) to largely tolerance (lower right).

These include, but are not limited to, possessing thickened cell walls and special wall ornamentation, increasing the capacity for external water uptake with dense rhizoidal mats, forming large external capillary spaces through which water can move and be stored without compromising gas exchange and photosynthetic potential, reducing surface to volume ratios, increasing the degree of overlap between leaves, and escaping through shortening the life cycle. Considering the structural features of taxa that typically inhabit sites with extended dry periods, several additional characters that promote desiccation tolerance through avoidance have also been postulated. These include slime secretions, especially over meristematic areas; highly divided, toothed, ciliated or highly rucked leaves; ventral water sacs; rhizomes or stolons; and perennating tubers or tuberous apices.

Leaf Insertions, Spacing, and Form

The leaves of liverworts are generally unistratose and in most cases are inserted laterally along an oblique line, in either a succubous (basal or basiscopic margin dorsal) or an incubous (apical or acroscopic margin dorsal) pattern. Typically, they overlap along their margins, resulting in a shingling arrangement. Experiments by Clee (1937) suggested that capillary water movement occurs from base to the apex in plants with succubous leaves and from apex to base in those with incubous leaves. Although this would seem to explain the predominance of incubous insertions in epiphytic taxa, this hypothesis does not seem to hold true (Mägdefrau, 1937; Schuster, 1966). It does appear, however, that water evaporates more quickly from plants with succubous leaves than plants with incubous leaves, a phenomenon that Schuster (1966) equates with the observation that succubous insertions allow more of the dorsal surface of the stem to be exposed. Incubous leaves, in contrast, are more tightly appressed and cover most of the exposed stem surface; *i.e.*, a tighter shingling is possible in incubous leaf arrangements. With a tighter, more closed leaf arrangement, the rate of water loss from internal tissues is decreased, and brief intermittent periods of drying, such as those found in epiphytic habitats, are better tolerated. According to Schuster (1966), plants with succubous leaves have little drought tolerance and are, therefore, most commonly found in moist, shaded habitats. Transverse leaf insertions occur primarily in plants of continuously moist habitats, but they are also found in *Herbertus* and *Cephaloziella*, two highly desiccation tolerant genera. In *Cephaloziella*, plants are extremely small, leaves are often papillose, distantly spaced, and tightly clasp the stem, which is the major photosynthetic structure of the plant. The plants are often darkly pigmented and typically form small, tightly interwoven mats on crusty soils, although they may also be interspersed throughout mats or cushions of other bryophytes. In contrast, *Herbertus* plants are robust, erect, radially symmetric cushion-forming plants in which the leafy shoots arise from a branched rhizome system that is densely covered with rhizoids. Water from below can be held in capillary spaces between the individual leafy shoots and atmospheric water can be held in the spaces between the erect, appressed, deeply bifid leaves and the stem. Rates of water uptake from the surroundings and short-term storage are also enhanced by elongate, thick-walled cells with large trigones and intermediate thickenings that form branched vittae in the lamina of each leaf and underleaf, and by the thick-walled cells of the stem.

Liverwort leaves vary greatly in form, but are usually divided into two or more equal or unequal lobes. Leaves that are highly divided and/or ciliated, such as those of *Ptilidium* and

Trichocolea, show slower rates of evaporation than undivided leaves of similar surface area, presumably because they create capillary spaces for water uptake and retention (Clausen, 1952). This may explain why perichaetial leaves and other structures, which surround the archegonia, are usually divided and toothed to ciliate even in taxa with undivided vegetative leaves. A few other modifications of leaf form that enhance survival in exposed harsh environments include the following: formation of broad channels, or pleats, on the adaxial surface of the highly crispate leaves of *Fossombronia*; polystratose leaves in the *Patagonian* taxa, *Herzogiara* (Pseudolepicoleaceae), and *Pachyglossa* (Lophocoleaceae); hyaline marginal and submarginal cells, with the larger photosynthetic cells restricted to lower parts of the leaf as in some species of *Gymnomitrion* (Gymnomitriaceae); and small completely hyaline, leaves covering branched stem paraphyllia, the only photosynthetic tissues in *Stephaniella* (Stephaniellaceae), a plant of dry, high elevation sites in the Andes.

Water Sacs and Ventral Appendages

The universal occurrence of inflated ventral lobules or water sacs in the Porellales was viewed by Goebel (1905) as evidence of their role as water-storing structures. Such a function is, in fact, inherent in them being called water sacs. Although this notion of water sac function persists, experiments on *Frullania* demonstrated that when wetted stems are air-dried at ambient temperatures, water quickly evaporates from the ventral water sacs (Blomquist, 1929); water will also rapidly re-fill the sacs when dried stems are wetted. Clausen (1952) postulated instead that ventral lobules, whether highly inflated or not, create capillary spaces that allow for rapid uptake of water when it is available, but her experiments also show that they are not water storage structures. It is more likely, as postulated by Herzog (1926), that they serve instead to capture and concentrate nutrients from throughfall.

Like water sacs, large ventral underleaves and foliar scales also increase capillarity along the ventral side of the stem in prostrate taxa. In erect, isophyllous genera they function in photosynthesis just like the lateral leaves, but in the prostrate taxa, they likely are not effective in photosynthesis although they still contain plastids. Underleaf insertion is always transverse, and many underleaves are divided or ornamented with slime papillae. Their presence creates capillary spaces along the ventral side of the stem and the papillae help protect the stem apex. In general, increasing the volume of capillary spaces promotes the maintenance of turgidity through brief periods of atmospheric drying, but is likely not a mechanism to avoid long-term drought (Clausen, 1952).

Rhizomes, Stolons, and Other Subterranean Structures

Rhizomes and stolons are branched subterranean stems. In hepaticology, the term stolon is reserved for the leafless, hyaline subterranean system of the Haplomitriales and the term rhizome is applied to underground stems bearing reduced leaves, thick-walled epidermal cells, and dense coverings of rhizoids. Rhizome systems can be extensive horizontal and vertical systems, with positively geotropic branches sometimes penetrating several centimeters into the soil (*e.g.*, *Lembidium* and *Wettsteinia*). Whether horizontal or vertical, these systems can absorb water from the upper layers of the substrate and move it into the leafy shoots by a combination

of capillarity and diffusion even when atmospheric moisture is limited. Rhizomes also function as perennating structures during periods of extended dryness, renewing production of leafy shoots from quiescent branch initials when moisture conditions return.

The modification of leafy stem apices into tubers is another means of persisting by perennation during drought or other unfavorable growing conditions. This avoidance mechanism is particularly well developed in *Fossombronia* and *Petalophylllum*. This developmental modification of normal stem growth is induced by drying. Tubers, like rhizomes, are densely covered with rhizoids and bear thick-walled epidermal cells, but they are fleshy, determinant in growth, and filled with starch-filled parenchyma cells.

Cell Wall Modifications

Liverworts of xerophytic habitats typically possess leaf and stem cells with especially large trigones, or corner thickenings, and nodular intermediate thickenings on their interior walls and/or protruding papillae on their dorsally exposed, superficial walls. Consisting primarily of hemicelluloses, pectins, and cellulose, such thickenings increase the potential for apoplastic water uptake, movement, and retention (Proctor, 1979). In addition, the depressions between papillae form a network of small capillaries that transport water over the surface of the leaf. When aqueous dye is used to wet papillose leaves, the dye solution quickly fills the depressions, but the tips of the papillae remain dry, allowing gas exchange to occur through them under otherwise saturated conditions. In contrast, taxa of moist, but intermittently dry habitats often have exposed, outer cell walls that are quite water-repellent. For example, in taxa with concave, overlapping leaves (*e.g.*, *Nowellia*, *Marsupella*, and *Solenostoma*) this is likely due to the physical effects of surface tension (Proctor, 2009), but in other taxa, these walls are coated with thin layers of epicuticular waxes (Duckett & Soni, 1972; Heinrichs et al., 2000). In *Anthelia*, these waxes are visible with a hand-lens as a layer of white crystalline threads or needles, but in other taxa waxy deposits are only visible in SEM preparations (Heinrichs et al., 2000). According to Heinrichs et al. (2000), the main component of the wax in *Anthelia* is a diterpene, while waxes in *Plagiochila* contain a mix of steryl esters, triacylglycerals, and free fatty acids. Neither these waxes nor various wall ornamentations are part of a cuticle, despite references to the contrary in taxonomic descriptions. In contrast to cuticle, which limits both water uptake and evaporation in vascular plant cells, the thin waxy deposits of liverworts do not prevent the plant from drying out. Rather, they help maintain an interface for gas exchange and also prevent wetting of fully dry plants when water is insufficient for recovery.

Pegged Rhizoids

One of the diagnostic characters of the complex thalloid lineage (Marchantiopsida, subclass Marchantiidae) is the occurrence of two types of unicellular rhizoids in most taxa: living, but highly vacuolated smooth rhizoids, and non-living rhizoids bearing internal wall thickenings termed pegs (Duckett et al., 2014). In general, smooth rhizoids emerge perpendicular to the thallus and grow downward into the substrate, while pegged rhizoids

extend in bundles parallel to the ventral thallus surface, where they are covered by the ventral scales. Bundles of pegged rhizoids also occur in internal furrows of the upright stalks of the gametangiophores and carpocephala. According to Duckett et al. (2014) the smooth rhizoids of complex thalloid liverworts function, as in all other liverworts, in anchorage and as fungal conduits and are not at all desiccation tolerant. Whereas smooth rhizoids will collapse when dried, pegged rhizoids, with their elastic walls and internal thickenings, do not. Dye studies show, conclusively, that pegged rhizoids form an extensive conducting system that is structurally unaffected by desiccation, which is especially important in the carpocephala. In addition, pegged rhizoids allow the vegetative thalli of taxa growing in periodically dry habitats (*e.g.*, *Mannia*, *Plagiochasma*, and *Targionia*) to resist desiccation (Duckett et al., 2014).

DESICCATION TOLERANCE IN HORNWORTS

Estimated to include approximately 250 species in 14 genera, hornworts are currently hypothesized to be sister to the tracheophytes, based on molecular phylogenetic studies (Qiu et al., 2006; Shaw et al., 2011). Their thalloid gametophores always possess ventral mucilage-filled cavities that harbor symbiotic cyanobacteria and their large plastids are unique among embryophytes in having channel thylakoids, with central pyrenoids present in many, but not all, taxa (Renzaglia et al., 2009). Hornworts typically occur on moist soil in fairly shaded habitats, with *Dendroceros* being the only epiphytic member of the group; however, some taxa, like *Phymatoceros*, are found in areas with persistent drought or long periods of seasonal dryness.

Whether any hornworts are actually desiccation tolerant, or are desiccation avoiders, is equivocal. In Oliver et al. (2000) hornworts are scored as desiccation tolerant on a phylogenetic tree, but no data are cited to support this conclusion. Alpert & Oliver (2002) cited this reference for the statement: 'There are also desiccation-tolerant hornworts.' However, Oliver et al. (2005) reconstruct hornworts as lacking vegetative desiccation tolerance, and further suggest that the earliest expression of desiccation tolerance in some of these early lineages was in spores, not in vegetative tissues. According to Wood (2007), *Dendroceros granulosus* was found to be desiccation tolerant by Proctor (personal communication), but the experimental proof of this has never been published, not even in subsequent publications written by Proctor (*e.g.*, Proctor et al., 2007; Proctor, 2009; 2010).

Some hornworts found in seasonally dry localities produce abundant swollen, marginal and/or apical tubers on their thalli (*e.g.*, several species of *Phaeoceros* from Washington, Oregon, and California), while the genus *Phymatoceros* produces long-stalked, subterranean ventral tubers. Tuber production of both types is initiated as sporophytes mature and the tubers persist in the soil crust or subterranean soil bank after the vegetative thallus deteriorates. When rains return, new plants germinate from these quiescent structures (Crandall-Stotler et al., 2006). At least in *Phymatoceros*, such tubers contain high levels of abscisic acid (Hartung et al., 1994), which has been shown to induce desiccation tolerance in other bryophytes (Pence et al., 2005), and personal observations confirm that both marginal and subterranean tubers can germinate after nine months of dryness. In addition to tuber production as a means of surviving periods of drought, hornworts also can have very short vegetative cycles, followed by rapid spore release, as in the case of *Notothylas*. Hornwort spores are able to germinate even after

many years of storage in dried herbarium packets (Renzaglia et al., 2009); these observations suggest that spores are likely desiccation tolerant, but this hypothesis has never been tested.

CONCLUSION

Species of mosses, liverworts, and hornworts occur in remarkably dry habitats. Most species of mosses and some species of liverworts tolerate drought by being tolerant to desiccation. At least in mosses it appears that this physiological tolerance to desiccation was present in the earliest lineages and in some of these lineages is a key feature for inhabiting extreme habitats. Many liverworts have less tolerance to desiccation, but there are notable exceptions. In addition to having a physiological tolerance to desiccation, some mosses and liverworts also have a suite of morphological features that enhance water retention and hence avoidance of drought, or have life history changes that allow the species to effectively escape drought. It is this combination of desiccation tolerance and drought avoidance (Figures 2, 15) that determines drought tolerance and is a key factor in determining habitat limitations for both moss and liverwort species.

ACKNOWLEDGMENTS

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