

BRYOLOGY

FOR THE
TWENTY-FIRST CENTURY



Edited by J.W. Bates, N.W. Ashton,
and J.G. Duckett



THE CENTENARY SYMPOSIUM OF
THE BRITISH BRYOLOGICAL SOCIETY

Bryology for the Twenty-first Century



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Bryology for the Twenty-first Century

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The British Bryological Society

The British Bryological Society exists to promote the study of mosses and liverworts. It was constituted in its present form in 1923, replacing the Moss Exchange Club founded in 1896.

Two field meetings of about a week's duration, are held every year at interesting venues in Britain, and Ireland and, increasingly, in other countries. An autumn weekend meeting brings members together for a presentation of papers and posters, the Annual General Meeting and a field excursion. In the early winter a weekend workshop provides instruction in the examination and identification of bryophytes or in related matters such as photography, cultivation, or recording and computing methods. Programmes of local excursions are organized to record bryophytes and encourage new members. A Tropical Bryology group exists to promote studies of bryophytes in poorly known regions.

Members receive the *Journal* and *Bulletin* free of charge and are entitled to borrow books, periodicals and reprints from the Society's library, to borrow specimens from the Society's herbarium and to consult the Society's panel of referees for assistance in the identification of specimens. There is a continuing scheme to map the bryophyte flora of Britain and Ireland in which members may also participate.

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Cover Illustration

Scanning Electron Microscope image of the gemmiferous protonema of the moss *Orthodontium lineare* (photo J. G. Duckett).

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Preface

This volume celebrates the one-hundredth birthday of the British Bryological Society which was originally founded as the Moss Exchange Club in 1896. The Centenary Symposium, consisting of sessions of papers from invited speakers and posters, took place in the Graham Kerr Building of the University of Glasgow, 5–7 August 1996. We are grateful to members of the BBS Centenary Committee (J. W. Bates, G. C. S. Clarke, J. G. Duckett, N. G. Hodgetts, R. E. Longton, M. E. Newton, A. R. Perry) for defining the emphasis of the symposium, and especially to Dr J. H. Dickson for making all the local arrangements. Dr Jim Dickson treated us to a memorable civic reception in the Satinwood Room of the City Chambers on the evening of the 6th. Here we first entered an impressive marble-lined hallway to the loud resonances of the highland pipes played by Mr William Park. Following a welcome by Baillie Jean Macey and an excellent reply by our Vice-President Dr Giles Clarke, around 90 delegates and their families thoroughly enjoyed a buffet meal with generous measures of assorted malt whiskies and other refreshments. The evening concluded with a delightful recital of Scots songs by Mrs Wilma McDougal. On the previous evening Dr Dickson organized a tour around the Glasgow Botanic Gardens. Here, particularly growing on the bases of the many mature tree ferns in the Victorian iron-and-glass Kibble Palace, we were shown large colonies of the tropical moss *Hypopterygium atrotheca* Dix., then known nowhere in the wild but since tentatively synonymized (by J. D. Kruijer, 1997, *Glasgow Naturalist* 23: 11–17) with the austral *H. muelleri* Hampe.

The organizers were determined that the symposium would be forward- rather than backward-looking, particularly with a new millennium as well as a new century in prospect. Therefore, the invited speakers were selected to emphasize areas of the study of bryophytes where recent and significant advances in understanding have been made. Parts 2–4 of this volume basically reflect the themes of the three one-day sessions of the symposium, although some rearrangements have been made to improve the integration of the chapters. Nevertheless, it is fitting to remember how we reached the present point and to this end Roy Perry sketches a brief history of the origins of the BBS in his *Welcome* to symposium delegates. In similar vein, Part 1 (*A Special Tribute*) contains a single chapter describing the unique contribution to British bryology of Professor Paul Richards who died in October 1995. Professor Richards' life spanned many of the most important years of the BBS and his inspiration has had a great influence on the direction of bryological research in Britain.

Part 2, dealing with *Origins, Evolution and Systematics*, differs strikingly from previous syntheses on bryophytes in two important respects. First, the conceptual approach known as *cladistics*, a stochastic method for deducing evolutionary relatedness of organisms based on shared and divergent characteristics, firmly underlies many of the contributions. Second, both ultrastructural and molecular characters are now being given considerable weight in unravelling phylogenetic trees. Cladistics is used here both to study the relations of the main bryophyte groups to algal and other land plant groups, and to infer relatedness of different bryophyte groups to each other. The distinctiveness of the hornworts from the other bryophyte groups continues to be emphasized by these analyses. More than one paper in this section reminds us that, powerful as cladistic analysis may be, everything depends on an intelligent interpretation and selection of characters. Corroborative results using different types of molecular, ultrastructural and morphological characters are recommended. This is the largest section of the book — a reflection of the intense activity in this area currently.

Morphogenesis and Cell Biology (Part 3) is another area where important new discoveries have recently been made, and one where there are promises of further breakthroughs in understanding in the near future. Several papers emphasize the importance of microtubular organelles in defining the positions of cell differentiation and also in effecting a previously unsuspected intracellular transport system in the mosses. The latter may explain the unusually elongated cells of many mosses and the prevalence of oblique cell walls in moss rhizoids, and it may also account for features such as apical dominance which have been poorly understood hitherto. Research with mutant strains of protonema has revolutionized our understanding of how gravitropism functions.

Part 4 (*Physiology, Ecology, Pollution and Global Change*) covers a range of interrelated topics involving function of bryophytes and interactions with their environment. In the first chapter Professor John Raven and colleagues explain that bryophytes are not all conventional C₃ plants as had previously been supposed. A carbon dioxide concentrating mechanism operates in Anthocerotes, again emphasizing their 'distance' from the other bryophyte groups. Updates on membrane function, photosynthetic pigments and internal redistribution of inorganic nutrients are provided by the first three chapters. Professor John Birks and co-authors (chapter 20) provide an instructive account of how the presently popular 'generalized linear models' approach may be applied to unravel bryophyte-environment relationships. The message in Professor Lars Söderström's chapter (21) is that bryophytes inhabit patchy environments of limited duration, so models of their population dynamics must also take account of the dynamics of the habitat patches. The final three chapters consider recent advances in our understanding of the effects on bryophytes of air pollution and of increasing atmospheric CO₂ and global climatic change. In the final chapter Professor Dicky Clymo presents a rather spine-chilling analysis of the carbon budgets of *Sphagnum*-dominated peatlands. He concludes that, contrary to popular understanding, peat systems may be net contributors to 'global warming' by releasing significant amounts of the 'greenhouse gas' methane despite their undisputed value in fixing the much less greenhouse-active CO₂.

We are indebted to all our authors for providing a collection of first rate papers that will undoubtedly stimulate further research into bryophytes. We also thank again those authors who acted as session chairmen for their invaluable contributions. The editors are deeply indebted to many of the authors and a number of other bryologists for refereeing the manuscripts prior to publication. It is inevitable in an undertaking of this type that a few contributions, for varying reasons, failed to reach fruition in this volume. A few papers were published elsewhere before this volume was completed. Two of us (JWB, JGD) would like to thank Dr Royce Longton for his help in planning the symposium and inviting speakers. He had originally intended to be an editor but withdrew to wear his other hat, that of local politician, as the 1997 British general election drew close. We are extremely glad that Neil Ashton agreed to join us at short notice. Royce Longton's contribution (*Reproductive biology and life-history strategies*) was published in *Advances in Bryology* (1997) but the summary of that paper is reproduced here as an Appendix by kind permission of the publishers. Finally, we gratefully acknowledge a grant from the New Phytologist Trust towards the costs of producing this volume.

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January 1998

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Welcome to the Centenary Symposium

A. R. Perry

*President of the British Bryological Society
National Museum & Galleries of Wales, Cardiff, U.K.*

We are here to celebrate the centenary of the Moss Exchange Club and I welcome you all.

The Moss Exchange Club (MEC) was founded on the initiative of Revd C. H. Waddell, a clergyman of the Church of Ireland and a native of Co. Down. Having noted that the lack of a Society devoted to the study of bryophytes was hindering the advance of bryology in the British Isles, he submitted notes at the end of 1895 to three scientific journals proposing the establishment of a Moss Exchange Club on lines similar to those already in existence for flowering plants. He thought the Club would promote greater knowledge of bryophyte distribution and would be of great assistance to beginners. Thirty active members would be needed to get the Club going and he proposed to contact 'some of our leaders in bryology'. H. N. Dixon warmly supported Waddell's proposal — he was soon to publish the first edition of his *The Student's Handbook of British Mosses* — though he showed some concern that the Club might lead to the extermination of our rarer species. Waddell, however, thought this could be prevented by judicious wording of the Rules of the Club and suggested that localities, especially near towns, would not be too definitely published.

Twenty-three members were enrolled in 1896 as a result of his advertisement and the first distribution of specimens took place that autumn, and consisted of over 2,000 packets. Dixon's book appeared that year too, and was a tremendous stimulus to the study of mosses, with its excellent descriptions and H. G. Jameson's clear drawings. It remained the moss bible for over 80 years until 1978 when A. J. E. Smith's comprehensive *The Moss Flora of Britain and Ireland* was published.

Liverworts, I sense, fared less well though Lett published *Hepaticas of the British Islands* in 1902 (which had a mixed popularity and was seemingly not widely used), and W. H. Pearson's bulky volume *The Hepaticae of the British Isles* (1899–1902) had no keys and was very expensive (it still is!). It was not until 1912 that S. M. Macvicar's user-friendly *The Student's Handbook of British Hepaticas* became available and this, a companion to Dixon's moss book, remained the most popular and useful work until it was superseded by A. J. E. Smith's *The Liverworts of Britain and Ireland* in 1990.

The MEC produced Reports annually but they were never much more than statements of the affairs of the Club. But in the early years they were accompanied by a Circulating Notebook, passed from member to member so that each could write comments on the specimens distributed in the exchange or on other matters of interest.

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A clear need for a Beginner's Section of the MEC became apparent early on. Section II, as it was usually called, started in 1901 and was an immediate success. From 1906 it also produced an annual report, the first of these recording a membership of 30.

From its early years one of the Club's aims was to compile reliable records of the distribution of British bryophytes on a vice-county basis. The results appeared in the first *Census Catalogue of British Hepatics* in 1903 and of *Mosses* in 1907. Several revisions of these catalogues have been forthcoming, and later this centenary year we are expecting an updated version of a new combined edition prepared by Tom Blockeel, David Long and Chris Preston.

The MEC held no meetings as we do these days, but continued to distribute specimens. However, its Senior Section declined and by 1922 Section II was the larger and more active part (and, incidentally, in its ranks was the 13-year-old Paul Richards, who had joined Section II two years before). Because of the divergence of the two sections it became strongly felt that the time had come to join them together and launch out as a much stronger, united Society. So members of the two sections were asked by letter to meet together, make acquaintance with one another, enjoy rambles together and form a new group. The replies showed general agreement of this and as a result several members from each of the two Sections met in August 1922 in Dolgellau, North Wales where there was an informal meeting at which Officers were nominated and Rules formulated. Dixon was elected the first President.

The following year, 1923, the first Annual Report of the newly formed BBS quoted a membership of 87, which included some foreign members. There was at the time the hope that many foreign members would join as the Society became more widely known. By 1934 the membership had risen to 100 and included such distinguished foreign members as Pierre Allorge, E. B. Bartram, Alexander W. Evans and Leopold Loeske.

Just after the Second World War a small group of members met for a weekend in a hotel in the Lake District to discuss reviving the Society. Later, in 1946, a formal Annual General Meeting was held in northern England and then, the same year, a Jubilee meeting was held in London to celebrate the 50th Anniversary of the MEC.

After this the Society expanded rapidly and by 1950 it had 250 members. There are probably several reasons for the upsurge. First there was a great expansion of interest in nature conservation; then there was an increase in developments of bryological research in the universities. But perhaps one of the most important reasons was the introduction of a new journal, *Transactions of the British Bryological Society*, the brainchild of Fred Sowter, who became its first editor. Ted Wallace supplemented the journal with a *Bulletin* from 1963 which has since grown from the early single duplicated sheets announcing future meetings of the Society and other short news items and advertisements, to a substantial printed publication containing in addition, reports of Officers and all meetings, short notes and papers of bryological interest and other relevant items.

One of the stimuli which has fuelled the interest of both our amateurs and professionals has been the compilation of reliable records of the distribution of bryophytes in the British Isles. This to some of our foreign members may seem rather parochial, but the exercise has been extremely popular and has resulted in a remarkable number of expert amateurs. Their records have appeared in the *Census Catalogues*, but since 1946 records

have been accepted only if supported by a correctly determined voucher specimen which is then deposited in the Society's herbarium in Cardiff. In 1960 the scheme to map the British bryophyte flora on the basis of the 10 km squares of the National Grid was set up, and A. J. E. Smith was elected Mapping Secretary, a post he held until the publication in 1991–94 of the magnificent 3-volume *Atlas of the bryophytes of Britain and Ireland*.

During the last few years of the existence of the Moss Exchange Club a very active role was played by its sole woman member, Miss Eleonora Armitage. In one of her reports as Distributor of specimens in the annual exchange, she wrote 'in the years to come may we look forward to pleasant gatherings of members in some choice botanical hunting-ground in these islands'.

This brings us to a theme that has run a hundred years. Our Society has succeeded through a cooperation of amateurs and professionals. This cooperation must continue! Miss Armitage would have been thrilled to join this 'pleasant gathering' in Glasgow and perhaps would not have become over-awed at the erudite subject matter that will be introduced. The choice botanical hunting-grounds will be the subject of the next two weeks' excursions.

In welcoming you all most warmly may I fervently hope that you will all derive a good deal of instruction and excitement from the papers that you will hear, and that those of you who go into the field later will see many wonderful plants. We held our Spring Field Meeting earlier this year at Dolgellau, the meeting place in 1922, if you remember, of the group of Moss Exchange Club members who decided to form the British Bryological Society. With the Spring Meeting and now this Symposium we have entered year 101 with a bang not a whimper; I hope you agree that this is a very auspicious start for the second hundred years.



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1. The life and work of Paul Westmacott Richards

December 19, 1908–October 4, 1995

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It is most fitting that this centenary volume of the British Bryological Society contains a tribute to Paul Richards, the man who more than any other was responsible for the rejuvenation of the Society after the Second World War and who translated it into its present form (Richards, 1983). The ethos of the Society perhaps embodies all that Paul lived and believed it should be; a collection of people from all walks of life drawn together as equals by a love of bryophytes. Thus the membership embraces amateur field bryologists, laboratory-based professionals, beginners, experts, the very young and the old(ish) all enjoying bryophytes together as friends.

It is not the intention here to provide a detailed chronology of the life and works of Paul, these can be found elsewhere (e.g. Willis, 1996; Stanley, Argent & Whitehouse, 1998), but instead to share with you some of my own memories and to try to put into a personal context a few of the many things he did in a very long, varied, happy and fulfilled life. In a sense Paul was an allegory of the multidimensional nature and the timelessness of the BBS. On the one hand he was an international scientist with the remarkable talent of being able to comprehend both the very large, from tropical forest biomes, to the minutiae of bryophyte taxonomy. He was a talented linguist, a literary man deplored split infinitives, verbalized nouns and the corruption of botany to plant biology, a lover of music (particularly Bach, Brahms and Mozart) and a man of unobtrusive wisdom with tremendous diplomatic awareness and foresight. On the other hand there was about Paul himself a timeless quality (Fig. 2a, b). Throughout the 30 years I knew Paul, he always looked, behaved and talked exactly the same; quiet, gentle and wise, with a keen, dry sense of humour (Fig. 3a), but not really up to much physically. As one of Paul's favourite anecdotes reveals, he always appeared frail. On being accepted for his first expedition to the tropics, British Guiana 1929, the leader, one Major R. W. G. Hingston, stated 'I think we will take young Richards but I doubt whether we will bring him back'. Just before retirement from the Chair of Botany at Bangor he was still climbing trees in the tropics (Fig. 5b) and long after continued to scamper around the most rugged crags of Snowdonia in much the same way as he must have done as a boy, never giving it a second thought that people of his age weren't supposed to do that sort of thing. As late as 1993, during the field meeting of the BBS at Ripon, the mid octogenarian Paul, supposedly with a worn-out hip joint, descended several 100 feet of muddy, slippery bank to observe *Orthotrichum sprucei* by the River Ure. Appropriately the previous day he had delivered a magical lecture on the life of Richard Spruce (Richards, 1994). Clearly Paul's frailty belied a robust constitution

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and indomitable spirit — undoubtedly one of the reasons why he achieved so much in wild and hazardous places.

Paul Richards enjoyed botany for over 80 years and much has been written about his travels, scientific publications and work for conservation, particularly of tropical forests. Collectively these achievements are best recognized by honours such as his Cambridge ScD in 1954, the CBE in 1974 and the Gold Medal of the Linnean Society in 1979. What is perhaps not so widely recognized is that Paul was born a botanist — he would have been a botanist regardless. But most fortunately he was born into a situation which enabled the full expression of his genotype.

Paul, the youngest of four sons of H. Meredith Richards (Fig. 2c) was born at Walton-on-the-Hill, Surrey on 19 December 1908. At that time his father was M.D., MOH for Croydon, but in 1911 moved to a senior post in the health service at Cardiff. From his earliest years Paul was encouraged by his father and his brothers, particularly Owain to pursue an interest in Botany for which he clearly had considerable talent. By the age of 8 he had a good working knowledge of the larger British flowering plants and was already looking towards smaller things — dune annuals and mosses. Regrettably I never asked him why he did such things, the answer would probably have contained a mixture of, an intrinsic curiosity about beautiful things, the love of wild places and the challenge — intellectual and physical. During these formative years Paul was also encouraged by the field botanist Eleanor Vachell and by Arthur Wade, then an assistant in the Herbarium at the National Museum of Wales. He joined the Botanical Exchange Club (the forerunner of the Botanical Society of the British Isles) in 1919 — soon publishing his first note (*Report of the Botanical Exchange Club*, 1919, 5: 682); *Allium sibiricum* L. Collected at Mullion; cultivated at Cardiff, it lost all its distinguishing characters and is indistinguishable from *Schoenopraesum* G.C. Druce then added 'This note from our youngest member is worth testing'. Membership of the Moss Exchange Club followed in 1920.

Some of his earliest herbarium specimens in the BBS herbarium (Fig. 1) clearly demonstrate that by his early teens Paul was already competent at bryophyte identification. One of the most significant events of these early bryological years was his first meeting with the distinguished bryologist D. A. Jones. In 1920, following what must have been a very adult letter, asking if he might visit Jones to discuss mosses, Paul took the train to Harlech, during a family holiday at Towyn, and duly arrived at Jones, doorstep, 'Where is your father?', 'But I am Paul Richards'. 'Oh Cambria stern and wild, meet nurse for botanic child' (ad hoc adaptation from Scott, *The Lay of the Last Minstrel* by Dr Pegler who was also present at this meeting). Needless to say they both became great friends — very much in keeping with the whole of Paul's life. He was a man who established enduring friendships with those from all walks of life. Occasionally however this non-hierarchical nature and disregard for rank, status and formal protocol landed him in trouble. After some three decades as Professor of Botany at Bangor, Paul decided that it was appropriate to write to his fellow Botany Professor at Leeds on first name terms. Professor Manton's reply was unequivocal, 'You must never write to me as Irene, the secretaries are bound to come to the wrong conclusion'.

The Richards' family returned to London in 1920 whereupon Paul was an active participant in lectures and field excursions from the South London Botanical Institute thereby establishing life-long friendships with other bryophilic schoolboys including

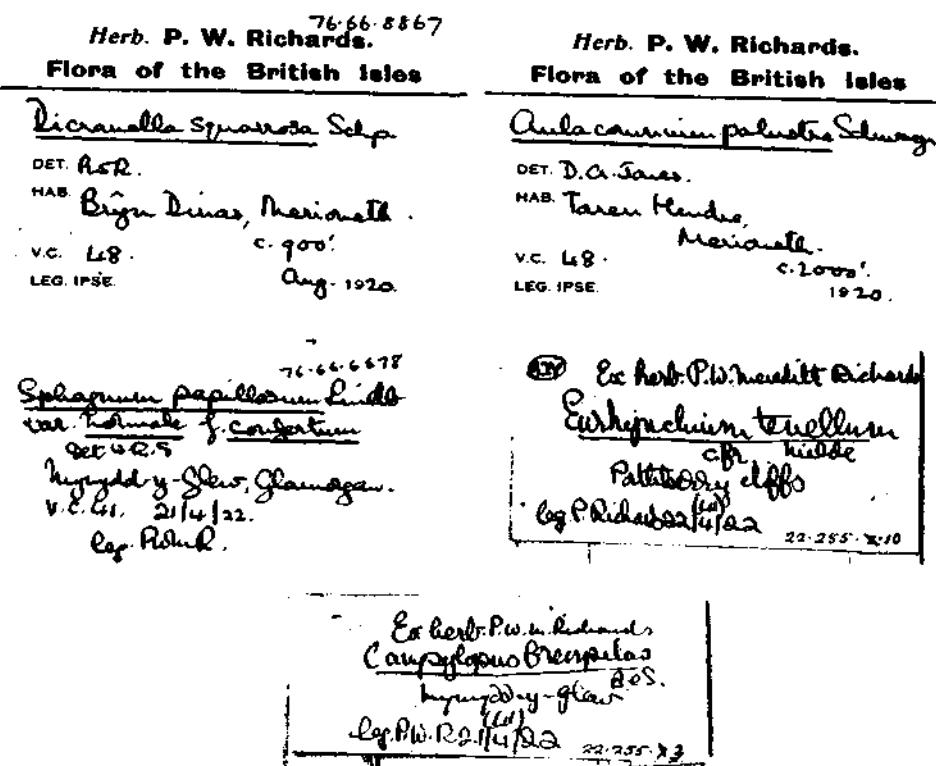


Figure 1. Specimens in the British Bryological Society Herbarium, National Museum of Wales, Cardiff reveal the teenage Paul as a more than competent moss taxonomist.

E. C. Wallace and D. G. Catcheside. He attended University College School from 1920–5 and University College London from 1925–7. Counter-balancing the paternal scientific influence his mother encouraged Paul's facility with languages. A four month visit to Zurich in 1925 not only gave him fluency in German (he was already competent in French via tuition from a French governess) but also led to a meeting with Carl Schroeter and further field excursions.

In 1927 Paul won an entrance scholarship to Trinity College Cambridge where he was an exact contemporary of T. G. Tutin and E. F. Warburg. He also became friends with many others who went on to outstanding careers. Perhaps the seminal event in his undergraduate career was taking part in an expedition to British Guiana organized by the Oxford Exploration Society. This expedition, followed by others to Sarawak (1932) and to Southern Nigeria (1935), laid the foundations for *The Tropical Rain Forest* (1952) started before World War II but not published until 1952 following further visits to Nigeria and British Cameroon. Opening my own copy of this book, purchased as an undergraduate in the 1960s, I was surprised to discover almost everyone of its 407 papers heavily annotated. Now, with the hindsight of my own visits to tropical forests I realise the supreme strengths of this volume. Paul made sense out of the most complex ecosystem in the world producing at the same time an enthralling read. This book also provided the scientific foun-

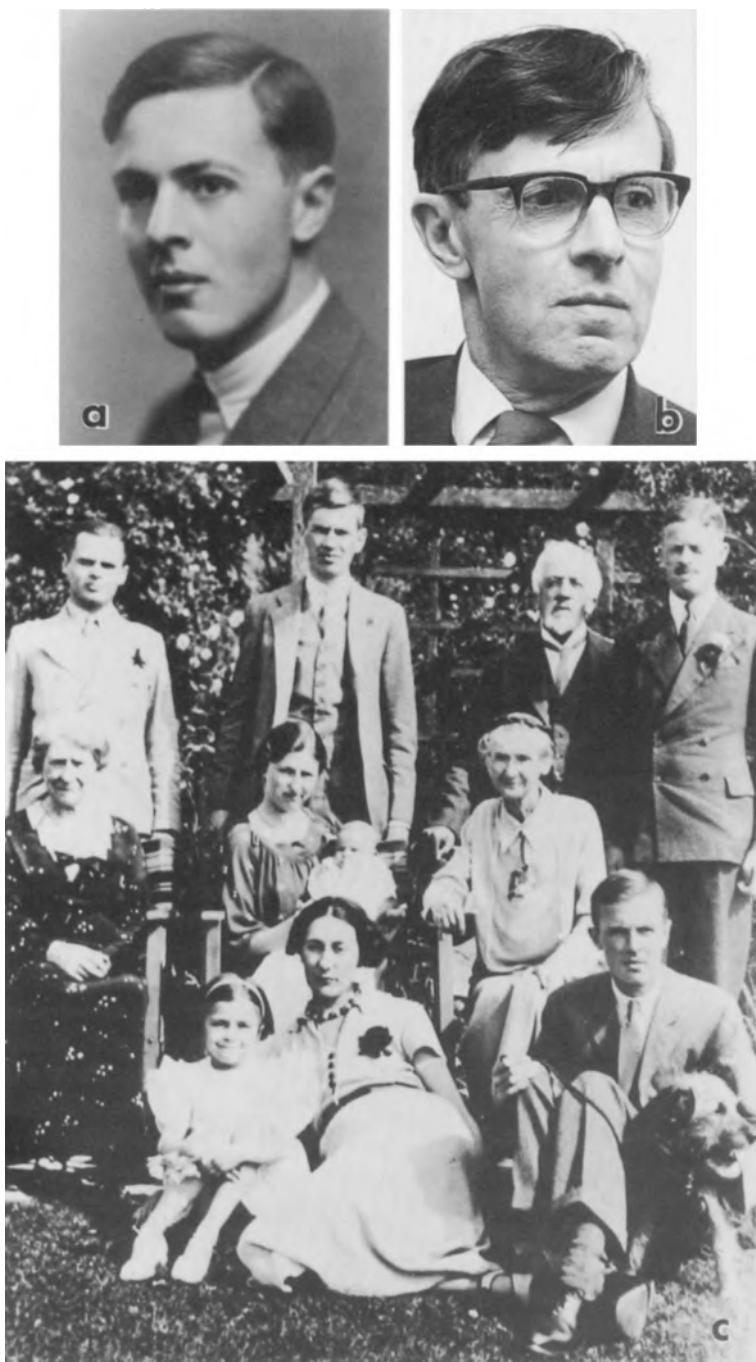


Figure 2. a, and b. Portraits of Paul as a research fellow, 1934 and on his retirement in 1976. c. The Richards family in 1935. Standing left to right; Brothers Gower, Owain, Father Harold Meredith and Brother Alan. Seated; Mrs Norris, Maud's mother, Maud (wife of Owain) with baby Gillian and Mother Mary Cecilia. On grass Daughter Ann, now Mrs Venables, Nina (wife of Gower) Paul and Bill (=dog).

dation for the conservation of tropical forest. TRF and the subsequent popular book *The Life of the Jungle* (1970) are written by a man whose heart is in the subject and by one who has a gift for describing forests exactly as they are. You feel and smell the growing jungle in Paul's works. Interestingly Paul's later bibliographic writing displays the same uncanny sensitivity at distilling the true essence of the people about whom he wrote.

After graduating with First Class Honours in both parts of the Natural Sciences Tripos, and the award of the Frank Smart Prize in Botany in 1931, Paul needed a means to live in Cambridge. Throughout his life he freely acknowledged that his greatest debt of gratitude was to Trinity College for the award of a research studentship followed by a fellowship thus enabling him to do what he really wanted namely to develop his tropical work, without the need to earn money and unencumbered by teaching and administration.

In 1930 Paul was introduced to Anne Hotham by Tom Harris whilst Anne was working on the Barnwell peat beds just before the 1930 Botanical Congress in Cambridge. Drawn together by the Biology Tea Club and a mutual love of music and plants the courtship survived the interruption of Paul's 1932 Borneo expedition until, at a summer camp at Studland in 1934, Anne announced that she had been offered a job in South Africa which prompted Paul to propose before leaving for 6 months in Nigeria. The couple became engaged in November 1934 and married in December 1935 after Paul's return from Southern Nigeria. This most happy marriage in some respects mirrored that of Paul's parents. Anne and Paul had four children (Fig. 3b) whom they took on numerous family holidays to beautiful and wild places (Fig. 4a). Apart from marriage, breeding and tropical expeditions, Paul travelled widely in Europe. Though much of the research was written up, in papers ranging from his early bryological works on the mosses of Glamorgan (1923), and Middlesex (1928) to his early tropical papers on the rain forests of British Guiana (1934) and Sarawak (1936), other treasures have been lost to science (Fig. 3a).

Paul began life as a PhD student in plant physiology (of *Prunus laurocerosus*) in 1931 under the supervision of F. F. Blackman, physiology then being the area most in vogue. Sensibly, and quickly, he realised that his interests and talents lay not in experimental work but in observation and description — 'the reading of vegetation'. After 2 weeks *Prunus* was forsaken in favour of comparative studies of rain forests under the watchful eye of Harry Godwin and with encouragement from Agnes Arber and E. J. Salisbury. Other catalysts towards this new direction were Schroeter's *Pflanzenzenleben der Alpen* and a symposium on European beech forests at the Cambridge Botanical Congress in 1930.

The war years interrupted work on *The Tropical Rain Forest* but were not entirely bereft of botany or unproductive. Paul as a University demonstrator (from 1938) was the youngest member of the Botany School not to be called up on condition that his vacations be devoted to Government work. Initially his instruction was to search England for *Frangula alnus* used to make fuses and previously imported from France; a car and petrol allowance were provided. When *Frangula* was superseded by electronic fuses in 1942 he worked for the Naval Intelligence Division contributing to Geographical Handbooks on how to get ashore and survive in alien lands. It is fortunate that these war works were so down to earth and did not involve equipment. Though Paul was clear thinking with matters organizational, and practical when it

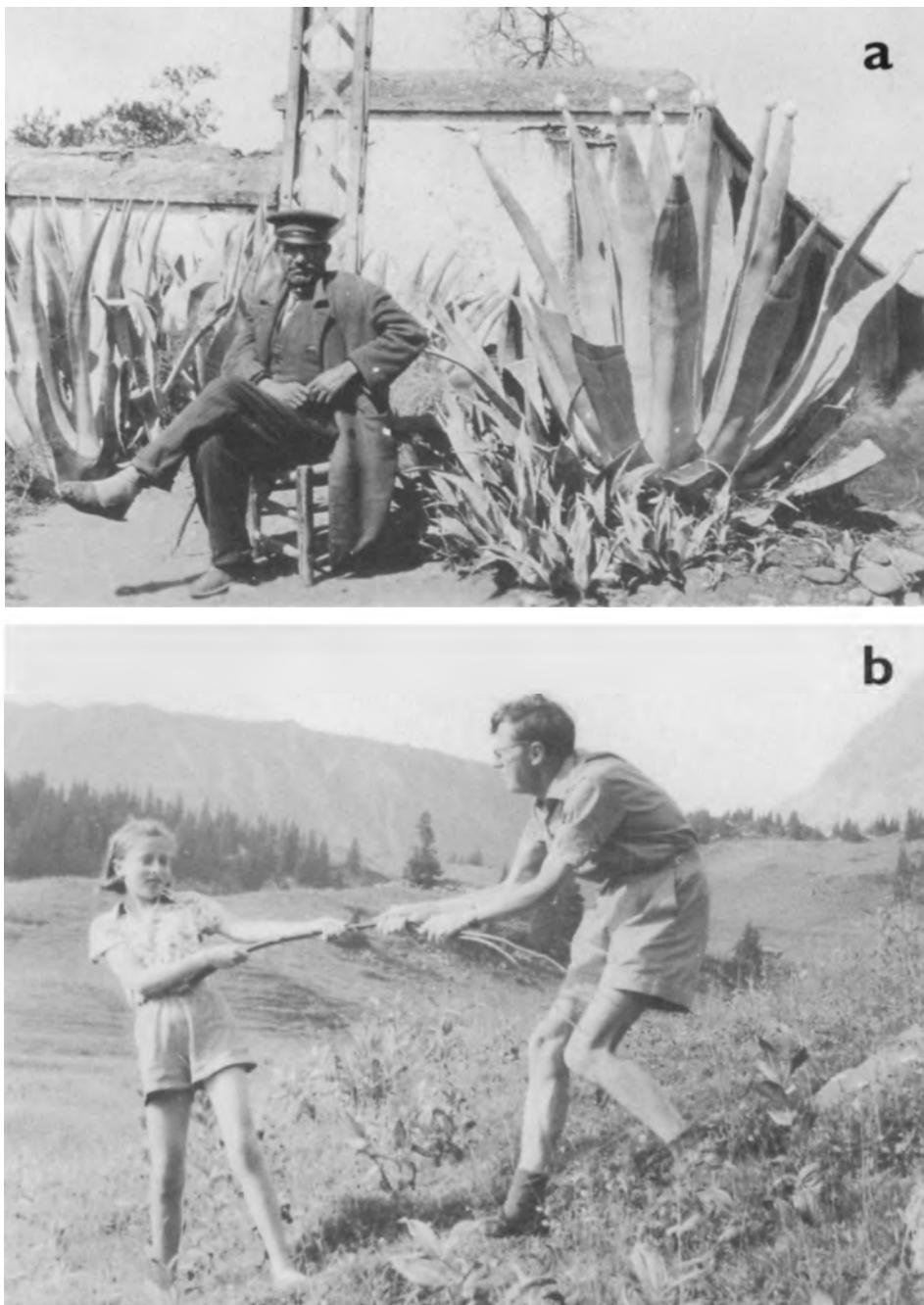


Figure 3. a. The 'egg' plant, (*Pseudoagave mirabilis* Nomen et planta fabricata), S. Spain with T. G. Tutin in 1931 – male left, female right. Paul travelled widely in Europe during the 1930s. Some of his more remarkable discoveries (un)fortunately were never published. The type specimen of this particularly plant was allegedly lost at sea on the return journey. b. Paul at play with daughter Sally on a family holiday, Körbersee, Vorarlberg, Austria, August 1961.



Figure 4. a. The summit of Plynlimmon Fawr, August 1947. Paul with Anne and their two eldest children, Martin and Catherine (Kate). b. Lecturing at a European Bryological Meeting, Hungary 1985.

came to plants and gardening, understanding machinery was not his forte. This minor failing is perhaps best illustrated by an incident involving the automobile. Paul drove throughout his life and on the open road never had an accident. He became proficient as a driver, according to his father, after only five of the six lessons recommended at a time before the days of compulsory driving tests. Reversing (lesson 6) into a garage however was not without its problems, whilst the workings of the machine remained a mystery and at times even the external features could be arcane in the extreme. During trips abroad Paul was in the habit of asking research students to look after his car and keep it in running order. One particularly conscientious student drove the car daily to and from the University. On the eve of the Richards' return to his horror he discovered a broken rear light. This was repaired and an immaculate vehicle returned to its owner. Invited to tea a few days later Anne remarked, 'I don't know what is coming over Paul these days—just before we went away he told me he had broken a rear light on the car whilst reversing into the garage but now there's nothing wrong with it!'

The war years also saw the birth of the *Biological Flora of the British Isles* — an idea conceived by E. J. Salisbury in 1928. In 1940 Paul, A. R. Clapham & W. H. Pearsall became the editorial committee with the first account, on *Juncus*, by Richards & Clapham following in 1941.

After the war Paul continued his multifarious botanical activities as a lecturer in Cambridge (1945–9) before moving to the University College of North Wales, Bangor to take up the Chair of Botany which he held until retirement in 1976. As Paul freely admitted, administrative duties sadly became increasingly detrimental to his botanical creativity. His period as Vice-Principal of the College from 1965–7 was particularly difficult as it coincided with the Principal experiencing serious health problems thus necessitating Paul's involvement as his deputy. Running the Botany department was also not without its difficulties: a dual problem of recruiting high quality new staff and an inheritance of others who were not an unqualified success. In the latter category was a nocturnal senior lecturer. Commenting many years later, about this singular individual Paul said, 'He tried my patience to the limit but in all honesty I cannot say that I have any ill feelings towards him'. This perhaps epitomizes Paul's generous nature; he was a man without rancour and bitterness, non-judgmental, accepting and tolerant of people as they were.

The Bangor years saw a heavy involvement in nature conservation in Britain. In addition to membership of the Nature Conservancy (later the Nature Conservancy Council), from 1954 to 1967 he was also Chairman of its Committee for Wales as well as working on the National Parks Commission from 1955–59. A founder member of the North Wales Naturalists' Trust he became its Chairman from 1969 to 1972. Hand in hand with conservation work was a major role in the British Ecological Society (joined 1926). He was editor of the *Journal of Ecology* from 1956 to 1963 and President from 1961 to 1963 during which time he founded the Tropical Ecology Group.

Paul did however manage to escape from time to time the trials of domestic university life and he visited many universities including Legon (Ghana,) Fourah Bay (Sierra Leone) and Ibadan (Nigeria) (Fig. 5a) as Visiting Professor. Other major overseas trips included the Royal Society Mato Grosso Expedition, Brazil (1967 & 1968) and a Bullard Fellowship at Harvard (1964–5). Tenure of the latter saw him teaching the first tropical botany fieldcourse in Costa Rica for 'The Organization for Tropical Studies' and the establishment of strong links with many Americans working in the tropics. Following on from this, and his knowledge of, and concern for, the biology of the tropics, in 1971 he was invited as one of two Britons onto a committee of the American Academy investigating the effects of the herbicides used in Vietnam. His visits to that country were the most distressing episodes of his life (Richards, 1984). Subsequently he refused to sign the official report on the grounds that it was an uninformed whitewash bearing little relation to the truth, but he and two others produced a minority document containing very different conclusions. Unfortunately the report was published the same week that the Watergate scandal broke and the impact it should have had was lost. The lasting legacy of Paul's commitment to and vision of tropical conservation was the establishment in 1986 of the Korup National Park in S. W. Cameroon, an area of primary rainforest containing over 400 tree species.

Finally home life. Although Paul was always heavily engrossed in some botanical



Figure 5. Paul in the tropics. a. Lake Bambalui, Nigeria, March 1995. Left to right; Charles Onochie, David Coombe, Geoffrey Berrie, Paul and a forest guard. b. Malaya 1974 still climbing trees just before retirement. c. Venezuela 1991; trainers have now replaced the sandals of earlier years.

activity or other, people always came first in his life. The Richards' house was always open to everyone and his generosity extended even into the garden. 'Come over for tea and a few cuttings' — usually meant you had to go back a second time because the donated boskage simply wouldn't fit into your car. Somehow the Richards' gardens, both in Bangor and subsequently in Cambridge, where Paul moved after retirement, increasingly took on the physiognomy of tropical forest; multistoried, biodiverse, always blooming, and most of all, rampant.

We will all miss Paul, a fine scholar, a man of immense wisdom, a generous man, an impractical man, a man free from rancour, a man with the highest ethical standards, a man who enjoyed life to the full at all levels from the intellectual to simply messing about in the countryside. Perhaps above all these things his real genius lay in the translation of the crystal-clear reading of nature at its most complex into the finest prose. It is most fitting and poignant therefore that one of the last acts of his life was correcting the galley proofs of the second edition of *The Tropical Rain Forest*.

ACKNOWLEDGEMENTS

My thanks are due to Anne and Martin Richards for loan of photographs and for background information on Paul's life. The interpretations are mine; I hope both these and the facts are a true and fitting picture of Paul. Other contributions from Arthur Willis, Mary Parker and many others amongst Paul's friends are gratefully acknowledged.

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2. The fossil record of early land plants and interrelationships between primitive embryophytes: too little and too late?

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2.1 SUMMARY

The megafossil record of early land plants is dominated by tracheophytes. The producers of the microfossils which extend much further back in the Palaeozoic (Llanvirn–Ordovician) remain conjectural, although spore configuration and ultrastructure suggest a bryophyte and more precisely hepatic affinity. In this paper, we review the evidence for this hypothesis, and present limited data on the morphology and anatomy of the spore producers. New research, particularly on exospore ultrastructure in dyads, reveals considerable diversity and demonstrates for the first time white-line-centred lamellae in a Lower Devonian fossil. Recent cladistic analyses suggest that liverworts, mosses and hornworts predate the tracheophytes. Here we bring together information on character states in a range of Silurian and Devonian plants, using information from conventional megafossils and more recently discovered mesofossils, that show exceptional cellular preservation in coalified fragments. Characters include features of the life-cycle, conducting tissues, macromolecules (associated with water conduction (lignin) and desiccation resistance), and sporangial anatomy including dehiscence and spores. It is concluded that a major barrier to the use of such fossils in phylogenetic interpretations is that their occurrences are in sediments deposited some tens of millions of years after the first microfossil evidence for life on land, and for the most part, the plants are at the tracheophyte grade. The anatomical potential of small, and, until recently, overlooked fossils prompts the search for further records in older rocks and presents fresh opportunity for more direct information on early bryophytes and the first tracheophytes, here hypothesised to have been very small plants.

KEYWORDS: Bryophytes, sporangia, spore ultrastructure, sporophyte and gametophyte generations, vascular tissue.

2.2 INTRODUCTION

The major advances of recent years in the descriptions and classification of megafossils of the earliest tracheophytes, including intensive collection from new localities and re-examination of existing taxa, have not been matched by discoveries in

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bryophyte palaeobotany. The megafossil record of bryophytes during critical phases of early terrestrialisation in the mid-Palaeozoic remains negligible, resulting in doubts on their very existence during this period and indeed, if they were to be found, their value in phylogenetic reconstructions.

It is therefore appropriate that the hundredth anniversary of the British Bryological Society should coincide with a major revival of interest in fossil bryophytes prompted by (1) the suggestion that the spores comprising the earliest evidence for land plants in the Ordovician derived from plants at a bryophyte level of organisation (e.g. Gray, 1985; Taylor, 1996) (2) the hypotheses produced by cladistic analyses of embryophytes and algae on the nature and interrelationships of the earliest bryophytes (e.g. Mishler & Churchill 1984; Mishler *et al.* 1994).

In the first part of this paper, the relevant microfossil evidence from Silurian to Lower Devonian rocks will be documented and the affinities of spores and other fragments, based on ultrastructure and *in situ* studies, reviewed. In the second part, character states recorded in the megafossils of the earliest tracheophytes, which have been used in cladistic analyses, will be surveyed. The diversification of tracheophytes began in the latest Silurian and continued with increasing intensity in the early Devonian, this being some 60 million years after the first Ordovician records of spores. It would be impossible to include all the relevant information here, and we therefore propose to concentrate on Lower Devonian (Pragian) Rhynie Chert fossils which show exceptional cellular preservation (e.g. rhizoids), and also on a relatively new source of data on early land plants, viz. semi-compressed, coalified mesofossils (Edwards, 1996). The latter derive from plants thought to be of approximately the same dimensions as bryophytes and as the first presumed tracheophytes in mid-Silurian times (*Cooksonia*: Edwards & Feehan, 1980).

2.3 THE MICROFOSSIL RECORD

The early land plant microfossil record comprises dispersed spores and fragmentary cuticles and tubes (Figure 1). There are two main lines of evidence suggesting that the spores derive from land plants: firstly, they are morphologically similar to spores of certain extant land plants; secondly, they are distributed in a similar range of depositional environments, including continental deposits, in which spores and pollen of extant land plants occur. The fragments are also believed to derive from land plants because they occur in continental deposits and, to a certain extent, resemble structures present in extant land plants, although their affinities are more controversial. Furthermore, resistant spore walls and cuticles appear to be adaptations for survival in the terrestrial environment, suggesting that the plants represented by these microfossils spent at least part of their life-cycle subaerially exposed.

2.3.1 Dispersed spore fossil record

Dispersed spores, which first appear in the Llanvirn (mid Ordovician) (Strother, Al-Hajri & Traverse, 1996), provide the earliest evidence for land plants (Gray & Boucot, 1971). The earliest forms, comprising monads and permanently united dyads and tetrads, are termed cryptospores, the name reflecting a lack of knowledge on

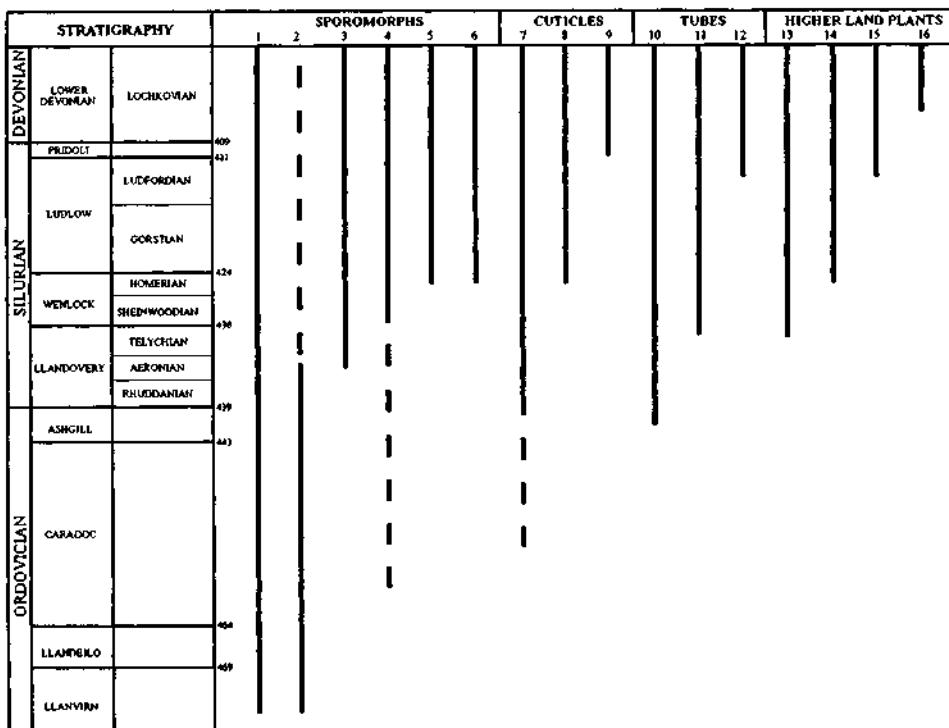


Figure 1. Stratigraphical distribution of early land plant fossils (microfossils and megafossils). (1) Naked cryptospores (monads, dyads and tetrads); (2) Envelope enclosed cryptospores (monads, dyads and tetrads); (3) Trilete spores; (4) Hilate cryptospores; (5) Sculptured trilete spores; (6) Sculptured hilate cryptospores; (7) 'Nematothallus' cuticles; (8) Higher land plant cuticles; (9) Stoma; (10) Laevigate tubes; (11) Banded tubes; (12) Tracheids; (13) Sterile axes; (14) Rhyniophyoids/Rhyniophytes; (15) *Baragwanathia*; (16) *Zosterophylls*

the nature of the producers (see Richardson, 1996a and references therein). They may be naked or enclosed within a thin, laevigate or variously ornamented envelope. The composition of the latter is unknown, but its persistence in such ancient rocks suggests that it was formed of sporopollenin or a sporopollenin-type macromolecule. Thus both the chemical nature and the homology of the envelope are unknown, although Gray points to similarities with perispore (Gray, 1991). The nature of cohesion between spores in permanently united dyads and tetrads is unclear. They are termed *unfused* if there is a superficial line of attachment between the spores and cohesion probably results from localised exospore links or bridges rather than large scale fusion. They are termed *fused* if there is no discernible line of attachment and cohesion probably results from fusion over all or most of the contact area. Cohesion may also be a consequence of enclosure within a tightly adherent envelope. Cryptospore assemblages are remarkably constant in composition throughout the Ordovician and earliest Silurian, suggesting that the earliest terrestrial vegetation was of limited diversity and possibly experienced little evolutionary change during this period, which extended over at least 40 million years (Gray, 1985; Wellman, 1996). However, in the late Llandovery (Early Silurian) trilete spores appeared, at

first in small numbers, and while envelope-enclosed cryptospores virtually disappeared, naked cryptospores (monads, dyads and tetrads) continued to dominate spore assemblages in terms of abundance. At approximately the same time, hilate cryptospores, deriving from dyads that dissociate prior to dispersal, are first recorded. Trilete spores and hilate cryptospores increased in abundance and diversity throughout the Silurian, but naked monads, dyads and tetrads remained important elements of the microflora (Richardson, 1996a). In the earliest Devonian, hilate cryptospore numbers began to diminish, and cryptospores became a rather minor component of spore assemblages as trilete spores continued to proliferate.

2.3.2 *Affinities of dispersed spores*

a) Comparative morphology. Based on analogy with the reproductive propagules of extant embryophytes, Jane Gray has argued persuasively that cryptospore permanent tetrads derive from plants at a bryophyte, most likely hepatic, grade of organisation (Gray, 1985, 1991 and references therein). The absence of body fossils in coeval rocks suggests that the producers, like bryophytes, lacked the recalcitrant polymers that enhance fossilisation potential. Gray noted that among extant free-sporing embryophytes, permanent tetrads are normally produced only by certain liverworts and mosses, citing *Sphaerocarpos* as a genus in which many of the tetrads are contained within an envelope, similar to those enclosing certain cryptospore tetrads. The affinities of cryptospore monads and dyads are more equivocal, and the relationship of the different cryptospore morphotypes unclear. Because cryptospore monads, dyads and tetrads occur enclosed within identical envelopes, it has been suggested that they are closely related, possibly deriving even from a single species. The occurrence of such dyad morphotypes is most comfortably explained by invoking normal meiosis, but with cytokinesis following the first meiotic division and sporopollenin deposition on the isolated pairs of the second. However, dyads rarely occur in extant non-angiosperm embryophytes and always as a result of meiotic irregularities (Fanning, Richardson & Edwards, 1991; Gray, 1993; Richardson, 1996b) and, in the absence of modern counterparts, the adaptive significance (if any) of their production by early land plants remains unknown. What is clear is that dyads are very common in the early land plant dispersed spore record, and their production was not confined to unusual circumstances. Trilete spores, on the other hand, have a clear counterpart among extant embryophytes. Their production is widespread among extant free-sporing tracheophytes, and also occurs, albeit less commonly, among bryophytes. The disappearance of dyads in the Lower Devonian and the persistence of trilete monads probably relates to other beneficial factors in the life-cycle of the producers of the latter, rather than to type of spore.

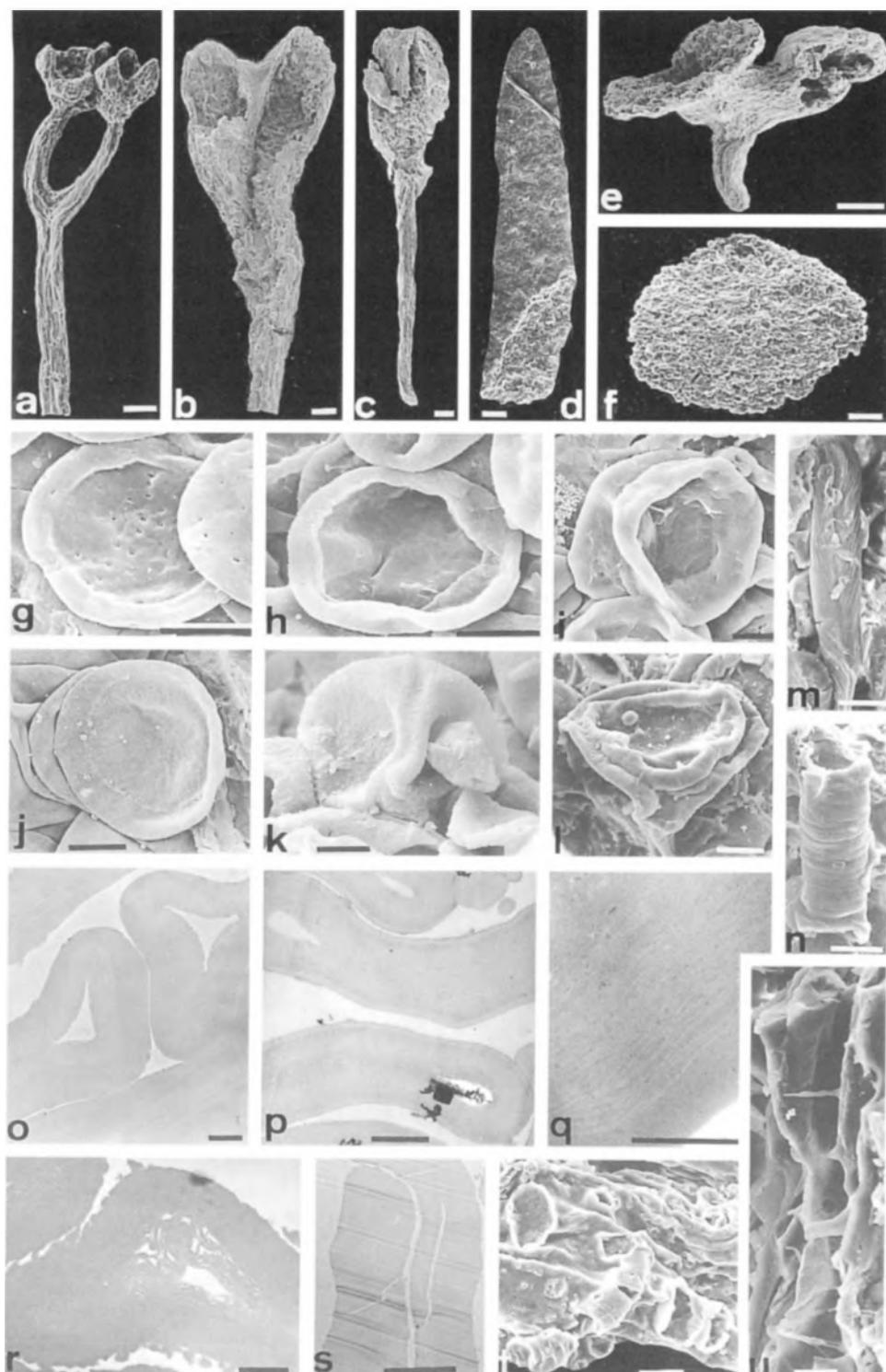
b) *In situ* cryptospores. Plant megafossils are unknown until the Silurian, when they are extremely rare, with restricted temporal and spatial distribution (Edwards, 1990), and are seldom sufficiently well preserved to permit useful analysis of *in situ* spores. The recently discovered assemblages of exceptionally preserved early land plant fossils, from uppermost Silurian and lowermost Devonian deposits from the Welsh Borderland, contain remarkably well preserved *in situ* spores (Edwards, 1996 and references therein). The mesofossils include spore masses, isolated sporangia, and

more complete plant fragments where sporangia terminate smooth usually isotomously branching or unbranched axes (Fig. 2b-f). Some contain tracheids. The majority lack evidence for tracheids and the fossils have been termed rhyniophytoid, because in gross morphology they resemble the simplest vascular plants.

Most of the sporangia, both rhyniophytoid (e.g. *Resilitheca*; Edwards *et al.*, 1995c) and tracheophyte (e.g. *Cooksonia pertoni*; Lang, 1937) contain single trilete spores (e.g. Fanning, Richardson & Edwards, 1988). However, cryptospores occur in a number of different types of mesofossil. Naked tetrads (Fig. 2l) resembling *Tetrahedraletes medinensis* Strother and Traverse emend Wellman and Richardson (1993), known from at least the Llanvirn (Ordovician) to the Lochkovian (Early Devonian), have been recorded in the terminal bifurcation of a small ?axial fossil (Fig. 2b) in which Edwards, Duckett & Richardson (1995b) detected some anatomical features reminiscent of hepatic. Such features included a single strap-shaped structure with herring-bone surface patterning located in the spore cavity (Fig. 2m), and cells with inward spine-like projections (Fig. 2u) or more regular ring-shaped thickenings (Fig. 2t). The latter were compared with rhizoids, but more recently have been considered as a fungal contaminant (Edwards, Abbott & Raven, 1996a). The only well-defined cells on the plant occur superficially in the depression between the sporangial cavities. An even less well preserved fragment comprises the cup-shaped basal regions of two sporangia (or a solitary bifurcating one) terminating a featureless short length of axis (Fig. 2e): the *in situ* spores are enveloped tetrads showing similarities with the dispersed taxon *Velatitetrads* (Fig. 2k). Further tetrads, both naked and envelope-enclosed, occur as discoidal masses or sporangia which provide even less information on the gross morphology of the plant.

Permanent dyads have been recovered from three specimens of earliest Devonian age. The specimen illustrated in Figure 2c comprises a cup-shaped cuticular sheath, containing naked dyads, and attached to an unbranched axis ca 1 mm in length. The dyads are naked, unfused and show distally invaginated walls (Fig. 2i). In contrast to the tetrad-containing plants with branched axes, the dyads occur in a sporangium terminating a short length of unbranched axis. The other specimens comprise dyads adhering to cuticular fragments that presumably represent sporangial cuticle. In one, the dyads are naked, unfused and with invaginated distal walls. In the other the dyads are unfused, with distally inflated walls, and covered in abundant extra-exosporal material. The dyads in all three specimens can be questionably assigned to the genus *Dyadospora* Strother & Traverse emend. Burgess & Richardson (1991) (Wellman, Edwards & Axe, in press).

Hilate cryptospores (*sensu* Richardson, 1996a) have been reported in spore masses, isolated sporangia and sporangia attached to short lengths of unbranched axis. They include laevigate and distally ornamented species. Laevigate hilate cryptospores (Fig. 2g, h, j) that would be placed in the dispersed spore taxon *Laevolancis divellomedia* (Chibrikova) Burgess & Richardson (1991) occur in spore masses (Fig. 2f), discoidal sporangia and elongate sporangia (Fig. 2d) of latest Silurian age, and in spore masses and discoidal sporangia of earliest Devonian age. Differences in the sporangial characteristics, coupled with subtle differences in the morphology and ultrastructure of the *in situ* spores, suggest that these morphologically similar spores may derive from a number of different plant types (see p. 22, Table 1; Wellman *et al.*, in press).



The uppermost Silurian and lowermost Devonian fossils with *in situ* cryptospores provide limited evidence regarding the nature of the plants that produced the different cryptospore morphotypes. However, the fossils are difficult to interpret, and their affinities and relationship with the trilete spore-producing rhyniophytoids and rhyniophytes remain obscure. It must also be emphasised that some of these cryptospore morphotypes, namely the permanent tetrads and dyads, were far less abundant in the dispersed spore record in the uppermost Silurian-lowermost Devonian than previous times (see pp. 17–18), and the plants with *in situ* examples must thus be viewed as relict taxa. Nevertheless they furnish some tantalising clues as to the gross morphology of the older cryptospore producers, the examples with branching axes indicating a more complex sporophyte than is present in bryophytes.

c) Spore wall ultrastructure. In an attempt to shed light on their affinities, Taylor (1995, 1996) has studied wall ultrastructure in Ordovician-earliest Silurian dispersed cryptospores. In the dyad *Dyadospora* sp. (uppermost Ordovician and lowermost Silurian: Ohio, USA) he reported a bilayered wall with an homogeneous outer layer (<1.5 µm in thickness) surrounding a clearly lamellate inner layer (1.4 µm in thickness). The lamellation consists of 10–15 lamellae which are 50–150 nm in thickness. Similarities with spore wall ultrastructure in extant sphaerocarpalean liverworts prompted Taylor to suggest that cryptospore dyads, and possibly other cryptospores, derive from plants with these affinities. However, Taylor found little or no evidence for layering or lamellation in other cryptospore morphotypes: the naked pseudodyad *Pseudodyadospora* sp. (uppermost Ordovician and lowermost Silurian: Ohio, USA) has an homogeneous exospore; the envelope-enclosed dyad *Segestrespora membranifera* (uppermost Ordovician: Ohio, USA) has an homogeneous “spongy” wall, with some irregular globular units and lamellae at the outside margin, and is surrounded by an envelope which is more electron dense than the wall, exhibits no structure and may be either attached or separated from the wall; the naked permanent tetrad *Tetrahedraletes medinensis* (uppermost Ordovician: Ohio, USA) has essentially homogeneous walls (Taylor, 1996).

Figure 2. Lochkovian (Lower Devonian) fossils from North Brown Clee Hill, Welsh Borderland: a–c, e, i–n, p, q, s–u; Pridoli (Uppermost Silurian) fossils from Ludford Lane, Ludlow: d, f–h, o, r. (a–n, t, u = SEMs; o–s = TEMs). a, branching sporophyte with terminal dehisced sporangia. NMW96.5G.6. Scale bar = 100 µm. b, bifurcating sporangium containing permanent tetrads. NMW94.76G.1. Scale bar = 100 µm. c, terminal sporangium containing laevigate dyads. NMW96.11G.6. Scale bar = 100 µm. d, fusiform, partially enveloped spore mass of dyads. NMW96.11G.2. Scale bar = 100 µm. e, bases of two sporangia containing enveloped tetrads. NMW96.11G.3. Scale bar = 100 µm. f, discoidal mass of dyads. NMW96.11G.1. Scale bar = 100 µm. g, example of a single hilate cryptospore (*Laevolancis divellomedia*). NMW96.30G.2. Scale bar = 10 µm. h, proximal surface of *L. divellomedia*. NMW96.30G.3. Scale bar = 10 µm. i, dyad from sporangium in (e). Scale bar = 10 µm. j, *L. divellomedia*, distal surface. NMW96.30G.4. Scale bar = 10 µm. k, enveloped tetrad from (e). Scale bar = 5 µm. l, tetrad from (b). Scale bar = 10 µm. m, strap-shaped structure in sporangium in (b). Scale bar = 10 µm. n, banded tube from (b). Scale bar = 10 µm. o, section through *L. divellomedia* from (f) showing bilayered exospore. Scale bar = 1 µm. p, section through *L. divellomedia* with bilayered walls from (j). Scale bar = 1 µm. q, white-line-centred lamellae in laevigate dyad. NMW96.30G.5. Scale bar = 0.5 µm. r, section through exospore with internal lamellae and extra-exosporal material from (h). Scale bar = 1 µm. s, homogeneous exospore in *L. divellomedia*. NMW96.30G.6. Scale bar = 5 µm. t, cup-shaped structures and banded tubes on surface of axis below bifurcating sporangium in (b). Scale bar = 10 µm. u, tube with needle-shaped ingrowths in axis of (b). Scale bar = 5 µm.

Cryptospore wall ultrastructure has also been studied in mesofossils from the uppermost Silurian and lowermost Devonian from the Welsh Borderland (work in progress). Naked permanent tetrads (cf. *Tetrahedraletes*) in a spore mass of Pridoli age are clearly unfused (*sensu* Wellman, 1996) and have homogeneous walls. Envelope-enclosed tetrads recovered from the Pridolin discoidal sporangium have homogeneous walls, surrounded by a less electron dense envelope that appears to be constructed of discrete granules that have been deposited over the tetrad and coalesced. Two Lochkovian specimens with intact sporangial bases (Fig. 2e) contain envelope-enclosed tetrads (Fig. 2k) with homogeneous walls, surrounded by a robust and continuous envelope that is also homogeneous but staining more darkly. Permanent dyads from the cup-shaped sporangium terminating an unbranched length of axis (Fig. 2c, i) have a bilayered wall comprising a thick spongy inner layer (*ca* 80% of the total thickness) and a narrow homogeneous outer layer (*ca* 20% of the total thickness). The two specimens with permanent dyads associated with fragments of ?sporangial cuticle contain dyads with walls that are homogeneous and spongy. Hilate cryptospore wall ultrastructure has been most extensively examined in the specimens containing the laevigate hilate cryptospore *Laevolancis divellomedia*. Wall ultrastructure reveals that at least five different types are present (Wellman, Edwards & Axe, in press). Pridoli spore masses and discoidal sporangia have spores with bilayered walls, the outer layer being more electron dense, with no extra-exosporal material (Fig. 2g, o). Pridoli elongate sporangia also have spores with bilayered walls, but the inner layer is strongly lamellated, and they are covered with abundant extra-exosporal material (Fig. 2h, r). Lamellae are present in the walls of spores from a Lochkovian spore mass (Fig. 2q). The walls are bilayered, the inner lamellated, and associated with extra-exosporal material. However, the lamellae are classic white-line-centred lamellae and are much narrower than the lamellae in the Pridolin specimens. Other Lochkovian examples occur in discoidal sporangia: some have homogeneous walls with no extra-exosporal material (Fig. 2s); others are bilayered with homogeneous walls, the thin outer layer being less electron dense, and are associated with extra-exosporal material (Fig. 2j, p). Similar diversity in wall ultrastructure is seen among trilete spores from the same locality (Rogerson *et al.*, 1993; Edwards *et al.*, 1995a, c; Edwards *et al.*, 1996b) and we have recently discovered trilete spores that clearly have lamellated walls (work in progress).

Such variation in cryptospore/trilete spore wall ultrastructure, as documented above, is not unexpected. Extant plants exhibit a bewildering diversity of spore wall ultrastructure (Tryon & Lugardon, 1991), resulting from variations in the contribution of different processes involved in spore wall development. However, the most primitive form of sporopollenin wall formation is believed to have involved deposition on lamellae (e.g. Blackmore & Barnes, 1987) and it seems likely that this process and the wall layers thus formed, are homologies in all groups of plants, including the earliest embryophytes (e.g. Blackmore & Crane, 1988). In most extant plants the lamellae are occluded to varying degrees by diverse processes during later ontogenetic stages, and are often not discernible at maturity. Nevertheless, in extant spores, lamellae remain visible in some mature spore walls, but these belong to a number of unrelated plant groups, thus limiting the value of this character in determining affinities in the fossils.

Studies of early land plant spore wall ultrastructure are still in their infancy, and

interpretation of such structure is complicated by the following: (1) the number of fossil taxa/morphotypes examined to date is relatively few, hampering recognition of patterns of character distribution; (2) interpretation of fossil spore wall ultrastructure is frustrated by problems associated with detection of taphonomic effects; (3) when dealing with fossils it may not be possible to recognise different ontogenetic stages; (4) when comparing spore wall ultrastructure in fossil and extant spores, it is unclear to what extent characters of ancient members of a group remain present in their descendants. However, ultrastructural studies are demonstrating extensive variation between *coeval* early land plant spore morphotypes and we anticipate that as more information becomes available, and patterns of character distribution are recognised, spore wall ultrastructure will become a major tool useful for ascertaining affinities among early land plants and testing phylogenetic relationships. Particularly noteworthy in this respect would be detailed comparative studies on dispersed permanent and non-obligate tetrads and dyads from the critical upper Llandovery time interval when the first trilete spores also appeared.

2.3.3 Dispersed fragments

Enigmatic fragments (cuticles and tubes) appear in microfossil assemblages in the Ordovician; sheets of cuticle are known from the Caradoc (late Ordovician), smooth tubes from the Ashgill (late Ordovician) and tubes with internal annular and spiral thickenings (banded tubes) from the Llandovery (Early Silurian) (Fig. 1). By the Wenlock (Late Silurian) ornamented cuticles, and cuticles clearly derived from higher land plants (tracheophytes), are present, as are a number of kinds of tubes, often occurring in complex associations. Recent reviews of the nature of these fragments and their stratigraphic distribution are provided by Edwards & Burgess (1990) and Edwards & Wellman (1996). The affinities of such fragments have been a focus of fierce debate (Gray & Boucot, 1977; Edwards, 1982, 1986; Edwards & Rose, 1984; Gray, 1985). It is now generally accepted that the cuticles derive from land plants. Certain Late Silurian-early Devonian forms, showing marked alignment of 'epidermal cells', clearly derive from the axes of higher land plants (rhyniophytoids and rhyniophytes), while others, with well-defined outlines and occasionally with attached spores, derive from their sporangia. However, the older forms, which persist into the Early Devonian, are often interpreted as deriving from nematophytes. These are an enigmatic group of putative land plants of uncertain affinity, with somatic organisation based on smooth ± banded tubes, and believed by Lang to possess a cuticular covering (Lang, 1937). The recent demonstration that such cuticle does not have the same chemical composition as those from coeval axial higher plants adds weight to the suggestion that they were fundamentally different, and raises major queries as to their functioning (Edwards *et al.* 1996a). The affinities of the tubular structures are even less clear. Many are often regarded as deriving from fragmentation of nematophytes. Others appear more like fungal hyphae and possibly derive from early terrestrial fungi (Sherwood-Pike & Gray, 1985). A recent discovery, lending weight to the suggestion of a fungal origin for certain tube types, is the report of banded tubes fused to, and apparently growing on, higher land plant fossils (Edwards *et al.*, 1996a). In addition, some of the associations resemble *Prototaxites* which has been attributed to the fungi (Hueber: Munster workshop, 1994). Finally, the recent

discovery of lichenised cyanobacteria in the Rhynie Chert (Taylor *et al.*, 1995), raises the possibility of a lichen source. Thus the affinities of the tubes, and to a lesser extent the cuticles, remain contentious. Indeed, it has recently been suggested that some of the cuticles and tubes derive from fragmentation of fossil bryophyte sporangia (Kroken, Graham & Cook, 1996). However, we find little support for this hypothesis (as regards size and symmetry) in our fossils.

2.4 THE MEGAFOSIL RECORD

2.4.1 *Alternation of generations*

The nature of the life-cycle in the earliest vascular plants is of particular interest not only in assessing hypotheses on the origin of the sporophyte generation but also on relationships between bryophytes and tracheophytes. Our best direct evidence comes from the Rhynie Chert, from plants which existed some 35 million years after the first records of vascular plants, where three taxa (*Aglaophyton*, *Horneophyton*, *Nothia*), unfortunately all somewhat difficult to place in existing classifications of early land plants, show isomorphic alternation of independent generations, with homoiohydric axial gametophytes (see later section). Tracheophytes, such as *Zosterophyllum* and *Huyvenia/Stockmansella*, are supposed to have had similar life cycles (Schweitzer, 1983). Rothwell (1995) has postulated that the small isotomously branching sporophytes of *Cooksonia*-like plants would have been held above the ground and remained permanently attached to the gametophyte. Evidence for the parasitic sporophyte comprises the small size of the cooksonias and their lack of rhizoids or roots. In our opinion the absence of anchoring organs is probably taphonomic: all Silurian examples were 'uprooted' and buried in marine sediments (Edwards, 1990).

2.4.2 *Sporophytic branching*

Kenrick & Crane (1991) recently created the informal grouping polysporangiophytes to include 'conventional' tracheophytes and those (e.g. *Aglaophyton*) where conducting cells do not have the same construction as tracheids. The numerous sporangia are produced on sporophytes showing branching. This ranges from predominantly isotomous \pm regular branching in axial fossils in the Silurian to highly diverse types in the Lower Devonian (Edwards, 1994; Remy & Hass, 1996). Problems arise in trying to establish *absence* of sporophytic branching in fertile material. The majority of our fertile mesofossils comprise a sporangium terminating a short length of unbranched axis. In longer specimens, branching is sometimes preserved. It would clearly be unrealistic to reach conclusions on branching on such fragmentary material unless very large numbers of varying size of specimens are available. However, in *Sporogonites exuberans*, large obovate to clavate sporangia terminate unbranched axes at least 10 cm long. The axes are straight and of such strikingly consistent width that they give the impression of great rigidity (Halle, 1916, 1936; Stockmans, 1940). Halle (1916) concluded that *Sporogonites* was a 'sporogonium of the Bryophyta of a "generalised type".' In contrast, the solitary, similarly-shaped sporangia of Upper Silurian *Tortilicaulis transwalliensis* terminate unbranched, much shorter, but twist-

ing, naked axes (Edwards, 1979). The axial twisting and absence of branching prompted favourable comparison with bryophyte sporangia, but unequivocal anatomical evidence for such affinities is lacking. Lower Devonian specimens with similar axial and sporangial gross morphology but with isotomous branching, have recently been placed in *T. offaeus*, but again absence of anatomy in the oldest material casts doubt that they are congeneric (Edwards, Fanning & Richardson, 1994).

2.4.3 Apices/Meristems

Two principal types of shoot apex occur in extant embryophytes—the pyramidal apical cell in bryophytes, Psilotales, *Equisetum*, ferns and *Selaginella* and the one or more layers of meristematic cells in seed plants and other lycophytes (Phillipson, 1990). He emphasised that the fundamental distinction is not in number of apical initials, but in subsequent planes of cell division, with periclinal division absent (except in the root) in the apical cell types—a distinction almost impossible to recognise in the fossil record. Indeed it would seem rather unlikely that ‘delicate’ meristematic cells would be preserved. Yet in the Rhynie Chert, Kidston and Lang described three apices of *Rhynia gwynne-vaughanii* with numerous closely packed isodiametric cells forming a cuticle-ensheathed, dome-shaped structure lacking any indications of an apical cell. Tangential sections show packets of four cells—the products of regular anticlinal divisions. Similar apices occur in *Aglaophyton* (Hass (Munster), pers. comm.), but in ‘lateral sub-apices’, Hass has found a central three-sided apical cell surrounded by derivatives, the meristem slightly raised above the apex surface. On the uppermost surfaces of rhizomes, the same shaped apical cells are thought to mark the position of incipient aerial axes. Hass recorded a similar pattern of meristems in *Rhynia*. Equally amazing is the demonstration of a single three-sided apical cell in a young gametophyte of *Aglaophyton* (Remy & Hass, 1996, Pl. I, Fig. 9) prior to the development of vascular tissue, and a single two-sided apical cell in a young sporeling of *Horneophyton* (Hass, pers. comm.). These and the observations of the sporophytic apical cells in lateral positions led Hass to suggest that the young sporophytes of *Rhynia* and *Aglaophyton* might have possessed an apical cell. Such changes during development possibly related to increase in size of the apex are not seen in extant plants (Phillipson, 1990).

In contrast, in two further sporophytes from the Chert, the lycophyte *Asteroxylon mackiei* has a flattened apex with a number of small central apical initials surrounded by leaf primordia (Hueber, 1992) and a similar but less well-preserved lateral apex is seen in the zosterophyll, *Trichopherophyton* (Lyon & Edwards, 1991).

2.4.4 Gametophytes

Remy and co-workers in Munster have recently described three types of unisexual gametophyte in the Rhynie Chert, which they relate to three sporophytic taxa on anatomical similarities in water conducting cells and on epidermal features (Remy, Gensel & Hass, 1993). The gametophytes share a basic architecture comprising an unbranched axis terminating in a cup-shaped gametangiophore with variously shaped sterile outgrowths. All possess vascular tissue and cuticle with stomata. Thus apart from the lack of axial branching in the gametophyte the generations are isomorphic.

Antheridia on the lobed gametangiophore of *Lyonophyton* (*Aglaoiphyton*) are spherical to reniform, columellate, possibly operculate and borne on short stalks (Remy & Hass, 1991a), while those of *Kidstonophyton* (*Nothia*) are clavate and conspicuously stalked (Remy & Hass, 1991b). The antheridial wall may be thin and single layered (*Kidstonophyton*) or possess 1–2 layers of cells (*Lyonophyton*) (Remy & Hass, 1991c), while the sperm (ca 2 µm diameter) are linear and spirally corked. *Langiophyton*, the gametangiophore of *Horneophyton*, has numerous irregular projections, some bearing archegonia. These have massive free necks with a wall composed of many tiers of cells (8–12 cells/tier) the basal layer possibly 2 cells thick in part, surrounding a long neck canal. The egg chamber is deeply sunken. Modification of tissue in the vicinity of the venter led Remy *et al.* (1993) to suggest that the archegonium might have been embedded in a 'radially diverging mass of conducting tissue'. So unlike the male organs, the archegonia have no direct counterparts in the embryophytes.

Branching axial structures bearing probable gametangiophores are preserved as compression fossils (gametangia have not been observed), but in the Lower Devonian *Calyciphyton* cup-shaped structures terminate lateral unbranched axes in a pseudomonopodial branching system (Remy, Schultka & Hass, 1991), while in *Sciadophyton*, the radiating vascularised axes which terminate in disc-like presumed gametangiophores occasionally branch (Schweitzer, 1983). Unlike in the previous examples, axes interpreted as sporophytic have been found attached to these discs in *Sciadophyton*, which Schweitzer believes to be the gametophyte of *Zosterophyllum*. Schweitzer's reconstruction of the life cycle shows a phase very similar to the illustration of the Cooksonioid Model of growth proposed by Rothwell (1995) which he considers a 'candidate for the archetype of primitive land plants'.

All the gametophytes so far recorded have been axial: thalloid or leafy forms have not yet been recognised. In their possession of cuticles and water conducting tissues, small fragments of such axial gametophytes would be impossible to identify as such, even in permineralised fossils.

2.4.5 Water conducting tissues

Recent researches have demonstrated a surprising diversity in presumed water-conducting cells when compared with extant 'pteridophytes' although few have been subjected to the same scrutiny by SEM as the fossils (Hartman & Banks, 1980; Kenrick & Crane, 1991; Kenrick, Edwards & Dales, 1991a; Kenrick, Remy & Crane, 1991b). Perhaps unexpectedly, the tracheids often associated with protoxylem in extant plants (viz. cellulose primary walls and distinct annular or spiral secondary thickenings) have not yet been recorded in early tracheophytes even in the Rhynie Chert where details of such tissue are difficult to elucidate (see *Aglaoiphyton*). Instead the wall between secondary thickenings appears lignified and pitted, even on the rare occasions where protoxylem is preserved (see Li & Edwards, 1995). Closest to extant forms are the G-type tracheids characteristic of zosterophylls and early lycophtyes (e.g. *Gosslingia*: (Kenrick & Edwards, 1988); *Baragwanathia*: (Hueber, 1983)) in which conventional annular or spiral secondary thickenings are connected by an additional presumed secondary wall with small (presumed) pits that vary in number, size and shape (Fig. 3a). An elaboration of this basic type is seen in *Psilophyton* where a perforated, presumed lignified sheet extends across the apertures of scalari-

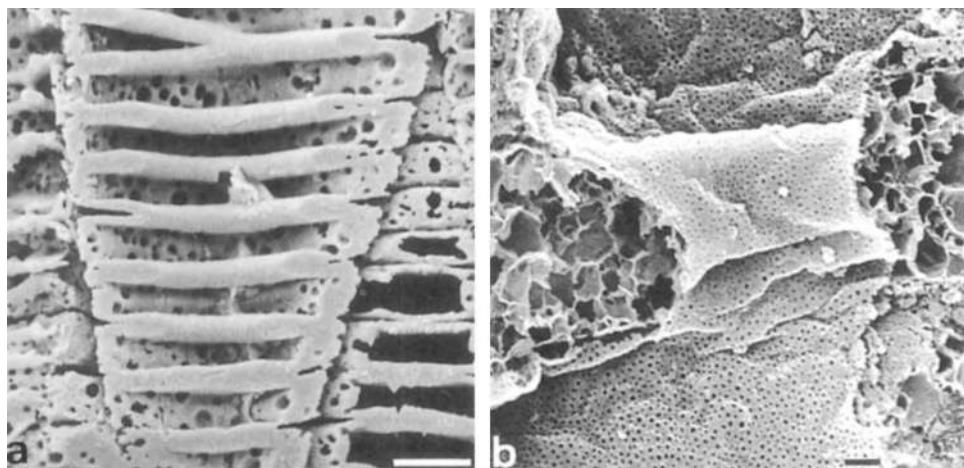
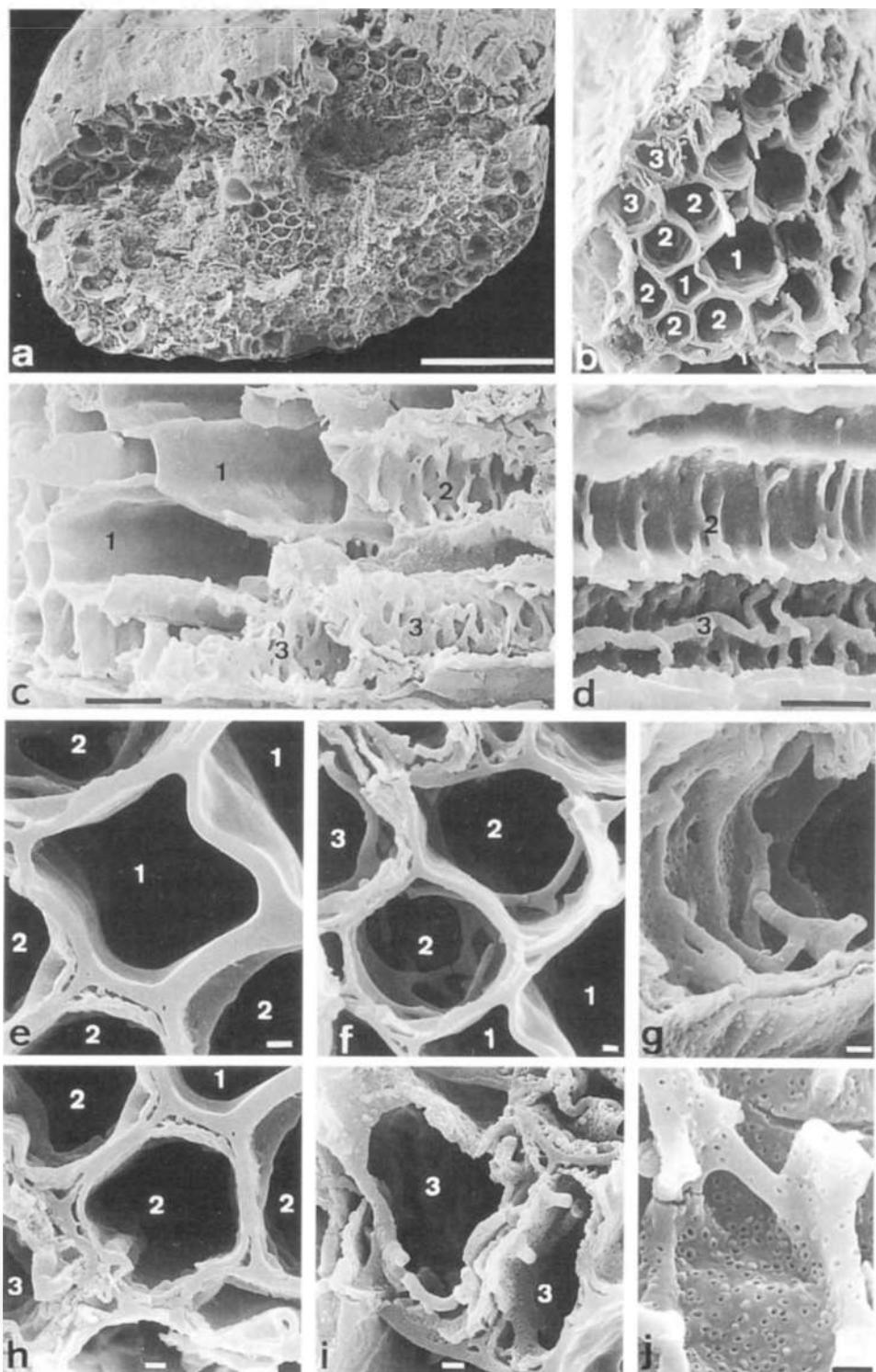


Figure 3. SEMs of internal surfaces of presumed water-conducting cells. **a**, demineralised G-type tracheid of *Gosslingia breconensis*, Pragian, Brecon Beacons Quarry, S. Wales. Stub 143. Scale bar = 10 µm. **b**, demineralised S-type tracheid of *Sennicaulis hippocrepiformis*, Pragian, Mill Bay West, Pembrokeshire, S. Wales. NMW90.42G.3. Scale bar = 1 µm.

form (bordered) pits (Banks, Leclercq & Hueber, 1975). S-types are less familiar in that the area conventionally occupied by cellulose primary wall and ligno-cellulose annular or spiral secondary thickenings has a honeycombed appearance and is overlain by a distinct thin layer with numerous pits or perforations the size of plasmodesmata (Fig. 3b) (40–200 nm diameter with a density of $16 \mu\text{m}^{-2}$). In that the layer persists in the fossils it is assumed to have been lignified. Such cells occur in *Rhynia gwynne-vaughanii*, *Stockmansella*, *Sennicaulis hippocrepiformis* and *Huvenia kleui* (Kenrick *et al.*, 1991b) and were used to unite these taxa into the family Rhyniaceae (Kenrick & Crane, 1991). Kenrick and Crane suggested that such perforations represent an intermediate state between mosses and vascular plants. Our systematic processing by SEM of axes of mesofossils from the Lochkovian have, to date, yielded two further types of conducting strands. In *Cooksonia pertoni* cells towards the centre of the axis are thick-walled with internal annular thickenings (Edwards, Davies & Axe, 1992). A far more complex arrangement has recently been found in a single parallel-sided sterile axis measuring 1.38 mm × 0.38 mm (Fig. 4a). There is a central strand approximately six cells wide which comprises roughly 2% of the volume of the axis. The outermost part of the axis comprises a sterome 1–3 cells thick, the two outermost layers having thick homogenised walls. The area between the sterome and the central strand had been completely filled by pyrite crystals as had all the cells in the sterome and the central strand itself, making it initially impossible to see any detail of the anatomy. The pyrite was therefore removed using fuming nitric acid during which process the axis fell apart. However the central strand remained intact as a cylinder although it fractured into two pieces transversely (Fig. 4b). This allowed the anatomy of these cells to be studied in detail, revealing three distinct types of cell (labelled 1–3 on Fig. 4b–i). There is a central zone comprising 3–4 cells (labelled 1 on Fig. 4) with diameters ranging from 8–15 µm, which may be as a result of tapering although this has not been seen in 100 µm lengths of the same cells exposed



in LS. These cells have completely homogeneous walls (Fig. 4e) and while in TS they appear to be finely corrugated internally, in fractured LS (Fig. 4c) they are completely smooth. Surrounding these cells is a single layer of cells (labelled 2 on Fig. 4) 8–12 µm in diameter forming a ring of approximately ten cells which share the homogeneous wall with the central cells but attached to this wall internally is a microporous layer which is extended into a bizarre array of thickened elements (Fig. 4d, 4f, 4g). The diameter of the pores ranges from 79–185 nm with a density of 0.8 µm⁻². This layer is only loosely attached in places giving the appearance of a layer that is 'sloughing off' (Fig. 4h). This may be due to shrinkage during fossilisation. The thickenings in places look annular but there are also numerous cross bars (Fig. 4g). On the outside of the strand is a layer (labelled 3 on Fig. 4) 1–2 cells thick of very variable appearance. Once again there appears to be a homogeneous wall but these cells are distorted and it is not always clear (Fig. 4i). Internally there is an even thicker layer which is either microporous or covered in tiny globules (*ca* 15–35 nm diameter) and forms a diverse array of thickenings (Fig. 4c, d). In a few rare examples there are globules adjacent to pores with rims suggesting that there is some relationship between them (Fig. 4j). In the distribution of central thin and encircling thicker-walled cells, this single specimen resembles *Aglaophyton* (see below), but the very different preservation status makes detailed comparison unrewarding, particularly in view of the controversy surrounding the nature of the wall-thickenings in the Rhynie plant. The microporous layer resembles that in S-type tracheids (Fig. 3b), where the internal thickenings are much more regular and cover a matrix with spongy appearance. It may be significant that the central cells in *Sennicaulis hippocrepiformis* are not preserved.

2.4.6 *Aglaophyton* major

Rhynia major (Kidston & Lang, 1920) was renamed *Aglaophyton major* (more correctly *A. majus*: MacGregor pers. comm.) by D. S. Edwards (1986) in recognition of its lack of conventionally thickened tracheids. He saw similarities in the three-zoned conducting strand with those in certain polytrichaceous mosses, although other anatomical and morphological features were those of coeval homoiohydric tracheophytes. The terete central conducting strand has two zones of thin-walled cells, the outer interpreted as phloem (see next section), separated by a thick-walled

Figure 4. SEMs of fractured coalified axial mesofossil, Lochkovian, North Brown Clee Hill, Shropshire, NMW96.30G.1. a, shows the axis before treatment to remove pyrite crystals which are infilling the cells. b-j fresh fractured surfaces revealed after treatment to remove pyrite. 1, 2, 3 are the three cell types referred to in the text. a, transversely fractured axis showing central strand and sterome. Scale bar = 10 µm. b, close up of ± intact isolated central strand. Scale bar = 10 µm. c, details of cells from (b) viewed obliquely showing central smooth-walled examples (1) with irregularly ornamented surrounding cells (2+3). Scale bar = 10 µm. d, cells in fractured LS showing the nature of the thickenings. Scale bar = 10 µm. e, central cell (1) from (b) with homogeneous wall, surrounded by cells with two-layered wall (2). Scale bar = 1 µm. f, detail of cells (2) from (b) which surround the central cells, showing intrusion of thickenings into the lumen. Scale bar = 1 µm. g, Detail of cells (2) from (c) to show microporous layer. Scale bar = 1 µm. h, detail of cells (2) from (b) to show the bilayered wall. Scale bar = 1 µm. i, Detail of cells (3) showing distorted bilayered walls and irregular thickenings. Scale bar = 1 µm. j, detail of microporous inner surface of wall with thickenings, showing globules adjacent to pores with rims. Scale bar = 1 µm.

Table 1. Summary of ultrastructure of cryptospores (dyads and tetrads) based on in situ and dispersed representatives. LL = Ludford Lane, Ludlow, Shropshire; NBCH = North Brown Clee Hill, Shropshire.

Locality/age	Description of mesofossil	In situ spores	Spore wall ultrastructure
LL Pridoli	Spore mass	Naked unfused permanent tetrads cf. <i>Tetrahedraletes</i>	Walls homogeneous
LL Pridoli	Discoidal sporangium	Envelope-enclosed permanent tetrads <i>Velatitetras</i> sp.	Walls homogeneous. Envelope constructed of discrete homogeneous granules that have coalesced
NBCH Lochkovian	Basal parts of two sporangia terminating a short length of unbranched axis	Envelope enclosed permanent tetrads <i>Velatitetras</i> sp.	Walls homogeneous. Envelope homogeneous but staining darker than the walls
NBCH Lochkovian	Cup-shaped cuticular sheath attached to an unbranched axis	Naked unfused permanent dyads <i>Dyadospora</i> sp.	Walls bilayered: thick spongy inner layer with narrow homogeneous outer layer
NBCH Lochkovian	Spores adhering to fragments of sporangial cuticles	Naked unfused permanent dyads <i>Dyadospora</i> sp.	Walls homogeneous and spongy
LL Pridoli	Spore masses and discoidal sporangia	Laevigate hilate cryptospores <i>Laevolancis divellomedia</i>	Walls bilayered: outer layer more electron dense. No extra-exosporal material
LL Pridoli	Elongate sporangia	Laevigate hilate cryptospores <i>Laevolancis divellomedia</i>	Walls bilayered: inner layer lamellate. Abundant extra-exosporal material
NBCH Lochkovian	Discoidal sporangium	Laevigate hilate cryptospores <i>Laevolancis divellomedia</i>	Walls bilayered: inner lamellated with white-line-centred lamellae. No extra-exosporal material
NBCH Lochkovian	Discoidal sporangium	Laevigate hilate cryptospores <i>Laevolancis divellomedia</i>	Walls homogeneous. No extra-exosporal material
NBCH Lochkovian	Discoidal sporangia	Laevigate hilate cryptospores <i>Laevolancis divellomedia</i>	Walls bilayered: inner thick, grainy and homogeneous, outer thin and homogeneous. Abundant extra-exosporal material
Ohio Ashgill (Taylor, 1995)	Dispersed spore	Naked unfused permanent tetrad <i>Tetrahedraletes medinensis</i>	Walls homogeneous, possibly with subtle lamellation
Ohio Ashgill/ Llandovery (Taylor, 1996)	Dispersed spore	Naked fused dyad <i>Pseudodyadospora</i> sp.	Walls homogeneous

Table I. (continued).

Locality/age	Description of mesofossil	In situ spores	Spore wall ultrastructure
Ohio Ashgill/ Llandovery	Dispersed spore	Naked unfused dyad <i>Dyadospora murusdensa</i>	Bilayered with lamellate inner layer and homogeneous outer layer
Ohio Ashgill	Dispersed spore	Envelope enclosed dyad <i>Segestrespora membranifera</i>	Homogeneous to somewhat spongy with irregular globular units and lamellae at the exterior. Envelope stains darker than wall

layer. All cells are elongate and intercellular spaces are absent. The central cells, angular in cross section and tapered longitudinally, have uniformly thin walls, sometimes with a faintly reticulate surface pattern. They are frequently degraded. The surrounding cells have thick, often darkly staining, walls ($<2\ \mu\text{m}$) comprising two layers with the inner showing a tendency to fragment and separate. D. S. Edwards (1986) described them as uniformly thickened, but in longitudinal section demonstrated a hexagonal pattern or irregular reticulum with occasional isolated circular structures on the outer wall. He thought these were artefacts (as did Lemoigne & Zdebska, 1980) produced by shrinkage of the wall during silicification (possibly associated with bacterial attack), because he found similar wall patterning in other tissues. However, Remy (Trelew lecture, 1994) reported completely spherical structures, limited by broken 'dotted' lines either lining the elongate cells in mature cells (metaxylem) or completely filling the lumens in younger tissue. Such vesicles are particularly apparent, sometimes following short chains, in cells described as trans-fusion tracheids (Remy & Hass, 1996: Plate VII, Fig. 2) associated with anomalous tissues in *Aglaophyton* rhizomes.

D. S. Edwards (1986) interpreted the outermost zone as consisting of leptoids, the central one of hydroids involved in water-conduction and the surrounding thick walled cells as support or possibly water-conducting tissue quite distinct from extant hydroids or stereids.

Central elongate cells completely lacking conventional tracheidal thickenings or ornament are also present in *Nothia aphylla*, whose fertile axes were originally thought part of *Asteroxylon mackiei* (Kidston & Lang, 1920). This remains the most enigmatic of Rhynie plants in that sporangia were borne laterally on short vascularised axes (stalks) in very variable arrangements in loose spikes (i.e. zosterophyll characters) but the presumed xylem is centrarch (El-Saadawy & Lacey, 1979).

2.4.7 Phloem

Except for *Psilophyton dawsonii* (Banks *et al.*, 1975) this is known only in the Rhynie Chert plants. It is identified on its circum-xylary position and its composition — elongate parenchymatous cells with oblique end walls and lacking intercellular

spaces. Although Kidston & Lang (1917) found no sieve areas in *Rhynia*, Satterthwait & Schopf (1972) described 'pock marks' (3–9 µm) tentatively identified as remnants of pit fields on the lateral walls of some cells, and a further wider type of cell with occasional areas of granular texture produced by circular subunits (<1 µm diameter). D. S. Edwards (1986) dismissed similar small-scale beading of the wall and granular appearances as a consequence of crystallisation of the matrix. He reported similar structures in the outermost zone of conducting cells in *Aglaophyton* which he identified as leptoids, because of their position and S-shaped end walls, a character seen in extant polytrichaceous mosses.

2.4.8 Lignin

From the construction of tracheids in early vascular plants and their resilience to decay it is assumed that they possessed lignified walls, but it has so far proved impossible to demonstrate unequivocally the presence of this macromolecule in their fossils (Ewbank, Edwards & Abbott, 1997). Instead, flash pyrolysis-gas chromatography-mass spectrometry of their coalified residues yields comparatively simple aromatic moieties such as alkylphenols and alkylbenzenes. Methoxyphenols and catechols, the anticipated biomarkers for lignin pyrolysates are absent. The reasons are taphonomic. During diagenesis, burial and subsequent thermal maturation, and possibly as a consequence of microbial decay, such compounds are broken down so that even their putative intermediate degradation products, dehydroxybenzenes disappear (or are not recorded), and are eventually converted into alkylphenols and alkylbenzenes (Hatcher *et al.*, 1988; Øygard, Larter & Sentfle, 1988; Ewbank *et al.*, 1997). However, while it would thus seem to be possible to use the presence of such relatively simple molecules as evidence for lignin, the latter is not their only source in embryophytes. Vascular plants possess a number of metabolic pathways involved in synthesis of phenolic compounds as diverse as flavonoids, alkaloids and terpenoids (Waterman & Mole, 1994) and ranging from simple phenolic acids (e.g. ferulic; Waterman & Mole, 1994) to extremely complex polyphenols (e.g. lignin and tannin) all of which might, on diagenesis, be anticipated to produce alkylphenols and alkylbenzenes. Many of these phenolics occur in bryophytes (e.g. Markham, 1988) although, with exceptions, the precise composition of more complex molecules coupled with their distribution in cells is unknown (e.g. Wilson *et al.*, 1989; van der Heijden, 1994). In a comprehensive anatomical and chemical investigation of *Sphagnum* shoots, both sub-fossil in peats and living, van der Heijden isolated sphagnic acid (p-hydroxy- β -[carboxymethyl]-cinnamic acid) linked to cell-wall polysaccharides in leaves and stems, a polyphenol resembling hydrolysable tannins from cell walls of leafy shoots, and also a suberin like polymer (based on isolation of phenols and phenolic compounds plus long and short chain fatty acids and hydroxy-fatty acids) from the stained cell walls of tissues surrounding the thin walled central cells of the stem. Van der Heijden concluded that the polyphenols conferred protection from decay, thus accounting for the abundance of *Sphagnum* in peats compared with plants e.g. *Calluna* with a preponderance of lignified tissues. In a recent study Kroken *et al.*, (1996) have demonstrated autofluorescent and acid-resistant cell walls in *Sphagnum* leaves and sporangial epidermes indicative of the presence of uncharacterised phenols: similar autofluorescence under UV or violet light having been used

as evidence for 'phenolic acids or lignin-like compounds' in bryophytes (Nordhorn-Richter, 1988). Lignin itself has not yet been demonstrated in bryophytes. Kroken *et al.* (1996) also related these resistant cell walls to the comparatively high fossilisation of *Sphagnum*.

2.4.9 Cuticle

Cuticles have been demonstrated anatomically on almost all compression and permineralised vascular plant fossils from the Lower Devonian, and also on axial stomatous and apparently astomatous axes from the Silurian (For reviews see Edwards & Edwards, 1986; Edwards *et al.*, 1996a). They have also been recovered from sporangia. We have also shown chemically by flash pyrolysis-gas chromatography-mass spectrometry that cuticles of *Psilophyton* sp., *Zosterophyllum myretonianum* and *Sawdonia ornata* yield a series of *n*-alkane/*n*-alkene doublets maximising between *n*-C₁₀ and *n*-C₁₄ and extending as far as *n*-C₂₇ (*Psilophyton*) indicating derivation from cutan, an insoluble non-hydrolysable, highly aliphatic polymethylenic polymer demonstrated in extant seed-plant cuticles (Nip *et al.*, 1986) which is far more resistant to decay than the polyester cutin (Tegelaar *et al.*, 1991). Unfortunately the wax component of the cuticle which largely influences its permeability is not preserved in fossils, but the combination of anatomy (including stomata) and chemistry is compelling evidence that the cuticle functioned in these axial vascular plants as it does in homoiohydric plants today. In contrast to such chemical analyses, '*Nematothallus*' cuticles have not yielded *n*-alkane/*n*-alkene doublets but are dominated by C₀-C₂ alkyl phenols, C₁-C₃ alkyl benzenes and C₀-C₂ alkyl naphthalenes (Edwards *et al.*, 1996a). The nature of the tissues and affinities of the plant bearing such cuticles remain contentious. Kroken *et al.* (1996) have added to the debate by suggesting that some 'pseudocellular' dispersed cuticles derive from sporangial walls of bryophytes. The differences in chemistry reported here suggest that the parent plants of certain Lower Devonian cuticles were not closely related to vascular plants. We need more detailed information on the chemistry of extant bryophyte cuticles to facilitate further comparisons. However, in some cases the cuticles we analysed (admittedly in bulk) were of the size suggestive of covering a thalloid organism rather than a sporangium.

2.4.10 Stomata

In all early axial plants, stomata had two guard cells (\pm reniform in surface view) and were surrounded by unmodified epidermal cells. Records of a single cell surrounding a pore are due to poor penetration of cuticle between guard cells (Edwards & Axe, 1992; Edwards *et al.*, 1996a). The dark colouration seen in guard cells of certain Rhynie Chert taxa and some compression fossils is strongly suggestive that, as in extant vascular plants (in contrast to the translucent epidermal cells) (Edwards, 1993) they possessed chlorophyll. Three dimensional studies are rare. Edwards & Axe (1992) working with isolated cuticles showed that the guard cells were often partially underpinned by adjacent epidermal cells, that their common walls were strongly impregnated with cuticle and that the outer wall of the guard cell, often missing in compression fossils, was lightly cuticularized. We thus inferred that

stomatal opening was achieved when the guard cell swelled outwards, a mechanism seen today in certain ferns (e.g. *Diplazium*) and mosses (e.g. *Polytrichum*: Ziegler, 1987). Stomata may be variously dispersed on sporangial walls (Edwards *et al.*, 1996a) as well as on widening axes immediately below sporangia.

2.4.11 Rhizoids

These are known only from sporophytes in the Rhynie Chert and are all unicellular. They range from short in *Trichopherophytion* (Lyon & Edwards, 1991) to elongate in *Rhynia* and *Aglaophyton* (Kidston & Lang, 1917, 1920).

2.4.12 Sporangial characters

Position. Here we concentrate on those solitary sporangia that terminate branching axial systems but omit those thought to be terminal on short lateral axes giving the appearance of sporangial stalks (see discussion in Niklas & Banks, 1990).

Wall characters. Rhynie Chert taxa, *Rhynia*, *Aglaophyton* and *Horneophytion* all show multilayered walls which persist at maturity, the latter inference based on their presence in empty, presumed dehisced, sporangia or where spores are mature. Their basic organisation is similar in that the outermost layer comprises thick-walled cells, and is separated from a presumed tapetum by a number of layers of thin-walled cells.

The latter are normally unrepresented in compression fossils, making it difficult to decide the significance of the single layer of thick-walled cells usually present (e.g. in *Cooksonia pertoni*, *Tortilicaulis offaeus*), i.e. whether it is taphonomic or a consequence of ageing.

In permineralised *Uskiella*, most of the sporangium wall is occupied by an outer layer of cells with massive thickening in outer periclinal and anticlinal walls, the sporangial cavity being surrounded by 3–4 layers of compressed thin-walled cells (Shute & Edwards, 1989), thus resembling the organisation in a number of our mesofossils (e.g. Edwards, 1996, Pl. II, Fig. 5), where the limiting layer remains the most prominent one.

Sporangia are usually covered by cuticle, revealed only when the sporangial wall has been removed by treatment with concentrated nitric acid or Schulze's solution (Edwards, 1996). Indeed sporangial cuticles, identified by their discrete shapes, aligned cell outlines and occasionally adhering spores, occur in the rocks of similar age to the earliest *Cooksonia* (Wenlock: Edwards & Feehan, 1980) and become increasingly frequent in younger sediments. Kroken *et al.* (1996) have suggested that some of the dispersed cuticles (e.g. of the *Nematothallus* complex) in older rocks might derive from sporangia of bryophyte affinity, because they have recovered similar fragments after acetolysis of liverwort and moss sporangia.

The presence of stomata on the sporangial surface of a number of Lower Devonian plants is suggestive of an underlying photosynthetic tissue, enclosing the developing spores, but evidence for an extensive aerating system is absent. Their distribution is variable (Edwards *et al.*, 1996a) but they are rarely concentrated near the base of the sporangium (cf. *Funaria*). There is some evidence for increased frequency in the subtending axis where it widens below the sporangium (e.g. *Cooksonia*; Edwards *et al.*, 1992).

In the mesofossils, individual sporangial cell walls are thickened, with differences in relative amounts on anticlinal and periclinal walls. The commonest situation is exemplified by *Tortilicaulis* where inner periclinal walls lack thickenings and the anticlinal ones are wedge shaped (Edwards, 1996: Pl. II, Fig. 9). We have never seen any differentially thickened walls in sporangial walls of plants comparable to those in sporangial stalks or peripheral stem tissue (e.g. *Tortilicaulis*; Edwards *et al.*, 1994; Edwards, in press) and thus have no evidence to support Kroken *et al.*'s (1996) hypothesis that some of the banded tubes found in Silurian and Devonian sediments derive from bryophyte sporangial walls. Indeed all our studies to date on isolated examples of unbranched tubes reinforce the concept of radially symmetrical tubular structures, which are often associated with wests of smaller tubes.

Tapetum. Evidence is mainly positional, but a number of Lower Devonian sporangia are lined by a layer of recalcitrant polymer (?cuticle or sporopollenin-impregnated), which may bear resilient granules similar to those found on and between spores (Edwards, 1996), or bear granules which are quite different. The lining layer is suggestive of the 'pollen sac' following completion of the activity of a secretory tapetum, but sporopollenin impregnated globules are produced by both secretory and periplasmoidal tapeta in pteridophytes (Lugardon, 1981). Whether the absence of obvious tapetal residues and the presence of 'extra exosporal' material, e.g. in *Cooksonia*, is evidence for a periplasmoidal tapetum as discussed in Edwards *et al.* (1995a) is equally conjectural. However, the very large numbers of spores produced in each sporangium argues against periplasmoidal activity (Pacini, 1990, p.21).

Perispore. Although details of *in situ* spores and sporangia are now known from a number of early land plants (Edwards & Richardson, 1996), unequivocal perispore as for example seen in extant moss spores has not yet been identified, although a loose outer coating (\pm ornamented) has been recorded in representatives of at least three lineages. In certain species of both *Zosterophyllum* (Edwards, 1969) and *Psilophyton* (Gensel & White, 1983), the spores lacking the layer would be attributed to *Retusotriletes*, but in *Uskiella*, a trilete mark has not yet been seen on the smooth spore beneath the layer which is conspicuously ornamented by granules, some being fused together (Shute & Edwards, 1989). In a number of cases, the outer covering more readily dissolves in concentrated nitric acid solution or Schulze's solution, a situation possibly reflecting ease of penetration of the acid rather than differences in chemistry. Our ultrastructural studies on *in situ* *Cooksonia* spores have demonstrated the presence of an acid-sensitive extra exosporal layer in a number of cases, but we cannot ascertain whether or not such peripheral material represents condensed locular fluid or perispore. The acid resistant envelope surrounding dispersed dyads and tetrads may represent a 'communal' perispore — in the vast majority of extant plants perispore is deposited on individual members of the tetrad after separation and thus covers the trilete mark. Our studies on enveloped *in situ* tetrads in Lower Devonian plants indicate that the layer is thinner and far more sensitive to acid treatment, and further the tetrads themselves tend to dissociate.

Elaters. There is just one possible record — comprising a single strap-shaped structure with surface herringbone pattern (Fig. 2m) in a sporangium filled with psilate tetrads (Edwards *et al.*, 1995b). There are none with 'conventional' spiral

thickenings, although if indeed the structure described above is an elater, then re-evaluation of the banded tubes is desirable, particularly those with thickenings that are narrow and closely spaced (e.g. Figs 48–50 and 52–54 in Burgess & Edwards, 1991, and *Porcatitubulus microannulatus* and *P. microspiralis* in Wellman, 1995). Kroken *et al.* (1996) report that elaters in extant *Conocephalum* and *Lophocolea heterophylla* survive acetolysis and autofluoresce (cited as evidence for phenolic acids and lignin-like compounds; Nordhorn-Richter, 1988). In view of their possession of such recalcitrant macromolecules it is surprising that isolated thickenings are not recorded in dispersed palynomorph assemblages.

Symmetry and dehiscence. Most taxa have terminal radially symmetrical sporangia with respect to the subtending axis. Bilateral symmetry is often imposed (and hence recognized) by a modification for dehiscence, compression making identification difficult where surface features are uniform or not preserved. The commonest form of dehiscence is into two equal valves, the dehiscence line extending around the longest margin (e.g. reniform type — *Resilitheca*, fusiform — *Salopella* sp.). In vertically elongate sporangia dehiscence is accompanied by twisting either before or after splitting. Thus in *Tortilicaulis offaeus* cells of the sporangial wall are spirally arranged with predetermined dehiscence (marked by a bevelled ridge) into two valves (Edwards *et al.*, 1994). In *Salopella* and in *Psilophyton* twisting probably occurred following dehiscence except that in the latter there is only one split per sporangium. D. S. Edwards noted a longitudinal split or splits in empty sporangia of *Aglaophyton* with wall cells uniformly twisted and aligned at 40–50°, and deliberated whether or not the twisting occurred during sporangial development at maturity or post dehiscence, but had no relevant information in intact sporangia. Marked spirally arranged striations characterise the fusiform sporangia of *Huvenia kleui* (Hass & Remy, 1991), such that the sporangia frequently appear to comprise discrete twisting bands united at the mucronate tip. However, dehiscence is reported as occurring along one dense spiral striation.

Both Silurian and Lower Devonian examples show modifications of the cell wall, in terms of cell shape, wall thickness, size or position in the vicinity of the dehiscence line (e.g. *Uskiella*; Shute & Edwards, 1989), but no evidence of a peristome or indeed operculum in the moss sense. In *Horneophyton*, cell arrangement in the sporangium apex has been described as operculum-like (Bhutta, 1972), while the circular dehiscence line recorded in the sporangial cuticle of a new taxon with sporangia of *Cooksonia* shape, but containing spores of *Apiculiretusispora* type, probably reflects the splitting of the sporangium wall for spore liberation.

Dehiscence into four valves has not been unequivocally observed, although the shape of some isolated sporangial cuticles suggests that more than two valves were present. A recent Lochkovian discovery of an as yet unnamed, bifurcating axis with terminal sporangia shows distal dehiscence with three well-defined lobes (Fig. 2a), the basal part of the sporangium remaining intact (Edwards *et al.*, 1996a).

Calyptra. This has not been recognised in early land plants. Sectioning of the dispersed cuticles mentioned above rarely shows an additional layer on the outside, but more work is needed to allow comments on its nature and significance.

Columella. Two examples are reported in early land plants. In *Horneophytton lignieri*, a central column of elongate thin-walled cells of fibrous appearance has an outermost layer continuous with the persistent 'tapetum' of the sporangium wall (Kidston & Lang, 1920). The sporangium itself may be up to four- or possibly five-lobed (Bhutta, 1972; Eggert, 1974) each with a columella, the sporangial cavity being continuous throughout the branching structure.

In *Sporogonites* the evidence for a columella is less secure. Although mentioned in the original description (Halle, 1916), Halle (1936) subsequently queried its presence, because its position was occupied by mineral matrix rather than cells. *Sporogonites* is also unusual in that the lower part of the terminal obovate to clavate sporangium is occupied by a solid mass of sterile tissue slightly less than half its length.

Certain Lower Devonian sporangia have a reniform outline which is produced by the projection of the subtending axis into the base of the sporangial region. Examples include *Cooksonia caledonica* and *Zosterophyllum*. Mishler & Churchill (1984) interpreted such a structure in *Lycopodium* (s.l.) sporangia of similar shape as the remnants of a dome shaped columella, although it is not a conspicuous feature of the earliest known fertile lycophytes, where (e.g. in *Drepanophycus qujingensis*) a shallowly convex line separates stalk from sporangium.

In descriptions of *Resilitheca salopensis* a new Lochkovian plant with terminal sporangia, morphologically similar to *Cooksonia caledonica*, Edwards *et al.* (1995c), interpreted bivalved structures found dispersed in the rock matrix as the resilient innermost layers of the sporangium wall. They have a reniform outline—the shape produced by the terminal expansion of the whole axis, but there is no anatomical information on the latter. However, a central pad of tissue composed of parenchyma and tracheidal cells protruding to varying extent into the base of the sporangial cavity has been anatomically recorded in *Rhynia gwynne-vaughanii* (D. S. Edwards, 1980). The tracheidal tissue is an expansion of that of the subtending axis and comprises shorter cells in which the thickenings are less distinct. The overarching parenchyma is continuous with the presumed axial phloem and laterally extends into the sporangium wall, but the tapetal layer, comprising cells with thicker radial and outer tangential walls, is absent over the pad. D. S. Edwards (1986) postulated that the degree of projection of the pad into the cavity reflects the amount of contraction of the sporangium base, possibly after spore release.

Timing of maturation. In all our studies on *in situ* spores and from illustrations of silicified sporangia assumed to have been fossilized at different ontogenetic stages, maturation appears to be synchronous. Dispersed elongate masses of tetrads of *Synorisporites downtownensis* show some abnormal tetrads where distal ornament is weakly defined and individual spores have collapsed or are represented by ?sporopollenin-impregnated straps or sheets (Edwards *et al.*, 1996b). In one specimen these are concentrated at one end leading to the possibility of prolonged spore production at the base of the sporangium. However in two other examples the abnormal tetrads occur to one side of the spore mass, suggestive of localized developmental failure.

Haptotypic features. In all early tracheophytes and in the rhyniophytooids, spores are trilete, although in *in situ* examples the trilete mark may be absent or difficult to observe (e.g. Edwards *et al.*, 1994; *Salopella*). Spores have usually separated into

monads, or more atypically are aggregated in 'loose' tetrads. Examples of monads with circular proximal contact areas (hilate cryptospores) that derive from dyads are described on p. 18 and illustrated in Fig. 2g, h, j.

Spore wall ultrastructure. Exospore ultrastructure from SEM and TEM has recently been described in cryptospores (see p. 21) and in trilete spores from tracheophytes and rhyniophytoids (Rogerson *et al.*, 1993; Edwards *et al.*, 1995a). For trilete spores, homogeneous, bilayered and lamellate walls have been recorded, and this character plus superficial features (e.g. sculpture), are proving useful in the circumscription and detection of affinities in such morphologically simple plants. However, such studies are in their infancy, and their potential for elucidating relationships among early embryophytes remains to be explored.

2.5 CONCLUSIONS

1. Various lines of evidence now support Gray's contention that the permanent tetrads in mid-Palaeozoic spore assemblages derive from embryophytes at a bryophyte and probable hepatic level of organization (Gray, 1985; Gray *et al.*, 1992). Limited vegetative biochemical adaptations, and hence low fossilization potential would explain the absence of body-fossils. Similar microfossils are very rarely recorded post Lower Devonian, indeed few palynomorphs have been unequivocally related to bryophytes until the Late Palaeozoic.
2. Direct 'megafossil' evidence for bryophytes is limited to species of *Sporogonites* and even less convincingly *Tortilicaulis transwalliensis*. The first unequivocal hepatic is *Pallaviciniites devonicus* (Hueber) Schuster, a probable metzgerialean from the Frasnian (Upper Devonian) of New York State (Hueber, 1961), and the earliest moss, *Muscites plumatus* (Thomas, 1972: Lower Carboniferous of southern England). It is surprising that despite the recent intensive investigations on the Rhynie Chert by Remy and co-workers at Münster, bryophytes have not yet been recognized in this deposit.
3. The micro and megafossil record in the late Silurian and early Devonian comprises morphologically simple axial fossils and some lycophytes. Almost all show a sophistication in tissue differentiation and sporangial characteristics that are more typical of tracheophytes than bryophytes. Evidence from the Rhynie Chert suggests that the life cycles of axial plants show isomorphic alternation of generations involving two independent homoiohydric plants. Thus the Siluro-Devonian megafossils derive from a time interval somewhat later than that which might be anticipated to encompass the first stages of cladogenesis of embryophytes based on cladistic analysis. The microfossil record suggests that the Upper Llandovery — Lower Wenlock rocks might hold the key to this critical phase in embryophyte evolution. However, in the near absence of body fossils, continued investigation of mesofossils such as those presented here, and searches for new assemblages might yield valuable insights into the earliest bryophytes and tracheophytes, even if in relict taxa. As Mishler & Churchill (1984) emphasised, an important outcome of cladistic analyses of green algae and embryophytes has been the prediction of the probable ancestral character combinations, with the implication that the earliest fossil bryophytes were simply

not recognised because we did not know what we were looking for. Such guidance in interpretation of our very simple fossils, coupled with mesofossils as a new source of information, augers well for progress in elucidating bryophyte-tracheophyte inter-relationships.

Finally, in view of the small size of the fossils, it is tempting to adopt the suggestion based on the invertebrate fossil record, that important events in cladogenesis occurred in organisms of small size. In relation to the apparent explosive radiation recorded near the base of the Cambrian, Fortey, Briggs & Wills (1996) wrote: 'Crucial phase in evolution of bauplan happened in animals of small size and may explain the paradox of mismatch between the fossil record and the evidence for Vendian cladogenesis'. Small size plus additional causes of poor preservation in the Ordovician and Lower Silurian might also contribute to the apparent explosive radiation of tracheophytes in the Siluro-Devonian.

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3. Bryophyte phylogeny and the evolution of land plants: Evidence from development and ultrastructure

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3.1 SUMMARY

Phylogenetic analyses were carried out based on 132 ultrastructural, biochemical and developmental characters to develop a hypothesis for the principal lineages of extant land plants, in particular the relationships among and within hornworts, liverworts, and mosses. The organisms included five hornwort, 11 liverwort, eight moss, five lycophyte and three genera of spore-bearing vascular plants. Gametophytic features (65 characters) and sporophytic features (67 characters) were analyzed separately and together. The combined data set resulted in 26 most parsimonious (MP) trees ($TL=293$) in which bryophytes were not monophyletic. Hornworts were the sister group to the remaining land plants, and mosses and liverworts were monophyletic, and formed the sister group to vascular plants. Hornworts showed a single arrangement with *Megaceros* as the apparent outgroup; however, decay analysis showed little support for this tree topology. MP trees for liverworts were variable in initial branch topologies, although most genera of Jungermanniales plus Metzgeriales formed a clade. Moss relationships were fully resolved, and *Sphagnum* was the outgroup to successive paraphyletic lineages for *Takakia*, *Andreaea* plus *Andreaeobryum*, *Polytrichum*, *Tetraphis* and the peristomate mosses. In trees one step longer, *Takakia* was the outgroup to the remaining genera. Relationships within vascular plants were poorly resolved, although *Selaginella* was the usual outgroup or it was part of larger lycophyte assemblages. These results suggest that there is extensive useful phylogenetic information at the base of the land plant clade in traditional morphogenetic combined with contemporary cytomorphogenetic approaches to systematic botany.

KEYWORDS: Land plants, development, phylogeny, hornworts, liverworts, mosses, ultrastructure.

3.2 INTRODUCTION

With their highly elaborate gametophytic generation and nonlignified walls, bryophytes have traditionally been considered the most primitive assemblage within terrestrial plants (Kenrick & Crane, 1991). Until relatively recently this concept was

not challenged by the fossil record and all ancient land plants were known only on the basis of sporophytic remains. More recently, with the characterization of nonligified organisms from the Devonian, with their gametophytic generation similar to the sporophytic one (e.g. Remy, 1982; Remy, Gensel & Hass, 1993), such a conclusion is questionable. A modern period of phylogenetic study for resolving the primary lineages of land plants and their relationships began with the seminal papers of Mishler & Churchill (1984, 1985). This work, based on morphological evidence, led to the hypothesis that bryophytes are paraphyletic with liverworts, hornworts and mosses forming successive branches from a clade where mosses are the sister to vascular plants. Subsequent analyses and theoretical discussions of the problem included Bremer *et al.* (1987), Kenrick & Crane (1991, 1997), Mishler *et al.* (1994), Lewis, Mishler & Vilgalys (1997), and these accounts largely supported the Mishler & Churchill (1984) hypothesis. However, Taylor (1988), Garbary, Renzaglia & Duckett (1993) and Hedderson, Chapman & Rootes (1996), Malek *et al.* (1996) suggested alternative arrangements based on differing morphological and molecular data sets.

In this paper we utilize a range of morphological and ultrastructural characters derived from classical literature of plant morphology and more recent data from cell biology. As much as possible, characters were selected and coded to allow for comparison across all plant groups used in the analysis. Character states based on development were incorporated wherever possible to enhance homology. To this end sporophytic and gametophytic characters were coded separately. This analysis poses several questions: 1) Can morphological and ultrastructural characters provide a reasonable hypothesis for the evolution of land plants? 2) Is the same phylogenetic signal given by characters from sporophytic and gametophytic life history stages? 3) What are relationships within the various bryophyte assemblages, and what are the relationships of these groups to each other and to the vascular plants?

3.3 MATERIALS AND METHODS

A total of 132 developmental, ultrastructural, morphological and biochemical characters were defined (Appendix 1), comprising binary and unordered multistate characters. The data were derived from standard plant morphology texts (e.g. Bierhorst, 1971; Gifford & Foster, 1988; Bold, Alexopoulos & Delevoryas, 1987), monographs of particular assemblages (e.g. Renzaglia, 1978; Bartholomew-Began, 1991; Schuster 1984b; Schofield 1985), previous phylogenetic analyses (Garbary *et al.*, 1993; Mishler & Churchill, 1984, 1985; Mishler *et al.*, 1994), a wide range of research papers (e.g. Renzaglia & Duckett, 1991), unpublished data, some of which is published in this volume (e.g. Ligrone, Duckett & Schmid, 1997; Vaughn & Renzaglia, 1997; personal communications from R. Ligrone, D. Whittier, J. Duckett, K. Vaughn) (Table 1). Features for gametophytic stages (characters 1–65) were coded separately from sporophytic characters (characters 66–132) (Appendix 1, Table 2). Some biochemical and ultrastructural characters were coded separately for both life history stages, although in the complete analysis each character was included only once for each taxon. Multistate characters were treated as unordered.

Phylogenetic analyses were carried out using PAUP v. 3.1 (Swofford, 1993)

Table 1. List of taxa and principal sources of information. General sources of information for many groups included: Campbell (1895), Goebel (1905), Smith (1955), Schuster (1966), Hébant (1977), Huneck (1983), Nehira (1983), Bold, Alexopoulos & Delevoryas (1987), Renzaglia & Duckett (1991), Garbary, Renzaglia & Duckett (1993), Ligrone, Duckett & Renzaglia (1993).

Genera	Assemblage, Class	References
<i>Coleochaete</i>	algae, Charophyceae	Graham <i>et al.</i> , 1991; Graham, 1993
<i>Anthoceros, Dendroceros, Megaceros, Notothylas, Phaeoceros</i>	hornworts, Anthoceropsida	Renzaglia, 1978; Renzaglia & Duckett, 1991; Schuster, 1966, 1984c; Schofield, 1985; Hassel de Menéndez, 1988; Brown & Lemmon 1988, 1990b; Vaughn <i>et al.</i> , 1992; Hasegawa, 1990, 1993, 1995; Hyvonen & Pippio, 1993; Vaughn & Hasegawa, 1993
<i>Bazzania, Blasia, Haplomitrium, Lejeunea, Marchantia, Monoclea, Pallavicinia, Pellia, Riccardia, Sphaerocarpos, Treubia</i>	liverworts, Hepatopsida	Renzaglia, 1982; Huneck, 1983; Schuster, 1966, 1984a, b; Schofield, 1985; Brown & Lemmon, 1988, 1990a, b; Bartholomew-Began, 1991; Renzaglia & Duckett, 1991
<i>Andreaea, Andreaeobryum, Aulacomnium, Hypnum, Polytrichum, Sphagnum, Takakia, Tetraphis</i>	mosses, Bryopsida	Crum & Anderson, 1981; Huneck, 1983; Vitt 1984; Schofield, 1985; Crandall-Stotler, 1986; Brown & Lemmon, 1988, 1990a, b; Renzaglia & Duckett, 1991; Smith & Davison, 1993; Renzaglia <i>et al.</i> , 1997
<i>Lycopodiella, Lycopodium, Palhinhaea, Phylloglossum, Selaginella</i>	lycophytes, Lycopodopsida	Bierhorst, 1971; Gifford & Foster, 1988; Brown & Lemmon, 1990a, 1991; Renzaglia & Duckett, 1991;
<i>Ceratopteris, Equisetum, Psilotum</i>	'ferns', Filicopsida, Equisetopsida, Psilopsida	Bierhorst, 1971; Gifford & Foster, 1988

implementing the heuristic search algorithm. The search routine used 300 replications under the random addition option in which only the shortest trees were kept during each replication, and the TBR (tree bisection-reconnection) option was used for tree swapping. In analyses of the complete data set and of the gametophytic characters *Coleochaete* was designated as the outgroup. For sporophytic characters there was insufficient memory to carry out the same analysis, and a reduced taxon number was used, with *Notothylas* designated as the outgroup. All resulting cladograms were reconstructed in MacClade for mapping of character traits (Table 3). Alternate phylogenetic hypotheses were then reconstructed in MacClade V. 3.01 (Maddison & Maddison, 1992) using the complete data set (Table 4).

3.4 RESULTS AND DISCUSSION

The three data sets (all characters, gametophytic features alone, sporophytic features alone) produced different phylogenetic hypotheses. The cladograms that intuitively provided the most satisfactory result came from the entire data set (Figs 1–2). The

Table 2. Character state coding for taxa in the analysis. See Appendix 1 for elaboration of characters and their states.

Genus	1-10	11-20	21-30	31-40	41-50	51-60
Coleochaete	0 0 0 0 ? 0 0 0 ? 0	0 0 ? 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	?	?	?
Notothylas	2 1 0 1 0 0 0 1 0 1	0 0 ? 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	2 ? 0 0 0 0 0 0 0 0
Phaeoceros	2 1 0 1 0 0 0 1 0 1	0 0 ? 0 0 0 0 0 0 0	0 0 0 0 0 0 0 0 0 0	0 0 1 2 0 1 0 0 1 0	0 1 1 0 0 1 2 2 0 0	0 0 0 0 0 1 0 0 1 2
Megacestis	2 1 0 1 0 0 0 1 0 1	0 0 ? 0 1 0 0 0 0 1	3 0 0 0 0 0 0 0 0 0	0 0 1 2 0 0 0 0 1 0	0 1 1 0 0 2 2 0 0 0	0 0 0 0 1 0 0 0 2
Dendroceros	2 1 0 1 0 0 0 1 0 1	0 0 ? 0 1 0 0 0 0 0	1 0 0 0 0 0 0 0 0 0	0 0 1 2 0 0 0 0 1 0	0 1 1 0 0 2 2 0 0 0	0 0 0 0 1 0 0 0 1
Anthoceros	2 1 0 1 0 0 0 1 0 1	0 0 ? 0 1 0 0 0 0 0	2 0 0 0 0 0 0 0 0 0	0 0 1 2 0 0 0 0 1 0	0 1 1 0 0 2 2 0 0 0	0 0 0 0 1 0 0 0 1
Haplomitrium	1 1 1 1 0 1 1 2 0 0	1 0 0 0 0 0 0 1 0 1	2 1 1 0 1 0 0 0 0 0	0 0 0 1 0 3 0 2 1 1	1 1 1 1 1 0 0 2 0 1 0	1 0 0 0 0 2 0 0 0 2 0
Monoclea	2 1 0 1 0 0 1 1 0	0 ? 0 0 0 0 1 0 1	2 1 1 1 1 0 0 0 0 0	0 0 2 1 0 3 0 0 1 1	1 1 1 1 1 0 2 2 1 0 0	1 0 0 0 0 2 0 0 0 2 0
Pellia	2 1 0 1 0 0 1 1 0	0 ? 0 0 0 0 1 0 1	2 1 1 1 1 0 0 1 0 0	1 0 0 1 0 3 0 0 1 1	1 1 1 1 1 0 2 1 0 0 0	1 0 0 0 0 2 0 0 0 2 0
Blasia	2 1 0 1 0 0 1 1 0	0 ? 0 0 0 1 0 1 0 1	2 1 1 1 1 0 0 2 0 0	1 0 0 1 0 3 0 0 1 1	1 1 1 1 1 0 2 1 0 0 0	1 0 0 0 0 2 0 0 0 2 0
Marchantia	2 1 0 1 0 0 1 1 0 1	0 1 1 1 0 1 0 1 0 1	2 1 1 1 1 0 0 2 0 0	1 0 2 0 0 3 0 0 1 1	1 1 1 1 1 0 2 2 1 0 0	1 0 0 0 0 2 0 0 0 2 0
Bazzania	1 1 0 1 1 1 1 2 0 1	0 ? 0 0 0 0 1 0 1	2 1 1 1 1 0 0 1 0 0	1 1 0 0 0 3 0 0 1 1	1 1 1 0 0 1 0 0 1 0	1 0 0 0 0 2 0 1 1 0
Lecidea	1 1 0 1 1 1 1 2 0 1	0 ? 0 0 0 0 1 0 1	2 1 1 1 1 0 0 1 0 0	1 1 0 0 0 3 0 0 1 1	1 1 1 1 1 0 0 1 0 0	1 0 0 0 0 2 0 1 1 0
Sphaerocarpus	2 1 0 1 0 0 1 1 0	1 0 0 0 0 0 0 1 0 1	2 1 0 1 1 0 0 2 0 0	1 0 0 1 0 3 0 0 1 1	1 1 1 1 1 0 2 2 0 0 0	1 0 0 0 0 2 0 0 0 2 0
Pallavicinia	3 1 0 1 0 1 1 1 0 1	1 0 0 0 0 0 0 1 0 1	2 1 1 1 1 0 0 1 0 0	1 0 0 0 0 3 0 0 1 1	1 1 1 1 1 0 0 2 1 0 0	1 0 0 0 0 2 0 1 1 0
Riccardia	3 1 0 1 0 1 1 1 0 1	0 0 0 0 0 0 1 0 1	2 1 1 1 1 0 0 1 0 0	1 0 0 0 0 3 0 0 1 1	1 1 1 0 0 2 1 0 1 0	1 0 0 0 0 2 0 1 2 0
Treubia	1 1 0 1 0 1 1 1 0 1	0 0 ? 0 0 0 0 1 0 1	2 1 1 1 1 0 0 1 0 0	1 0 0 1 0 3 0 0 1 1	1 1 1 0 0 2 1 1 1 0	1 0 0 0 0 2 1 2 2 0
Andreaea	1 2 1 0 2 1 1 3 0 1	0 0 0 0 0 0 1 1 0	2 0 0 1 3 0 0 1 0 1	0 1 4 0 0 3 0 0 1 1	1 1 1 0 1 2 1 2 0 0	2 1 2 0 0 2 1 1 2 0
Takakia	1 2 1 0 2 1 0 3 ? 0	1 0 0 0 0 0 1 1 1	2 1 0 1 0 0 2 0 1 1 0	0 1 4 0 0 3 0 0 1 1	1 1 1 0 1 2 2 0 1 0	2 1 2 0 0 2 1 1 2 0
Andreaeobryum	1 2 1 0 2 1 1 3 1 2	0 ? 2 0 0 0 1 1 1	2 1 0 1 3 0 0 1 1 0	0 1 4 0 0 3 0 0 1 1	1 1 1 0 1 2 1 2 0 1	2 1 2 0 0 2 1 1 2 0
Polytrichum	1 2 1 0 2 1 1 3 1 2	2 1 0 0 0 0 1 1 0 1	2 1 0 1 3 0 0 1 1 0	0 1 4 0 0 3 0 0 1 1	1 1 1 0 1 1 2 0 0 0	2 1 1 0 0 2 1 1 2 0
Sphagnum	1 2 1 0 2 1 1 3 0 0	0 1 2 0 0 0 0 1 0 1	2 1 0 1 3 0 0 1 1 0	0 1 4 0 0 3 0 0 1 1	1 1 1 0 1 1 2 0 0 0	2 2 1 0 0 2 1 1 2 0
Tetraphis	1 2 1 0 2 1 1 3 1 2	2 1 0 0 0 0 1 1 0 1	2 1 0 1 3 0 0 1 1 0	0 1 4 0 0 3 0 0 1 1	1 1 1 0 1 1 2 0 0 0	2 1 1 0 0 2 1 1 2 0
Hypnum	1 2 1 0 2 1 1 3 1 2	2 1 0 0 0 0 1 1 0 1	2 1 0 1 3 0 0 1 1 0	0 1 4 0 0 3 0 0 1 1	1 1 1 0 1 1 2 0 0 0	2 1 1 0 0 2 2 0 2 0
Aulacomnium	1 2 1 0 2 1 1 3 1 2	2 2 0 0 0 0 1 0 0 1	2 1 0 1 3 0 0 1 1 0	0 1 4 0 0 3 0 0 1 1	1 1 1 0 1 1 2 0 0 0	2 1 1 0 0 2 2 0 2 0
Scleropodium	4 9 2 0 2 0 1 3 1 2	0 0 ? 0 0 0 0 1 1 1	2 1 0 1 2 0 3 0 3 0 1	2 2 3 0 2 0 3 0 0 1 1	0 2 2 0 0 2 0 0 0 0	2 0 1 0 2 0 0 0 2 0
Lycopodium	4 9 1 0 2 0 1 3 0 1	0 0 ? 0 0 0 0 0 0 1	2 1 0 1 2 0 1 0 0 1	0 0 3 2 0 3 0 0 0 1	0 2 1 0 0 7 2 0 0 0	2 0 0 1 2 0 0 0 2 0
Palhinhaea	4 9 1 0 2 2 2 1 2 1	0 0 ? 0 0 0 0 0 0 1	2 1 0 1 3 0 1 0 0 1	0 0 3 2 0 3 0 0 0 1	0 2 1 0 0 7 2 0 0 0	0 0 0 0 2 0 0 0 1 0
Phylloglossum	4 7 0 1 3 2 1 2 1	0 0 ? 0 0 0 0 0 0 1	2 1 0 1 3 0 1 0 0 1	0 0 3 2 0 3 1 0 0 2	0 1 3 0 0 7 2 0 0 0	0 0 0 0 2 0 0 0 1 0
Lycopodiella	4 7 0 1 3 2 1 2 1	0 0 ? 0 0 0 0 0 0 1	2 1 0 1 3 0 1 0 0 1	0 0 3 2 0 3 1 0 0 1	0 1 1 0 0 7 2 0 0 0	0 1 0 0 2 0 0 0 2 0
Equisetum	4 7 0 0 0 2 2 1 2 1	0 0 ? 0 0 0 0 0 0 1	2 1 0 1 3 0 1 0 0 1	0 0 3 2 0 3 1 1 0 2	0 1 2 0 0 9 2 0 0 0	2 0 0 0 2 0 0 0 2 0
Psilotum	1 1 1 1 2 0 1 2 1 2	2 2 0 0 0 0 0 0 0 1	2 1 0 1 3 0 1 0 0 1	0 0 3 2 0 3 1 1 0 2	0 1 2 0 0 9 2 0 0 0	2 0 0 0 2 0 0 0 2 0
Ceratopteris	2 9 0 0 2 9 1 1 2 1	0 0 ? 0 0 0 0 0 0 1	2 1 0 1 3 0 0 0 0 1	0 0 3 2 0 3 1 1 0 2	0 1 2 0 0 0 2 0 0 0	2 0 0 0 2 0 0 0 2 0

Genus	61-70	71-80	81-90	91-100	101-110	111-120	121-130
<i>Coleochaete</i>	0? 0?? ? ? 0? 0?	? ? 0? 2? 2? 0? ? 0	0? 0? 7? ? 2? 0? 0	?? ? ? 0? 2? 0? 0?	?? ? ? 0? 2? 0? 1?	0? ? ? 0? 3 0? 2? 2?	0? ? ? 0? 2? 0? 0? 0
<i>Nostothylas</i>	0 0 0 1 2 0 0 0 3	0 1 0 0 1 0 0 0 1	1 2 0 2 0 0 1 0 0 1	1 1 1 1 0 0 1 1 0	2 0 0 1 0 2 0 0 1 0	0 1 0 0 0 3 0 2 4 0	0 0 0 0 0 0 0 0 0 0
<i>Phasoceros</i>	0 0 0 0 2 0 ? 0 0 3	0 1 0 0 1 1 0 0 1	1 2 0 2 0 0 1 0 0 1	1 1 1 1 0 0 1 1 0	2 0 0 1 0 2 0 0 1 0	0 1 0 0 0 3 0 2 4 0	0 0 0 0 0 0 2 0 0 0
<i>Megaceros</i>	0 0 0 0 2 0 ? 0 0 3	0 1 0 0 1 0 0 0 1	1 2 0 2 0 0 1 0 0 1	1 0 2 1 1 0 0 1 1 0	2 0 0 1 0 2 0 0 1 0	0 1 0 0 0 3 0 2 4 0	0 0 0 0 0 2 1 3 0 0 0
<i>Dendroceros</i>	0 0 0 0 1 0 2 0 0 3	0 1 0 0 1 0 0 0 1	1 2 0 2 0 0 1 0 0 1	1 0 2 1 1 0 0 1 1 0	2 0 0 1 0 2 0 0 1 0	0 1 0 0 0 3 0 2 4 0	0 0 0 0 0 1 0 0 0 0
<i>Anthoceros</i>	0 0 0 0 1 0 2 0 0 3	0 1 0 0 1 1 0 0 1	1 2 0 2 0 0 1 0 0 1	1 1 0 1 0 0 1 1 0	2 0 0 1 0 2 0 0 1 0	0 1 0 0 3 0 2 4 0	0 0 0 0 1 0 2 0 0 0
<i>Haplomitrium</i>	0 1 1 2 2 1 0 0 1 1	0 0 0 0 1 0 1 0 0 2	0 0 0 0 1 0 0 0 0 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 0 0 1 1 0	1 1 0 1 0 3 0 2 4 0	0 0 1 1 2 1 1 1 0
<i>Monodelta</i>	0 1 1 2 2 1 0 0 1 1	0 0 0 0 1 0 1 0 0 2	0 0 0 1 1 0 0 0 0 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 0 0 1 1 0	1 1 0 2 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Pellia</i>	0 1 1 2 2 1 0 0 1 1	0 0 0 0 1 0 1 0 0 2	0 0 0 2 1 0 0 0 0 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 1 0 1 1 0	1 1 0 2 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Blasia</i>	0 1 1 2 2 1 0 0 1 1	0 0 0 0 1 0 1 0 0 2	0 0 0 1 0 0 0 0 0 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 2 0 1 1 0	1 1 0 2 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Marchantia</i>	0 1 1 2 2 1 0 1 1 1	0 0 1 0 1 0 1 0 0 2	0 0 0 1 1 0 0 1 1 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 0 1 1 1 0	1 1 0 2 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Bazzania</i>	0 1 1 2 2 1 0 1 1 1	0 0 0 0 1 0 1 0 0 2	0 0 1 1 0 0 0 0 1 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 1 0 1 1 0	1 1 0 2 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Lejeunea</i>	0 1 1 2 2 1 0 1 1 1	0 0 0 0 1 0 1 0 0 2	0 0 2 1 0 0 0 0 1 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 1 0 1 1 0	1 1 0 2 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Sphaerocarpos</i>	0 1 1 2 2 1 0 1 1 1	0 0 0 0 0 1 0 0 2 2	0 2 0 1 1 0 0 1 1 0	2 0 2 ? 1 0 1 0 1 0	3 0 0 1 0 0 0 2 1 0	1 1 0 3 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Pallavicinia</i>	0 1 1 2 2 1 0 0 1 1	0 0 0 0 1 0 1 0 0 2	0 0 0 1 0 0 0 0 1 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 0 1 1 1 0	1 1 0 3 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Riccardia</i>	0 1 1 2 2 1 0 0 1 1	0 0 0 0 1 0 1 0 0 2	0 0 0 1 1 0 0 1 1 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 0 1 1 1 0	1 1 0 2 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Treubia</i>	0 1 1 2 2 1 0 0 1 1	0 0 0 0 1 0 1 0 0 2	0 0 2 1 0 0 0 0 1 0	1 0 2 ? 1 0 1 0 1 0	2 0 0 1 0 0 2 0 1 0	1 1 0 0 0 3 0 2 4 0	0 0 1 0 2 1 2 1 1
<i>Audrasa</i>	0 0 0 2 ? 1 2 1 1 2	1 1 0 0 1 0 1 0 0 2	0 2 1 0 0 0 0 0 0 0	0 2 ? 2 1 0 0 1 0 1	2 1 0 1 0 2 0 0 1 0	0 1 0 0 0 3 0 2 4 0	0 0 1 2 1 2 1 0 1
<i>Takakia</i>	0 0 1 2 2 1 2 1 1 2	0 1 0 0 1 0 1 0 1 2	0 1 1 0 0 0 0 0 0 1	0 2 ? 2 1 1 1 1 0 1	2 0 0 1 0 2 0 1 1 0	0 0 0 0 0 3 0 2 4 0	0 0 0 1 2 1 2 1 0 0
<i>Audrasobryum</i>	0 0 2 ? 2 1 2 1 1 2	0 1 0 0 1 0 1 0 1 2	0 1 2 0 0 0 0 0 0 0	1 2 0 ? 2 1 0 1 0 1	2 1 0 1 0 2 0 1 1 0	0 1 0 0 0 3 0 2 4 0	0 0 0 1 2 1 2 1 0 1
<i>Polytrichum</i>	0 0 0 2 ? 2 1 2 1 1 2	0 1 0 0 1 2 1 1 0 1 2	0 1 1 0 0 0 0 0 0 0	0 2 ? 2 1 1 1 1 0 1	1 0 1 0 1 2 0 1 0 1 0	0 0 0 0 0 3 0 2 4 0	0 0 1 2 1 2 1 0 1
<i>Sphagnum</i>	0 0 0 2 ? 2 1 2 1 1 2	0 1 1 0 0 2 1 1 0 0 1	0 2 0 2 0 0 0 0 0 0	0 2 ? 2 1 1 1 1 0 1	1 0 1 0 1 0 2 0 0 1 0	0 1 0 0 0 3 0 2 4 0	0 0 1 0 2 1 2 1 0 1
<i>Tetraphis</i>	0 0 2 ? 2 1 2 1 1 2	0 1 0 1 2 1 1 0 1 2	0 1 0 0 0 0 0 0 0 0	0 2 ? 2 1 1 1 1 0 1	1 0 1 0 1 0 2 1 1 1	0 0 0 0 0 3 0 2 4 0	0 0 1 2 2 1 1 0 1
<i>Hypnum</i>	0 0 1 2 2 1 2 1 1 2	0 1 0 2 2 1 1 0 1 2	0 1 0 0 0 0 0 0 0 0	0 2 ? 2 1 1 1 1 0 1	0 1 0 1 0 2 1 1 1	0 0 0 0 0 3 0 2 4 0	0 0 1 2 2 1 1 0 1
<i>Aleurochitnum</i>	0 0 2 ? 2 1 2 1 1 2	0 1 0 2 2 1 1 0 1 2	0 1 0 0 0 0 0 0 0 0	0 2 ? 2 1 1 1 1 0 1	0 1 0 1 0 2 1 1 1	0 0 0 0 0 3 0 2 4 0	0 0 1 2 2 1 1 0 1
<i>Selaginella</i>	1 0 1 2 2 1 2 1 1 2	0 0 0 0 1 2 1 1 1 2	0 2 0 0 2 1 0 1 0 2	0 2 ? 2 1 0 0 0 0 1	7 0 1 1 1 2 0 1 1 0	0 1 0 1 0 2 0 2 1 1	2 1 0 0 2 1 1 0 1
<i>Lycopodium</i>	1 0 1 2 2 1 2 2 1 1	0 0 0 0 1 2 1 1 1 2	0 2 0 0 2 1 0 1 1 2	0 2 ? 2 1 1 1 1 0 1	7 0 2 1 1 2 0 1 1 0	0 1 0 0 1 0 2 0 2 1	2 1 0 0 2 1 1 0 1
<i>Palhinhaea</i>	1 0 1 2 2 1 2 2 1 1	0 0 0 0 1 2 1 1 1 2	0 2 0 0 2 1 0 1 1 2	0 2 ? 2 1 1 1 1 0 1	7 0 2 1 1 2 0 1 1 0	0 1 0 0 1 0 2 1 2 1	2 1 0 0 2 1 1 0 1
<i>Phylloglossum</i>	1 0 1 2 2 1 2 2 1 1	0 0 0 0 1 2 1 1 1 2	0 7 0 2 1 0 2 2 ?	0 7 ? 2 1 0 2 2 ?	7 0 2 1 1 2 0 1 1 0	0 1 0 0 1 0 2 0 2 1	2 1 0 0 2 1 1 0 1
<i>Lycopodiella</i>	1 0 1 2 2 1 2 2 1 1	0 0 0 0 1 2 1 1 1 2	0 2 0 0 2 1 0 2 2 ?	0 2 ? 2 1 0 2 2 ?	7 0 2 1 1 2 0 1 1 0	0 1 0 0 1 0 2 1 2 1	2 1 0 0 2 1 1 0 1
<i>Equisetum</i>	1 0 1 2 2 1 2 2 1 1	0 0 0 0 1 2 1 1 1 2	0 2 0 0 2 1 0 2 1 0	0 2 ? 2 1 0 0 0 0 1	7 0 2 1 1 2 0 1 1 0	0 1 0 0 1 0 2 1 2 1	2 1 0 0 2 1 1 0 1
<i>Psilotum</i>	1 0 1 2 2 1 2 2 1 1	0 0 0 0 1 2 1 1 1 2	2 2 2 2 2 2 2 0 2 0 0	0 2 ? 2 1 0 1 0 1	7 1 1 1 1 2 0 1 1 0	0 1 0 0 0 0 0 3 1 1 3 2	1 1 1 2 2 1 1 0 1
<i>Cratopeltis</i>	1 0 1 2 2 1 2 2 1 1	0 0 0 0 3 2 1 1 1 2	0 2 0 0 3 3 0 1 1 0	0 2 ? 2 1 0 1 0 1	7 0 1 1 1 2 0 1 1 0	0 1 0 0 1 1 3 0 3 4	1 2 1 0 2 1 1 0 1

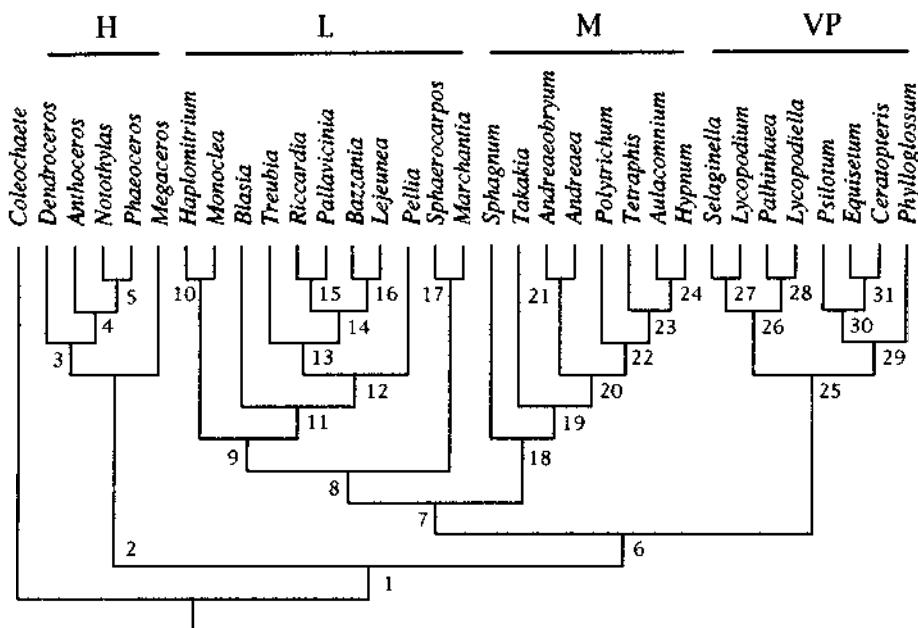


Figure 1. One of 26 most parsimonious cladograms ($TL = 293$; $C.I. = 0.66$) from analysis of complete data set. Cladogram from PAUP analysis showing relationships. See Table 3 for primary character changes on tree. In all cladograms (Figs 1–4) **H** = hornworts; **L** = liverworts; **M** = mosses **VP** = vascular plants.

gametophytic characters produced a cladogram with similar implied relationships of liverworts, mosses plus liverworts and vascular plants; however, there was a major anomaly within the moss plus liverwort assemblage in which mosses were shown as a crown group within a highly paraphyletic liverwort assemblage (Fig. 3). In the analysis of sporophytic characters where *Notothylas* was designated as the outgroup, highly anomalous tree topologies resulted in which *Sphagnum* was the sister to the remaining taxa in the analysis and liverworts were the sister to vascular plants (Fig. 4). Thus a stronger phylogenetic signal appears to come from the gametophytic characters and this is 'fine tuned' by the sporophytic characters. Subsequent discussion deals only with the cladograms generated from the combined data set (Figs 1–2).

The heuristic search of the combined data set resulted in 26 most parsimonious (MP) trees with treelength (TL) of 293 ($C.I. = 0.66$; $H.I. = 0.33$; $R.I. = 0.87$; $R.C. = 0.58$) which had the same basic tree topology (Figs 1–2). Four major clades were resolved: hornworts, liverworts, mosses and vascular plants. All trees had hornworts as the sister group to the remaining land plants. In all MP trees mosses and liverworts formed a sister group to vascular plants. The absence of monophly for bryophytes as a whole was reasonably supported, and two additional steps are required to support the hypothesis of bryophyte monophly (Table 4). The basic tree topology in Figs 1–2 is the same as that reported by Hedderson *et al.* (1996) who used 18S rRNA sequences.

Hornwort relationships were fully resolved (Figs 1–2); however, there was only

Table 3. *Apomorphy hypotheses for internal nodes of land plant cladogram shown in Fig. 1. For branch number 1 character changes are assumed to be from 0 to 1; however, further outgroups are required to demonstrate this in the analysis. Unless indicated otherwise, character changes are from 0 to 1. See Appendix 1 for details of characters and states.*

Branch number	Assemblage or clade	Apomorphy Hypotheses
1	land plants	1 (0<->2); 2 (0<->1); 4 (0<->1); 8 (0<->1); 42 (0<->1); 43 (0<->1); 77 (0<->1); 95 (0<->1); 104 (0<->1); 131 (0<->2)
2	hornworts	27 (0->2); 28 (0->2); 36 (3->1); 90; 98; 99
3		125 (2->0)
4		59; 92; 93 (2->1)
5	<i>Notothylas/Phaeoceros</i>	15 (1->0); 81; 132
6	land plants (minus hornworts)	3; 40; ?126 *; 128; 130
7	mosses plus liverworts	18; 30 (1->0); 34 (2->0); 41; 44; 51 (0->1 or 2)
8	liverworts	45; 51; 62; 82 (0); 84; 99; 111; 114 (0->2); 129; 131 (2->1)
9	liverworts minus <i>Marchantia/Sphaerocarpos</i>	33 (1->0); 34; ?247 (0); ?267 (0)
10	<i>Haplomitrium/Monocleae</i>	28 (0); 31 (1->0) ?85 (0)
11		28(1); 45 (1->0); ?48 (1); 89
12		1 (2->1); 6
13		34 (1->0); ?58
14		1 (1->3)
15	<i>Riccardia/Pallavicinia</i>	5; 8 (1->2); 32; 67; 106
16	<i>Bazzania/Lejeunia</i>	28 (2); 67 (1); 88; 89
17	<i>Sphaerocarpos/Marchantia</i>	1 (2-1); 2 (1->2); 3; 4; 5 (0->2); 6; 8 (1->3); 10 (1->0); 28 (1); 29; 32; 33 (1->4); 46; 53; 57; 58; 70 (1->2); 96; 98
18	mosses	53 (1->2); 97 (1->0); 101 (2); 11 (0->2); 74; 75; (1->2); 76; 101 (0); 107; 110; 124 (2); 57 (2); 58 (1->0); 83 (1->0); 74 (1-2)
19	mosses minus <i>Sphagnum</i> 52 (1); 68; 79; 82 (1); 83; ?112 (1->0); 17; 102;	1 (2->0); 27; 39 (0); 48 (2->0); 61/105; 68 (0->2); 72 (0); 76 (0->2); 78; 79; 85 (0->2); 86; 103 (0->2); 105; 115; 116 (2->0); 117 (0->2); 118 (2->0); 119 (4->2); 120; 121 (0->2); 122
20		3
21	<i>Andreaea/Andreaeobryum</i>	
22	peristomate mosses	
23		
24	<i>Aulacomnium/Hypnum</i>	
25	vascular plants	
26	lycophytes minus <i>Phylloglossum</i>	
27	<i>Selaginella/Lycopodium</i>	7 (1->0); 88 (1); 123 (1->0); 131 (2)
28	<i>Palhinhaea/Lycopodiella</i>	118; 131 (3)
29		37; 40 (1->2);
30	non-lycophytes	38; 103 (2->1); 117 (2->1); 121 (2->1); ?131 (3)
31	<i>Equisetum/Ceratopteris</i>	4 (1->0); ?243 (2); 66 (1->0); 89; 116; 122 (1->2)

* Assumes separate loss of pyrenoids in *Megaceros*.

weak support for relationships within the assemblage. Decay analysis allowing for cladograms one step longer than the most parsimonious essentially reduced hornworts to a polytomy. Proskauer (1960) and Schofield (1985) considered *Notothylas* to be a highly specialized and reduced form within hornworts, and this

Table 4. Treelengths for alternate phylogenetic hypotheses. Initial TL (i.e. hypothesis 1) from PAUP analysis; length penalties based on restructuring of trees in MacClade.

Phylogenetic Hypothesis	Treelength	Length Penalty
1. Hornworts sister to remaining land plants, with mosses plus liverworts sister to vascular plants (Fig. 1)	293	
2. Bryophytes monophyletic with mosses sister to liverworts (Garbary <i>et al.</i> , 1993)	295	+2
3. Bryophytes paraphyletic with hornworts sister to remaining land plants	296	+3
4. Bryophytes paraphyletic with liverworts sister to remaining land plants (Mishler & Churchill, 1984)	295	+2
5. Hornworts sister to vascular plants and with mosses and liverworts forming a clade	296	+3
6. Bryophytes paraphyletic with liverworts sister to remaining plants and with hornworts in a clade with vascular plants (Lewis <i>et al.</i> , 1997)	300	+7
7. Hornworts plus mosses sister to tracheophytes (Waters <i>et al.</i> , 1992)	301	+8
8. As in Fig. 1 but with <i>Takakia</i> associated with <i>Haplomitrium</i> in liverwort clade	319	+26

is consistent with our tree. When *Folioceros* was included in the analysis (not shown), virtually no resolution of relationships was apparent. Our lack of confidence of resolved hornwort relationships reflects the fact that features characterizing the genera show little consistency in distribution, and there are numerous conflicting characters. Further studies of the complex, including more comprehensive sampling of taxa for molecular sequencing are in order.

Land plants, excluding hornworts, were characterized by the presence of axial gametophytes (character 3), the loss of pyrenoids (character 20), the absence of channel thylakoids (character 22), the occurrence of staggered flagella (character 40) and the presence of flavonoids (character 108) (Table 3). All of the MP trees showed a topology in which mosses and liverworts formed a separate clade and were the sister group to the vascular plants (Fig. 1). The four apomorphies for this lineage were the presence of slime papillae (character 18), the superficial archegonia (character 30), dimorphic basal bodies (character 41), and the presence of an aperture associated with the spline (character 44). That mosses and liverworts are sister taxa was very strongly argued by Schuster (1984a), and was also supported by Garbary *et al.* (1993) based on male gametogenesis. In addition, molecular studies by Hedderson *et al.* (1996) using sequencing of the 18S rRNA gene and Malek *et al.* (1996) using the mitochondrial *cox3* gene support this topology. Contrasting views regarding the homology of morphological and cytological traits between mosses and liverworts (and hornworts) were expressed by Crandall-Stotler (1980, 1984). Based on 18S rRNA sequences Capesius & Bopp (1997) reported cladograms quite different from those of Hedderson *et al.* (1996) and our results in Figs 1–2; however, these differences may be attributed to problems associated with taxon selection by Capesius & Bopp (1997). Lewis *et al.* (1997), using the chloroplast-encoded gene *rbcL*, also reported quite different tree topologies (e.g. with hornworts sister to vascular plants).

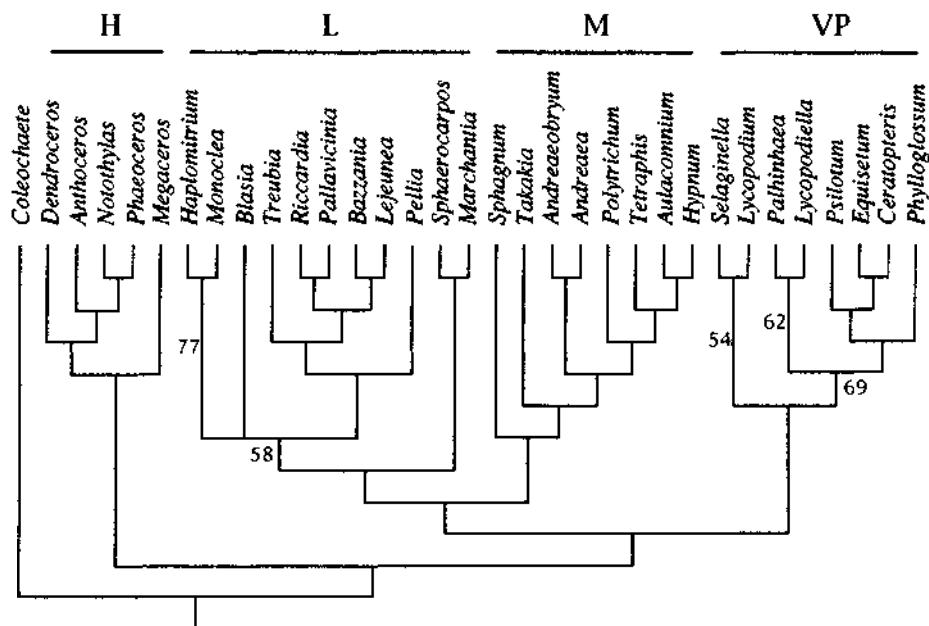


Figure 2. Majority rule consensus tree of 26 cladograms from overall analysis. Numbers above branch indicate percentage of trees with this topology from among the most parsimonious. Note: Unless indicated otherwise, values are 100%.

Their results may reflect limitations on the use of *rbcL* on these deep divisions in the land plant clade.

Relationships among liverworts (Fig. 1) were poorly defined by the characters in this study. The majority rule consensus tree (Fig. 2) suggested four distinct lineages: 1) *Haplomitrium* plus *Monoclea*, 2) *Blasia*, 3) *Marchantia* plus *Sphaerocarpos*, and 4) the *Pellia*, *Bazzania*, *Lejeunea*, *Pallavicinia*, *Riccardia*, *Treubia* assemblage. The last assemblage includes the bulk of the Metzgeriales in the analysis (minus *Blasia*), and it is characterized by the absence of a notch in the lamellar strip (character 45). In some MP cladograms *Blasia* is linked to this assemblage with which it shares the presence of multiple layers in sporangial walls (character 85). This group of taxa (either with or without *Blasia*) does not support recognition of Treubiales and Jungermanniales as distinct from Metzgeriales, nor does it support Schuster's (1984b) concept of the subclass Jungermannidae. Two of the MP cladograms, however, distinguish the liverwort genera into the subclasses Jungermannidae and Marchantiidae as characterized by Schuster (1984a, b) with the exception of the inclusion of *Haplomitrium* and *Blasia* in the Marchantiidae. Schofield (1985) suggested similarities between Sphaerocarpales and Marchantiales based on similar trends in sporophytic reduction. Characters that indicate a relationship between these orders include reduction of antheridial stalks to one or two rows (character 34), similarities in embryo development (character 67) and the absence of SMC lobing (character 88).

The basal branches of the vascular plant clade were poorly resolved by our analysis and formed two groups of tree topologies. In the first group, there was successive

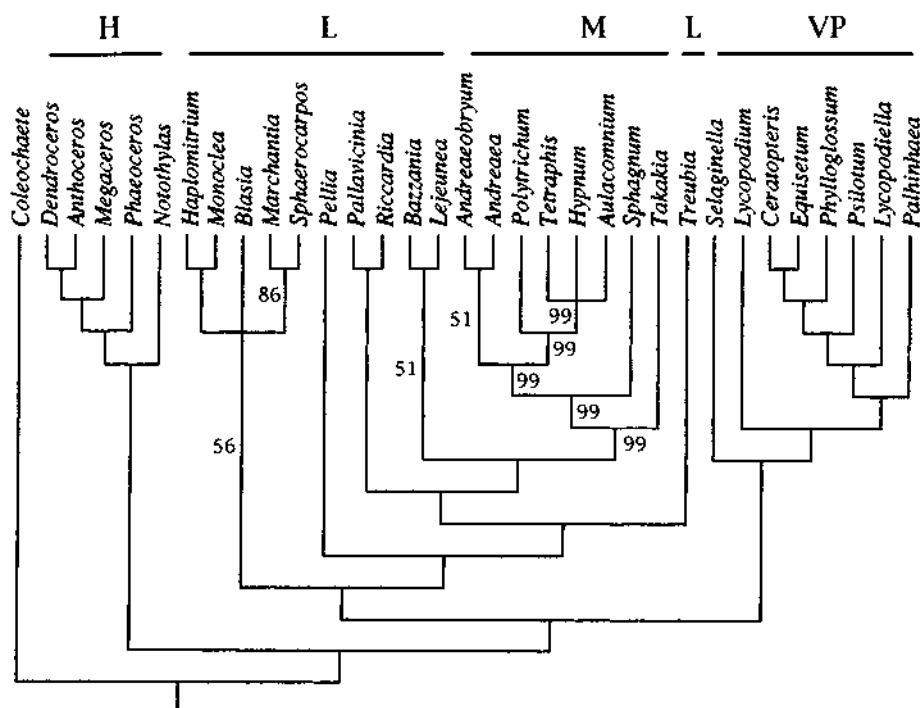


Figure 3. Majority rule consensus tree of 85 most parsimonious cladograms from gametophytic characters only (TL = 150; C.I. = 0.67). Note: Unless indicated otherwise, all values are 100%.

paraphyly of lycophyte genera with *Selaginella* being basal or associated with *Lycopodium*. In none of the MP trees did all lycophytes form a monophyletic group. In the second major tree topology (Fig. 1), lycophytes other than *Phylloglossum* formed one clade. The remaining genera formed a clade in which *Phylloglossum* was always the sister group to *Ceratopteris*, *Equisetum* and *Psilotum*. Other than the position of *Phylloglossum* (a lycophyte), the second tree topology is congruent with other analyses on *Lycopodiella lateralis* and *Selaginella* sp. (Maden *et al.*, 1997; Renzaglia *et al.*, unpublished data) using a character set based on male gametogenesis. This arrangement supports the preliminary analysis of Garbary *et al.* (1993) in which male gametogenesis was used as the sole basis for phylogenetic reconstruction.

Until recently molecular sequence data has only resolved the phylogenetic relationships of land plants poorly. The analyses of 5s rRNA sequences (e.g. Van de Peer *et al.*, 1990) were considered unsuitable for this problem, although the cladogram based on this analysis bears close similarity with that based on male gametogenesis (Garbary *et al.*, 1993). The *rbcL* sequences have proven useful for resolving relationships within more recently derived groups; however, such sequences have not been effective for deep branching in the land plant assemblage (Manhart, 1994). The early 16s, 18s and 23s rRNA sequences of Chapman & Buchheim (1991), Waters *et al.* (1992) and Mishler *et al.* (1994) provided no compelling resolution. Analyses of large numbers of taxa using complete sequences of the 18s rRNA gene were recently published by Hedderson *et al.* (1996), Hedderson & Chapman (1998) and Bopp &

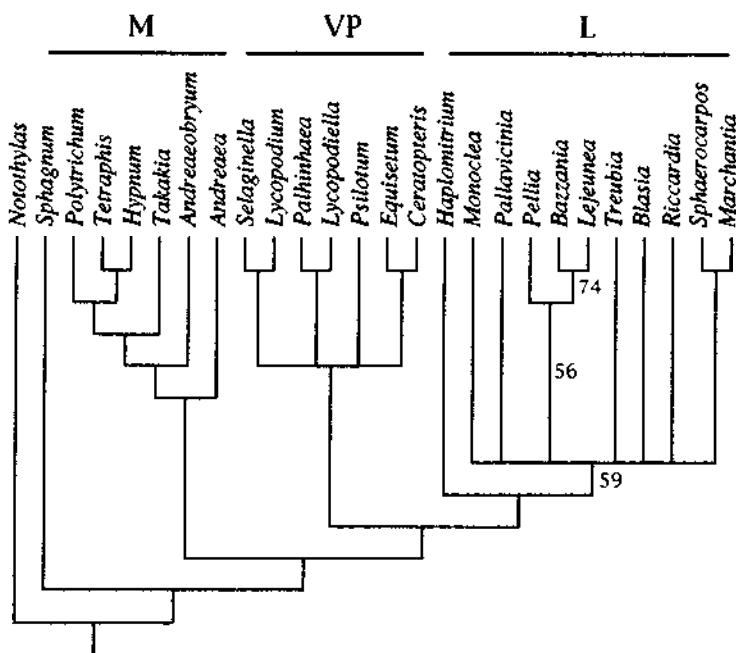


Figure 4. Majority rule consensus tree of 1446 cladograms based on sporophytic characters only (TL = 130). Note: *Notothylas* designated as outgroup, and cladogram with reduced number of taxa relative to Figs 1–3. Note: Unless indicated otherwise, all values are 100%.

Capesius (1995, 1996). The resulting cladograms were dramatically different both from previous studies and from each other. Hedderson *et al.* (1996) and Hedderson & Chapman (1998) presented cladograms more or less consistent with the analysis presented here. Bopp & Capesius (1995, 1996), however, produced a radically different tree in which Marchantiopsida was the sister group to the remaining bryophytes. In addition, Jungermanniales was the sister group to hornworts, with the Jungermanniales assemblage as sister group to the mosses. The different hypotheses that resulted from these studies may reflect the taxon selection by Bopp & Capesius with large numbers of bryopsid mosses and leafy liverworts and few representatives of basal lineages being selected. In addition, vascular plants were not included, and this may reflect their anomalous results. Our basic arrangement of the hornwort, liverwort, moss and vascular plant clades to each other are congruent with the tree in Fig. 1 of Hedderson *et al.* (1996). Within the liverwort and moss clades the results are dramatically different from ours, and may be caused by limited taxon sampling within the potentially primitive moss and liverwort lineages (i.e. absence of *Sphagnum*, *Blasia* and *Monoclea* from their analysis).

The position of hornworts as the sister group to other land plants is consistent with the fact that hornworts are the only group of land plants with a pyrenoid (Vaughn *et al.*, 1992). Ultrastructurally and physiologically (Vaughn *et al.*, 1990, 1992; Smith & Griffiths, 1996a, b) pyrenoids of hornworts are the same as those of green algae. Smith & Griffiths (1996b) argue that this is associated with the physiology of carbon-concentrating mechanisms, and that it supports the notion of

hornworts as being the most primitive of extant land plants. The position of hornworts on our cladogram (Fig. 1) is consistent with this hypothesis, and the evolutionary speculations of Schuster (1984c and earlier) who supported an independent origin of hornworts and other bryophytes. The analysis of male gametogenesis (Garbary *et al.*, 1993) showed that hornworts were isolated from other bryophytes. This was further elaborated by Vaughn & Renzaglia (1998) in their studies of bicentriole origin in spermatogenous cells of hornworts, and by processes associated with microtubule organization during mitosis and meiosis (Brown & Lemmon, 1990b, 1993).

The analysis produced some MP trees topology in which *Haplomitrium* was the basal lineage in liverworts and *Takakia* was close to the base of the mosses (only one additional step is required for *Takakia* to be the outgroup for mosses). Although this is not overwhelming support, if correct, it suggests that the similarities between *Takakia* and *Haplomitrium* are plesiomorphies, and that the classical taxonomic interpretations of Schuster (1984b) and Schofield (1985) (i.e. with both genera included in Calobryales) had a morphological basis that can be recognized in the current analysis. The identification of *Takakia* as a moss is now based on a wide variety of evidence including sperm architecture and sporophyte development (Garbary *et al.*, 1993; Smith & Davison, 1993; Renzaglia, McFarland & Smith, 1997), and 18s rRNA sequences (Hedderson *et al.*, 1996; Hedderson & Chapman 1997). With these data and the general developmental and ultrastructural features utilized here, the historically problematic assignment of *Takakia* is now resolved (*cf.* Crandall-Stotler, 1986). Key structural similarities between *Takakia* and *Haplomitrium* include the lateral origin of branches (character 6), the absence of rhizoids (character 10), the formation of water conducting cells via plasmodesmata (character 11), the absence of food conducting cells (character 12), and the early timing of transfer cell development (character 84). These are intriguing similarities, even if many are joint absences of character states. In spite of these similarities, our analysis clearly demonstrates that *Takakia* is in the moss clade, and 26 extra steps are required to align *Takakia* artificially with the liverworts.

The position of *Takakia* near the base of the moss clade is supported by recent investigations of the sporophyte (Renzaglia *et al.*, 1997). The single spiralled suture line in the capsule is reminiscent of *Tortilicaulis* from the Lower Devonian (Edwards, Fanning & Richardson, 1994; Edwards, 1996). This may be the primitive mechanism for sporangial opening, although the occurrence of two equal valves in *Salopella* (Edwards, 1996; Edwards *et al.*, 1994) suggests an early radiation of sporangial dehiscence mechanisms. The association of the dehiscence mechanism in *Takakia* with the absence of any differentiated suture cells further highlights the primitiveness of *Takakia* within mosses.

Is there a possibility that mosses are sister group to land plants as proposed by Mishler & Churchill (1984, 1985), and supported as the favoured tree topology in a number of subsequent studies (e.g. Kenrick & Crane, 1991, 1997; Mishler *et al.*, 1994)? Trees only two steps longer than the MP trees allow for this possibility (Table 4). The perine layer in spores of mosses and vascular plants provides an apparent synapomorphy between these groups, if they form a clade. Given the tree topology shown in Fig. 1, one must either hypothesize that this is a convergence or that the character was lost in liverworts. This tree topology is also associated with

the interpretation of stomatal homology in all land plants, and whether or not the paired cells in the capsules of *Sphagnum* are vestigial stomata. If these structures are not homologous then stomata are likely to have had an independent origin in mosses and vascular plants. The possibility of a third origin for stomata is raised by the fact that stomata are absent in *Notothylas*, and are present in only one of the five hornwort genera considered here (i.e. *Anthoceros*). Thus if the *Sphagnum* structures are not stomata, then a moss/vascular plant clade requires a minimum of five losses of stomata (i.e. at least once in hornworts, once in liverworts and three times in mosses), versus three separate origins for these structures. Clearly the 'stomata' of *Sphagnum* require further analysis. A single origin for stomata at the base of the land plant clade and their subsequent loss in various lineages would be the simplest explanation for the distribution of this character.

Most orders of liverworts (i.e. Calobryales, Monocleales, Marchantiales and Sphaerocarpales) are represented only by single genera; thus, it is only ordinal concepts for Metzgeriales and Jungermanniales that can be evaluated with our analysis. *Bazzania* and *Lejeunea* are sister groups, thus supporting the Jungermanniales. Renzaglia (1982) argued that similarities in vegetative development in Metzgeriales supported a natural assemblage; however, this order is not supported in our cladograms. Thus, there are at least three separate paraphyletic lineages in a clade (Fig. 2) with Jungermanniales as well as an apparently unrelated *Blasia*. Relationships of *Blasia* are difficult to resolve because of missing data (e.g. characters 28, 48, 67, 106). The clade that included *Treubia*, *Pellia*, *Riccardia*, *Pallavicinia*, *Bazzania* and *Lejeunea* was present in all of the most parsimonious trees. The relationship between Metzgeriales and Jungermanniales was discussed by Schofield (1985) although he concluded that they were independent evolutionary lineages. Our inability to distinguish these orders might reflect the generalized nature of our characters, and their inability to deal with the extent of variation in this assemblage. The problematic nature of the Metzgeriales is also highlighted in the liverwort cladogram of Hedderson *et al.* (1996) based on 18s rRNA sequences. Taken together, these two studies suggest a need for a major reexamination of Metzgeriales.

Prior to Hedderson *et al.* (1996), Malek *et al.* (1996), Hedderson & Chapman (1998) and this paper, several cladistic analyses had suggested that the hornworts were the sister group to the remaining land plants. Mishler *et al.* (1994) showed that the general morphological characters (i.e. no sperm data) could support such a tree topology (their Fig. 3) as well as the 18s data alone (their Fig. 7). Thus the general isolation of hornworts among land plants that has been suggested by many authors (e.g. Proskauer, 1960; Schofield, 1985; Brown & Lemmon, 1993; Vaughn & Renzaglia, 1998; Hedderson *et al.*, 1996; Hedderson & Chapman, 1998) is confirmed by our analysis. Whether or not there is (or ever can be) sufficient morphological evidence from nonvascular land plants in the fossil record (e.g. Remy *et al.*, 1993; Edwards, Wellman & Axe, 1998) to incorporate those taxa into a more complete analysis remains to be demonstrated. The extensive studies of Remy *et al.* (1993) of Devonian gametophytes of land plants have reasonably established that the interpolation model for the origin of the land plant life history is questionable. The isomorphic life histories in these organisms supports the hypothesis that this feature arose prior to the origin of extant embryophyte groups (except possibly hornworts) (*cf.* Hemsley, 1994). The discovery of intermediates between organisms with

isomorphic life histories from the Devonian and earlier, and those with a bryophyte life history, provides an important challenge for paleobotanists. Should such data be recoverable it will provide a critical test for the phylogenetic hypothesis presented here.

The general features of the cladogram that we present for land plants (Figs 1–2) provide a reasonable hypothesis for the relationships of land plants, especially since this tree is supported by molecular sequencing of 18s rRNA. Several alternative arrangements are only slightly less parsimonious (Table 4), however, and will require careful consideration in any future analyses of this problem. As further developmental and ultrastructural studies are carried out, character state homology should be better defined and lead to more definitive answers with respect to the phylogeny of land plants.

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APPENDIX 1 LIST OF CHARACTERS

Note: Characters 1–65 refer to gametophytic characters. Characters 66–132 refer to sporophytic features. In analyses of complete dataset repeated characters were omitted.

1. Apical cell geometry: 0, dome or tip cell; 1, tetrahedral; 2, wedge-hemidiscoid; 3, lenticular; 4, meristem
2. Segmentation of the gametophyte: 0, transverse; 1, parallel; 2, oblique
3. Gametophyte morphology: 0, dorsiventral; 1, axial
4. Gametophytes with fungi: 0, absent; 1, present
5. Development of leaves or wings: 0, central wedge; 1, two apical cells; 2, one apical cell
6. Branch origin: 0, dichotomous; 1, lateral
7. Monoplasticidic meristematic cells: 0, present; 1, absent
8. Growth habit: 0, filamentous; 1, thallose; 2, leafy (liverwort); 3, leafy (moss)
9. Veins in leaves or wings: 0, absent; 1, present
10. Rhizoids: 0, absent; 1, unicellular; 2, multicellular
11. Development of water conducting cells: 0, absent; 1, associated with plasmodesmata; 2, not associated with plasmodesmata
12. Food conducting cells: 0, absent; 1, microtubule associated; 2, phloem
13. Pegged rhizoids: 0, absent; 1, present
14. Air chambers: 0, absent; 1, present
15. Mucilage cavities: 0, absent; 1, present
16. Ventral scales: 0, absent; 1, present
17. Paraphyses: 0, absent; 1, present
18. Slime papillae: 0, absent; 1, present
19. Terete phylids: 0, absent; 1, present
20. Pyrenoids: 0, present; 1, absent
21. Pyrenoid subunits: 0, irregular; 1, irregular with inclusions; 2, lens shaped; 3, absent
22. Channel thylakoids: 0, present; 1, absent
23. Oil bodies: 0, present; 1, absent
24. Grana end membranes: 0, absent; 1, present
25. Spindles in vegetative cells: 0, from centrioles; 1, polar organizers; 2, plastid based; 3, membrane based
26. Charasomes: 0, absent; 1, present
27. Antheridial initial: 0, superficial; 1, epidermal/subepidermal; 2, endogenous; 3, endosporic
28. Number of primary androgones: 0, single; 1, paired; 2, four; 3, NA
29. Gametangial apical cell: 0, absent; 1, present
30. Archegonial morphology: 0, superficial; 1, sunken
31. Liverwort pyramidal axial cell architecture: 0, yes; 1, no
32. Acrogyny: 0, absent; 1, present
33. Tiers of primary androgones: 0, one tier; 1, two tiers; 2, more than two tiers; 3, NA; 4, alternate
34. Antheridial stalks: 0, uni- or biserrate; 1, fourserrate (or more); 2, NA
35. Antheridial jacket cells tiered: 0, absent; 1, present
36. Number of antheridia per chamber: 0, one; 1, two to four; 2, numerous; 3, NA
37. Biflagellate: 0, present; 1, absent
38. Monoplasticidic sperm cells: 0, present; 1, absent; 2, one or two
39. Sperm with two mitochondria: 0, absent; 1, present
40. Flagella staggered: 0, absent; 1, present; 2, continuous stagger

41. Dimorphic basal bodies: 0, absent; 1, present
42. Lamellar strip orientation: 0, 90°; 1, 45°; 2, variable
43. Centriole origin: 0, always present; 1, bicentrioles; 2, blepharoplast
44. Aperture on spline: 0, absent; 1, present
45. Notch in lamellar strip: 0, absent; 1, present
46. Callose in sperm cells: 0, present; 1, absent
47. Leaf insertion: 0, oblique; 1, transverse
48. Number of cell in archegonial neck: 0, four; 1, five; 2, six
49. Oil body cells: 0, absent; 1, present
50. Beaked papillae: 0, absent; 1, present
51. Calyptra: 0, absent; 1, intact; 2, apical calyptra
52. Protonema: 0, absent; 1, present; 2, thallus
53. Protonemata cylindrical: 0, NA; 1, absent; 2, present
54. Subterranean gametophytes: 0, absent; 1, present
55. Cuticle: 0, absent; 1, present
56. Coiling of sperm: 0, uncoiled; 1, coiled left; 2, coiled right
57. Antheridia in leaf axils: 0, NA; 1, present; 2, absent
58. Transfer cells in gametophyte: 0, present; 1, absent
59. Transfer cells labyrinthine (hornworts): 0, simple; 1, complex
60. Hornwort phenolics: 0, absent; 1, present
61. Lignin: 0, absent; 1, present
62. D-lunularic acid: 0, absent; 1, present
63. Flavonoids: 0, absent; 1, present
64. Plastoglobuli (hornworts): 0, absent; 1, present
65. Hornwort phenolics (megacerotinin): 0, absent; 1, present
66. Zygote division: 0, vertical; 1, horizontal; 2, free nuclear
67. Embryo development (liverworts): 0, hypobasal haustoria only; 1, hypobasal more; 2, NA
68. Apical cell in embryo: 0, absent; 1, present; 2, more than one
69. Endothecium: 0, columella; 1, sporogenous tissue plus columella
70. Intercalary meristem: 0, NA; 1, absent; 2, embryo; 3, continuous
71. Pseudopodium: 0, absent; 1, present
72. Columella: 0, absent; 1, present
73. Archegoniophore: 0, absent; 1, present
74. Peristome: 0, absent; 1, whole cells; 2, articulated
75. Line dehiscence: 0, absent; 1, present; 2, operculum; 3, lip cells
76. Stomata: 0, absent; 1, sporangium; 2, vegetative cells
77. Sporophyte generation: 0, absent; 1, present
78. Branched sporophyte: 0, absent; 1, present
79. Conducting tissue: 0, absent; 1, present
80. Transfer cells in sporophyte: 0, NA; 1, absent; 2, present
81. Placental protein crystals: 0, absent; 1, present
82. Seta elongation: 0, cell elongation; 1, cell division; 2, no elongation
83. Thickened gametophyte placental walls: 0, absent; 1, present
84. Timing of transfer cell development: 0, early; 1, late; 2, continuous
85. Sporangial wall: 0, multiple layers; 1, single layer; 2, eusporangiate; 3, leptosporangiate
86. Sporangial location: 0, solitary and terminal; 1, microphylls; 2, caulinne associated enations; 3, megaphylls; 4, sporangiophores
87. Synchronized sporogenesis: 0, present; 1, absent
88. Spore mother cell lobed (bryophytes only): 0, present; 1, absent
89. Monoplasticid spore mother cells: 0, present; 1, absent
90. Axial microtubule system: 0, absent; 1, present
91. Sporogenous tissue: 0, all cells; 1, elaters; 2, nurse cells
92. Elater mother cell division: 0, absent; 1, present
93. Pseudoelators: 0, absent; 1, not spiraled; 2, spiraled
94. Elater autofluorescence: 0, absent; 1, present
95. Spore wall: 0, absent; 1, present
96. Aperture: 0, absent; 1, present
97. Exine: 0, nontripartite; 1, tripartite
98. Callose in sporocyte wall: 0, present; 1, absent
99. Primexine: 0, absent; 1, present
100. Perine in spore wall: 0, absent; 1, present
101. Moss spore walls: 0, foundation layer; 1, two exine layers; 2, spongy exine
102. Trilete mark: 0, present; 1, absent
103. Apical growth: 0, absent; 1, single cell; 2, seed plant
104. Cuticle (sporophyte): 0, NA; 1, present
105. Lignin: 0, absent; 1, present
106. Pinguis-sesquiterpenes (liverworts): 0, absent; 1, present
107. Cylindrical sporogenous layer: 0, absent; 1, present
108. Flavonoids: 0, absent; 1, present
109. Polyphenolics with sex: 0, absent; 1, present
110. Apophysis: 0, absent; 1, present
111. Single haustorial cells: 0, absent; 1, present
112. Foot shape: 0, long-tapering; 1, bulbous; 2, conical-slightly elongate; 3, ovoid; 4, cup-shaped; 5, spheroidal
113. Palisade in foot: 0, absent; 1, present

- 114. Thickenings in capsule walls: 0, NA; 1, longitudinal; 2, transverse; 3, absent
- 115. Roots: 0, absent; 1, present
- 116. Stele: 0, protostele; 1, siphonostele; 2, NA
- 117. Embryo development: 0, NA; 1, exoscopic; 2, endoscopic (with suspensor); 3, other
- 118. Protocorm: 0, absent; 1, present; 2, NA
- 119. Sporangial morphology: 0, synangium; 1, elongated; 2, reniform to globose; 3, other; 4, capsule
- 120. Microphylls: 0, no leaves; 1, spiralled; 2, whorled; 3, prophylls; 4, megaphylls
- 121. Tapetum: 0, absent; 1, plasmoidal; 2, secretion
- 122. Xylem maturation: 0, NA; 1, exarch; 2, endarch
- 123. Monoplastidic meristem: 0, present; 1, absent
- 124. Development of water conducting cells: 0, absent; 1, associated with plasmodesmata; 2, not associated with plasmodesmata
- 125. Chloroplast number: 0, one; 1, two; 2, > two
- 126. Pyrenoids: 0, present; 1, absent
- 127. Pyrenoid subunits: 0, irregular; 1, irregular with inclusions; 2, lens; 3, absent
- 128. Channel thylakoids: 0, present; 1, absent
- 129. Oil bodies: 0, present; 1, absent
- 130. Grana end membranes: 0, absent; 1, present
- 131. Spindles in vegetative cells: 0, from centrioles; 1, polar organizers; 2, plastid based; 3, membrane based
- 132. Plastoglobuli (hornworts): 0, absent; 1, present



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4. Bryophytes and the origins and diversification of land plants: new evidence from molecules

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4.1 SUMMARY

We analyse complete or nearly complete small subunit rRNA gene sequences for 82 taxa representing all the major lineages of green plants. Parsimony and maximum-likelihood analyses of these sequences resolve two main lineages, one comprising the Charophytes and the land plants, and one comprising the remainder of the green algal groups. Within the former lineage, a clade comprising *Coleochaete*, *Klebsormidium*, and the land plants is very strongly supported (bootstrap and jackknife analyses), with the first two taxa forming a moderately well-supported clade. The hornworts are strongly supported as the sister group to the remaining land plants, while the mosses and liverworts each form monophyletic groups that are weakly supported as sister taxa and together form the sister group to a well supported tracheophyte clade. Within the tracheophyte lineage non-lycopod taxa form a well supported group. Psilotales are weakly supported as sister to the eusporangiate ferns, and this combined clade is resolved as sister to a very strongly supported seed plant clade. Other relationships among tracheophytes are not, or only weakly, supported by our analyses.

KEYWORDS: 18s rRNA, Anthocerotopsida, Hepatopsida, Bryopsida, *Psilotum*, *Coleochaete*, green plants.

4.2 INTRODUCTION

The evolution of the land plants, making possible the subsequent evolution of much of modern terrestrial biodiversity (Behrensmeyer *et al.*, 1992; Graham, 1996), was clearly among the most significant of incidents in the Earth's history. Understanding this event and subsequent diversification of the main lineages of land plants is important from a number of perspectives. For example, insight into the evolutionary transformations associated with adopting terrestrial modes of existence and with further diversification of life histories and breeding systems would be much increased by further comprehension of these events (Hedderson, Chapman & Rootes, 1996); Graham (1996) gives detailed treatment to three additional areas where the possession of phylogenetic knowledge is critical for future advances.

Some features of this problem do indeed appear to be approaching resolution.

For example, a substantial suite of characters, including molecular, biochemical, ultrastructural and morphological information, indicates that the land plants are monophyletically derived from algae, and probably share a common ancestor with some member of the Charophyceae (Graham, Delwiche & Mishler, 1991; Garbary, Renzaglia & Duckett, 1993; Graham, 1993, 1996; Mishler *et al.*, 1994; McCourt, 1995; Hedderson *et al.*, 1996). Similarly, a considerable body of data provides strong evidence for the monophyly of the seed plants (Stewart & Rothwell, 1993). Nonetheless, the evolutionary origins of land plants, and especially their major patterns of diversification, remain imperfectly known. In particular, the relationships of the morphologically and biologically diverse lineages that constitute the 'bryophytes' and the 'vascular cryptogams' remain controversial. These are precisely the groups that are likely to be most crucial in relation to understanding the processes mentioned above.

The question of how the three main bryophyte lineages (i.e. hornworts, mosses and liverworts) are related to each other and to other land plants has been the subject of several recent analyses using morpho-anatomical, biochemical or spermatogenesis data sets alone or in various combinations. These have yielded diverse results. Some suggest monophyly of the bryophytes (Garbary, Renzaglia & Duckett, 1993), others complete paraphyly, with mosses alone sister to the tracheophytes and the hepaticas sister to the remaining land plants (Mishler & Churchill, 1984, 1985; cf. Bremer *et al.*, 1987; Kenrick & Crane, 1991; Mishler *et al.*, 1994), and yet others suggest that hornworts are sister to the remaining land plants while mosses and liverworts form a monophyletic group that is sister to the tracheophytes (Garbary & Renzaglia, this volume).

Interpretations of relationships among the vascular cryptogams have been similarly controversial. For example, the Psilotales (*Psilotum* and *Tmesipteris*) have frequently been considered the earliest-diverging tracheophytes, largely on the basis of their supposed similarity (in lacking leaves and roots, and their simple stem anatomy) to the Devonian Rhyniophyta (e.g. Eames, 1936; Parenti, 1980; Minkoff, 1983; Bremer *et al.*, 1987). Other evidence, including data from anatomy, ultrastructure, and cpDNA organization suggests that the psilotophytes are probably not a basal divergence among tracheophytes, and are more likely to be related to either the eusporangiate or leptosporangiate ferns (Bierhorst, 1971; Gensel, 1977; Raubeson & Jansen, 1992; Garbary *et al.*, 1993). Similarly, relationships among the eusporangiate and leptosporangiate 'ferns', and the placement of the Sphenopsida have been the subject of considerable controversy (Stewart & Rothwell, 1993).

Recently, DNA sequence data have been used widely in attempts to resolve relationships among anciently diverging lineages (e.g. Olsen & Woese, 1993). Analyses of *rbcL* sequences appeared unable to resolve among the land plant lineages — presumably because of substitutional saturation and subsequent loss of phylogenetic signal at such high taxonomic levels in this relatively rapidly evolving sequence (Manhart, 1994). A number of studies have used the much more conserved small subunit rRNA gene sequence to address questions of land plant phylogeny, with sometimes widely divergent results. For example, the various available analyses of the bryophytes (Waters *et al.*, 1992; Bopp & Capesius, 1995, 1996; Capesius, 1995; Kranz *et al.* 1995; Hedderson *et al.* 1996) have suggested quite disparate relationships. In one study (Hedderson, *et al.*, 1996) the hornworts were resolved as a basal

lineage, with mosses and liverworts forming a monophyletic group sister to the tracheophytes. Other analyses have resolved the hepaticas as sister to the remaining land plants, with the hornworts sister to the mosses (Waters *et al.*, 1992). A recent study (Bopp & Capesius, 1996), using a large number of bryophyte taxa and a single species of *Chara* as an outgroup, resolved the hepaticas as non-monophyletic, with a hornwort-Jungermannidae clade sister to the mosses, and with the thalloid hepaticas emerging as the earliest-diverging lineage. However, if treated as unrooted, the topological relationships among the bryophytes in this tree are the same as those found by Hedderson *et al.* (1996), except for the placement of *Chara*. Since the thalloid hepaticas and the outgroup taxon both exhibit considerable sequence divergence, there is reason to suspect that a 'long branch' problem (Felsenstein, 1978) may exist; such problems can render any method of phylogeny reconstruction inconsistent. As we show below, using 'closer' outgroups (e.g. *Coleochaete*; Mishler & Churchill, 1984, 1985; Graham *et al.*, 1991) alters the rooting point, hence interpretation of the phylogenetic relationships among the taxa. Other potential sources of discrepancy among the different studies include differences in taxon sampling, as well as variation in the quality of sequence data as available laboratory techniques have improved.

Studies using the ssu rRNA gene for examining land plant relationships have rarely incorporated many representatives of the vascular cryptogam lineages. A recent analysis (Kranz & Huss, 1996), using nine pteridophyte sequences, provided support for the placement of the lycopods (*Lycopodium*, *Selaginella*, *Isoetes*) rather than the psilophytes at the base of the tracheophyte clade; *Psilotum* was resolved as sister to the seed plants. *Equisetum* was resolved as sister to the *Psilotum*-seed plant clade, while the leptosporangiate ferns were resolved as a monophyletic group. The eusporangiate ferns were not represented.

In this paper we present analyses of an extensive set of ssu rRNA gene sequences. All of the major lineages of green plants are represented, but our coverage of the land plants is especially dense. The main objectives of this analysis were to 1) reassess whether land plants are monophyletically derived, 2) identify the nearest sister group to the land plants among the green algae, 3) determine the relationships among the main bryophyte groups and their relationships to other land plants, and 4) determine the relationships of the various lineages of 'pteridophytes'.

4.3 METHODS

The 82 taxa used in this analysis are listed by broad taxonomic group in Appendix 1 along with their Genbank accession numbers. As noted above, all major groups of green plants are represented, with the sampling being dictated to a certain extent by availability of published sequences. Sampling concentrates explicitly on the cryptogamic land plant lineages, and as far as possible we have included representatives of all the major groups previously recognized within each of these. Our sampling includes two hornworts, 20 mosses, eight liverworts, eight lycophytes, six leptosporangiate ferns, a eusporangiate fern, one species of *Equisetum*, both extant genera of Psilophyta and 13 seed plants. In addition, we have included eight representatives of the Charophyta in an attempt to address the critical issue of identifying the root-

node of the land plant clade. Analyses including all green plant lineages used representatives of the glaucophytes and chlorachniophytes as outgroups.

For taxa sequenced in this study, we used standard methods to extract DNA from either freshly collected field material or from herbarium specimens (Edwards, Johnstone & Thompson, 1991), and to prepare sequencing templates *via* the polymerase chain reaction (Saiki *et al.*, 1988). Templates were sequenced using internal primers (Hamby *et al.*, 1988) in conjunction with the ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit (P. E. Applied Biosystems). Sequencing products were resolved on an ABI (model 373, stretch) automated sequencing machine.

Assembled sequences were aligned manually using Megalign (Lasergene System Software, DNASTar Inc.). Alignments were optimized by explicit reference to a secondary structural model (Huss & Sogin, 1990; Neefs & De Wachter, 1990; Neefs *et al.*, 1993). Some positions, especially in the V2 and the V4 variable regions (terminology follows Neefs & De Wachter, 1990, and Neefs *et al.*, 1993), could not be aligned unambiguously. These, along with *ca* 45 positions from either end of the molecule for which many taxa had missing information, were excluded from subsequent analyses.

Parsimony analyses were run in PAUP* (D. L. Swofford, unpublished) mounted on a Macintosh PowerPC. The g_1 statistic (Huelsenbeck, 1991), based on the length distribution of 50,000 trees generated by the RANDOM TREES procedure, was used to test for the possible presence of significant phylogenetic signal in the data set. Because of the large number of taxa involved, heuristic search procedures were employed. These were implemented with random taxon addition (300 replicates) to improve our search of the tree space. Support for particular nodes was assessed by Bootstrap (Felsenstein, 1985) and Parsimony Jackknife analyses (Farris *et al.*, 1995). For the bootstrap analyses, 3,000 replicates were used, while the jackknife percentages were based on 10,000 replicates.

Maximum-likelihood estimates of phylogeny were also made in PAUP*. The General Time-Reversible Model (Yang, 1994) of nucleotide substitution was used with rate variation among sites assumed to approximate a gamma distribution. A discrete approximation to gamma was used, based on four rate categories. Substitution probabilities, as well as the shape parameter of the gamma distribution, were estimated empirically from a randomly chosen parsimony tree. In all cases, neighbour-joining was used to generate starting trees for the likelihood analyses. Heuristic searches were performed with TBR branch swapping. Time constraints precluded the calculation of support indices (i.e. bootstrap, jackknife) for nodes resolved in the likelihood analyses.

4.4 RESULTS

4.4.1 *The green plants*

The overall alignment, including the outgroup taxa, is 1998 nucleotides long. Because of alignment difficulties, mostly involving the outgroup taxa, 264 positions have been excluded from analysis in addition to those excluded from the ends. Of the remaining 1644 positions, 834 are variable and 529 are parsimony-informative. The distri-

butions of 50,000 random trees are highly significantly left-skewed ($g_1 = 0.75$; $p < 0.0001$), suggesting the presence of substantial phylogenetic signal in the data set.

Under the parsimony criterion 58 equally optimal trees (Length = 2675; CI = 0.48; RI = 0.70) were identified, occupying a single tree island. This island was not found until replicate number 116, and was hit only four times in 300 random-addition replicates. The trees found differed only in arrangements within the larger clades and were in complete agreement with respect to relationships among the higher-level lineages (Fig. 1). The maximum likelihood tree also had an identical topology at these levels.

In all trees the green plants are strongly resolved into two major clades (Fig. 1). One of these clades contains the Ulvophycean, Chlorophycean, Trebouxiophycean and Prasinophycean taxa. The Ulvophyceae form a well-supported monophyletic group as do the combined Chlorophyceae-Trebouxiophyceae. The only representative of the Prasinophyceae (*Tetraselmis*) is placed sister to the latter lineage. The second clade comprises Charophyceae and the land plants. The Characeae and the land plants are each robustly supported as monophyletic, and a group containing *Coleochaete* and *Klebsormidium* is moderately well supported. Characeae is placed sister to the rest of the taxa in this clade, while the *Coleochaete-Klebsormidium* clade is well supported as sister to the land plants. *Mougeotia* is weakly resolved as sister to the land plant-Coleochaetalean lineage.

4.4.2 The land plants

For analyses of relationships within the land plants, the two protist outgroups have been excluded, along with most of the green algae. This reduced sampling allows more of the data to be used since more regions can be aligned without ambiguity after removal of some taxa. *Coleochaete*, *Klebsormidium*, *Chara* spp. and *Lamprothamnium* have been retained from the Charophyceae, along with the chlorophycean taxa *Chlorella fusca*, *Muriella aurantiaca* and *Chlamydomonas reinhardtii* which are used to root trees. This produced an alignment 1954 positions long, from which 92 positions were excluded (in addition to those excluded from the ends of the molecule) because of alignment difficulties involving alignment between the land-plant/charophyte clade and the more distantly related chlorophycean algae. Of the remaining 1772 positions, 781 are variable and 475 are parsimony informative. Again, the distribution of random trees was significantly left-skewed ($g_1 = -0.72$; $p \ll 0.001$).

Under parsimony, 41 equally optimal trees ($L = 2390$; $CI = 0.41$; $RI = 0.66$), were recovered from a single tree island. As in the overall analysis, this island was not hit until relatively late in the search (replicate no. 104), and was found only 52 times in 300 replicates. The single tree found under the likelihood criterion was identical to one of the MPTs. The strict consensus of the 41 trees is shown in Fig. 2.

As in the overall analysis, a clade containing *Coleochaete*, *Klebsormidium* and the land plants is well supported, with moderate support for a sister-group relationship between the first two. Each of the three main bryophyte lineages is resolved as monophyletic, with the hornworts resolved as sister to the remaining land plants and the liverworts and mosses forming a clade which is sister to the tracheophytes. The monophyly of the hornworts and their position sister to the remaining land

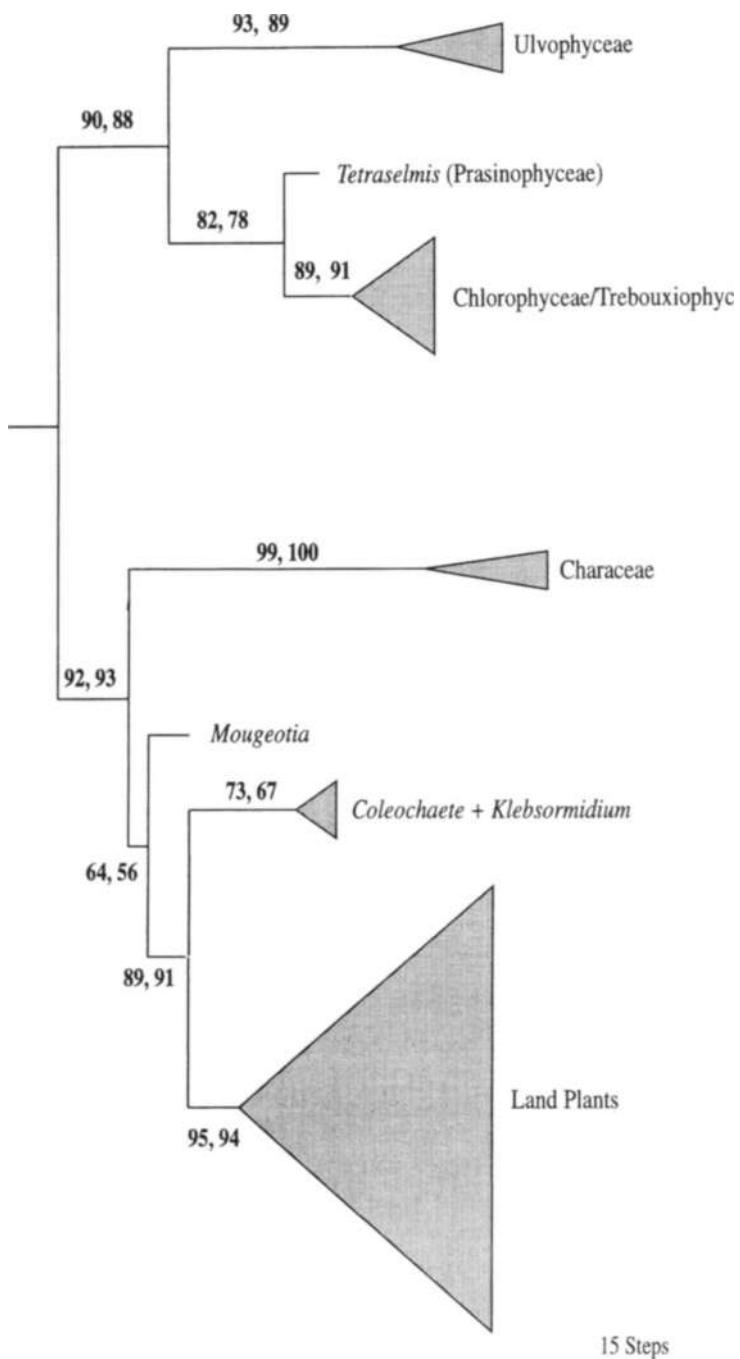


Figure 1. Summary of 58 MPTs ($L = 2675$; $CI = 0.48$; $RI = 0.70$) found in 300 random-addition searches of the green plant 18s gene sequence alignment. Fundamental trees differed only with respect to the placement of terminals within the larger taxonomic groups shown here. Numbers given at each node are, respectively, bootstrap and jackknife percentages.

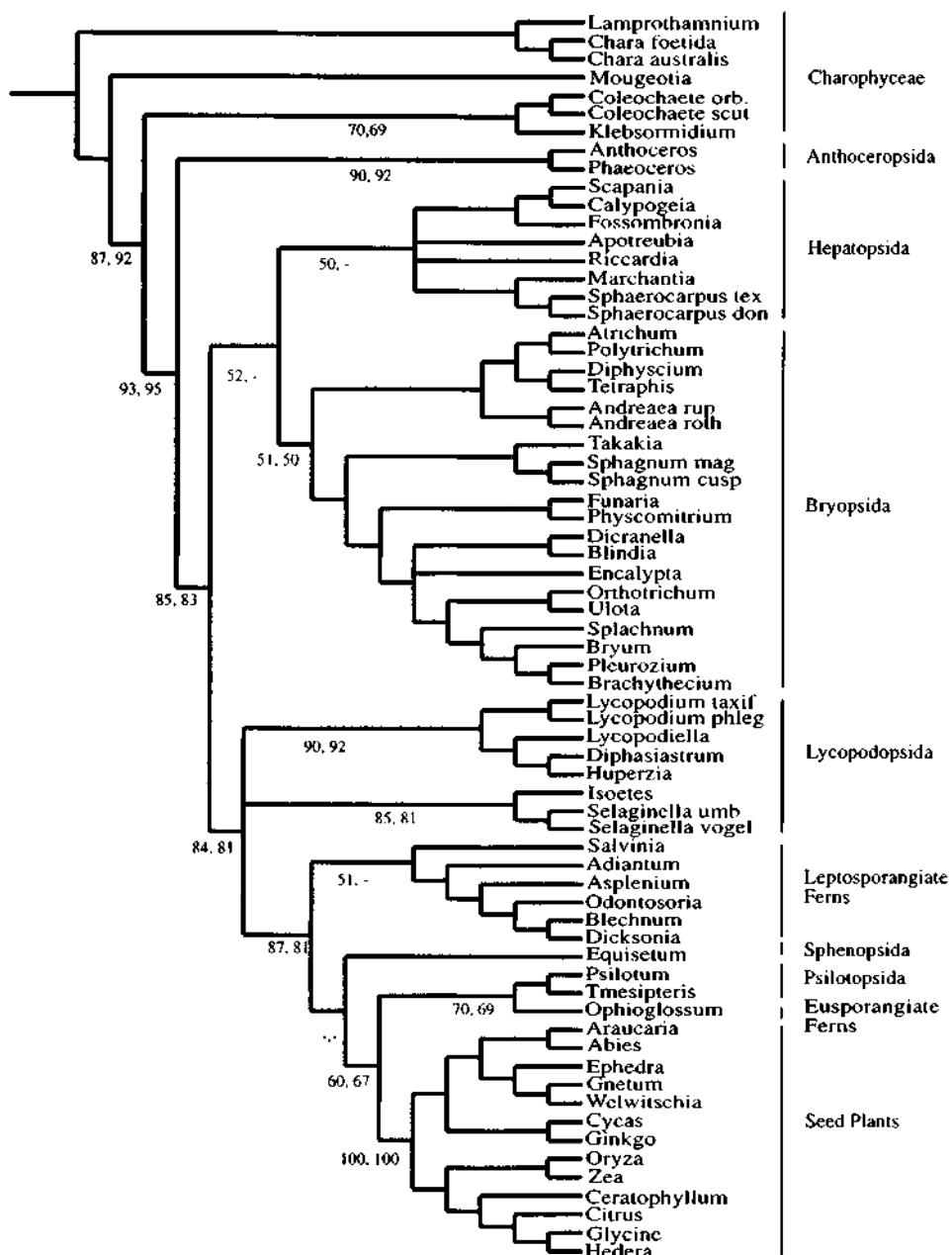


Figure 2. Strict consensus of 41 MPTs ($L=2390$; $CI=0.41$; $RI=0.66$) found in 300 random-addition searches of the $18s$ data set realigned over the land plants. Trees are rooted to the Chlorophycean taxa *Chlamydomonas reinhardtii*, *Chlorella fusca* and *Muriella aurantiaca*. Bootstrap and jackknife percentages are given for nodes of interest. The maximum-likelihood topology was identical to one of the MPTs.

plants are strongly supported by the 18s data. However, there is no strong support for the monophyly of either the mosses or liverworts, or for their sister-group relationship. For example, in many of the trees one or two steps longer, a *Takakia-Sphagnum* lineage is resolved sister to the tracheophytes, while a lineage comprising the nematodontous mosses + *Andreaea* emerges from within a grade of leafy liverworts basal to all land plants except the hornworts.

There is strong support in the data for the monophyly of the tracheophytes. While internal relationships are well resolved, most of the groupings are not especially strongly supported. A clade comprising all the tracheophytes except the Lycopodopsida is well supported, but the latter group comprises two clades (one comprising the elgulate, and the other the ligulate genera), whose relationship to each other and to the remaining vascular plants is not resolved. The leptosporangiate ferns (including *Salvinia*) are weakly resolved as a monophyletic group. The Psilotales are moderately well supported as the sister group to *Ophioglossum*, and this joint clade emerges with moderate support as the sister taxon to the seed plants (a very well supported clade); *Equisetum* is weakly resolved as the sister group to the seed plant-Psilotales/*Ophioglossum* clade.

4.5 DISCUSSION

The overall 18s phylogeny presented here provides strong evidence for the existence of two distinct lineages within the Chlorobionta (*sensu* Bremer, 1985) — one comprising the majority of green algal groups, and the other the 'Charophycean' green algae plus the land plants. The existence of this fundamental split among green plants was originally suggested by Pickett-Heaps & Marchant (1972) and Pickett-Heaps (1975), predominantly on the basis of ultrastructural features (e.g. cell division processes, and the structure of reproductive cells (see also Graham, 1993; McCourt, 1995)). This idea has been supported subsequently by a number of studies using diverse data sets (see reviews in Chapman & Buchheim, 1992; Mishler *et al.*, 1994; Graham, 1996).

Although the limited number of taxa used here for the 'classical' green algal clade precludes extensive comparison with previous studies, it is noteworthy that the Ulvophyceae and the Chlorophyceae (including Pleurastrophytes and Trebouxiophyceae) are both strongly resolved as monophyletic. This result was obtained in earlier analyses (Mishler *et al.*, 1994). The placement of the Ulvophyceae sister to the Chlorophyceae is also consistent with former analyses based on morphology and ultrastructure (Mattox & Stewart, 1984; O'Kelly & Floyd, 1984).

Given the body of ultrastructural, biochemical and molecular data accumulated in its favour (e.g. Mishler & Churchill, 1985; Graham *et al.*, 1991; Chapman & Buchheim, 1992; Mishler *et al.*, 1994; Graham, 1996), the hypothesis that the land plants share a most recent common ancestor with some member of the Charophyceae is no longer controversial. The Charophyceae, however, constitute a diverse assemblage of plants ranging from unicellular desmids to the complex and highly organized Charales and Coleochaetales. The exact group of Charophyceae with which land plants share ancestry has therefore been the subject of some debate (Mishler & Churchill, 1984, 1985; Sluiman, 1985; Bremer *et al.*, 1987; Graham *et al.*, 1991; Mishler *et al.*, 1994).

The present analyses provide rather strong support, as indicated by bootstrap and jackknife analyses, for a clade containing *Coleochaete*, *Klebsormidium*, and the land plants. *Coleochaete* has long figured prominently in discussions of land plant origins (see, for example, summaries in Pickett-Heaps, 1976, 1979; Melkonian, 1982; Graham, 1996). Non-molecular characters linking this group to land plants include parenchyma, placental transfer cells, polyphenolics associated with the $n/2n$ boundary, multicellular antheridia and the retention of the zygote on the haploid plant through sporogenesis (Delwiche, Graham & Thomson, 1989; Graham, 1982, 1984; Graham & Wilcox, 1983; Graham *et al.*, 1991). Inclusion of *Klebsormidium* in this clade seems rather at odds with the ultrastructural features noted above. Indeed, most previous analyses have generally resolved this genus in a more basal position within the charophycean grade (e.g. Mishler & Churchill, 1985; Graham, *et al.*, 1991; McCourt *et al.*, 1996), although some analyses based on partial ribosomal sequence data have also included *Klebsormidium* and *Coleochaete* within the same clade. It is worth noting that the sister-group relation between *Klebsormidium* and *Coleochaete* is only moderately well supported, and in some trees one step longer than the MPTs (not shown) the former genus is resolved as sister to a *Coleochaete*-land-plant clade.

The relationships found among the three bryophyte lineages are completely congruent with those found in a previous analysis of 18s sequences for these groups (Hedderson *et al.*, 1996) as well as with analyses based on a large suite of ultrastructural, biochemical and developmental data (Garbary & Renzaglia, *this volume*). As in these previous analyses, one of the best supported results is the position of the hornworts as the sister taxon to the remaining land plants. This is consistent with a number of features of this unique group of plants including the presence and physiological functioning of the pyrenoid (Vaughn *et al.*, 1990; Smith & Griffiths, 1996), as well as features of male gametogenesis (Garbary *et al.*, 1993; Vaughn & Renzaglia, 1997).

Our data are less convincing (given the low values for both support indices) with respect to the relationships among the mosses, liverworts and tracheophytes. However, the relationships as resolved here are compatible with many features of morphology, anatomy and biochemistry (see Hedderson *et al.*, 1996 and Garbary & Renzaglia, *this volume* for detailed discussion). Furthermore, Hedderson *et al.* (1996) demonstrated that the alternative relationships among these three lineages (assuming that each is monophyletic) all have significantly lower likelihoods. Sequence divergence within these three groups is high, whilst levels of divergence among the lineages is low (data not shown). This may mean that the three lineages diverged very rapidly so that most observable sequence variation was accumulated since this divergence and would not be expected to resolve relationships convincingly. Such a scenario would be consistent with the odd combinations of characters found in the fossil record (e.g. Kenrick & Crane, 1991, 1997; Remy, Gensel & Hass, 1993; Hemsley, 1994; Edwards, *this volume*). As noted above and by Garbary & Renzaglia (*this volume*), departures between the present study and several previous 18s analyses (Bopp & Capesius, 1995, 1996; Capesius, 1995) are difficult to evaluate given the choice of outgroups in those studies and the absence of tracheophyte representatives. Relationships found within the moss clade depart considerably from those found in earlier analyses. These, however, are not a primary focus of this work, and are discussed in more detail in a separate paper (Hedderson *et al.*, *in press*).

The data available at present provide little strong evidence of relationships among the main tracheophyte lineages. However, it is clear that the psilophytes do not represent a 'primitive' tracheophyte group, despite their supposed similarity to Devonian Rhyniophyta. Instead, they represent a highly derived group of ferns as anticipated by Bierhorst (1971; c.f. Gensel, 1977; Raubeson & Jansen, 1992; Garbary *et al.*, 1993), albeit they appear more closely related to the eusporangiate Ophioglossales rather than the leptosporangiate groups as that author suggested. The moderately well supported relationship between eusporangiate ferns (including Psilotales) and the seed plants may mean that ideas regarding seed plant origins need serious re-evaluation.

Our data provide strong evidence that the lycopods represent an early divergence, or divergences, among the tracheophytes. This is entirely consistent with their long fossil record — Lycopodiales were present at least 370 million years BP — and with a large suite of other molecular and morphological characters (Crane, 1990; Raubeson & Jansen, 1992; Garbary *et al.*, 1993; Mishler *et al.*, 1994; Kenrick & Crane, 1997).

In summary, the 18s data provide high levels of resolution among the major lineages of green plants. Many of these phylogenetic hypotheses are internally robust, but support for others is quite weak. However, it appears that there may be considerable congruence between the molecular data and other data sources, and these give us reason to be optimistic about future progress. We can only re-emphasize the need to combine a diversity of data sets, including non-molecular as well as molecular information, and perhaps especially data from the fossil record. As such data sets accumulate, they will continue to provide critical tests of the hypotheses advanced here.

4.6 ACKNOWLEDGEMENTS

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APPENDIX 1

List of taxa used with GENBANK accession codes. Voucher specimens (deposited at RNG) are cited for taxa that we have sequenced for this study. na, code not available, contact authors for details.

OUTGROUPS: **Glaucophyta** *Glaucocystis nostochinearum* X70803; **Chlorachniophyta**; *Euglypha rotunda* X77629.

CHLOROPHYCEAE/TREBOUXIOPHYCEAE *Characium perforatum* M62999; *Chlamydomonas moewusii* U41174; *Chlamydomonas reinhardtii* M32703; *Chlorella fusca* var. *fusca* X73995; *Mantoniella squamata* X73999; *Muriella aurantiaca* X91268; *Trebouxia impressa* Z21551; *Volvox carteri* X53904.

ULVOPHYCEAE *Acetabularia acetabulum* Z33461; *Cladophora pellucida* Z35314; *Ernadesmis verticillata* Z35321; *Valonia utricularis* Z35323.

PRASINOPHYCEAE *Mantonella squamata* X73999; *Nephroselmis olivacea* X74754; *Tetraselmis striata* X70802.

CHAROPHYCEAE *Chara australis* U05260; *Chara foetida* X70704; *Coleochaete orbicularis* M95611; *Coleochaete scutata* X68825; *Klebsormidium flaccidum* X75520; *Lamprothamnium macropogon* U18508; *Mougeotia scalaris* X70705; *Nitella flexilis* U05261.

ANTHOCEROTOPSIDA *Anthoceros agrestis* X80984; *Phaeoceros laevis* U18491.

HEPATOPSIDA *Apotreubia nana*; *Calypogeia arguta* X78439; *Fossombronia pusilla* X78341; *Marchantia polymorpha* X75521; *Aneura pinguis* X85095; *Scapania nemorea* X80983; *Sphaerocarpos donellii* X85094; *Sphaerocarpos texensis* U18522.

BRVOPSIDA *Andreaea rothii*; *Andreaea rupestris* U18490; *Atrichum angustatum* U18492; *Blindia acuta* AF023681; *Brachythecium rutabulum* AF023713; *Bryum caespiticium* AF023703; *Dicranella heteromalla*; *Diphyscium foliosum* na; *Encalypta rhabdocarpa* AF023680; *Funaria hygrometrica* X74114; *Orthotrichum lyellii* na; *Physcomitrium pyriforme* U18517; *Pleurozium schreberi* na; *Polytrichum commune* U18518; *Sphagnum cuspidatum* X80213; *Sphagnum magellanicum* na; *Splachnum luteum* AF023680; *Takakia lepidozoides* U18526; *Tetraphis pellucida* U18527; *Ulothrix phyllantha* AF023692.

PSILOPSIDA *Psilotum nudum* U18519; *Tmesipteris tannensis* U18103.

LYCOPODIOPSIDA *Diphasiastrum tristachium* U18511; *Huperzia lucidula* U18505; *Isoetes durieui* X83521; *Isoetes engelmanii* U18506; *Lycopodiella inundata* U18512; *Lycopodium phlegmaria* X81964; *Lycopodium taxifolium* X83522; *Selaginella vogelii* X75517; *Selaginella umbrosa* X83520.

EQUISETOPSIDA *Equisetum hyemale* X78890.

PTEROPOPSIDA *Adiantum raddianum* X78889; *Asplenium nidus* D85303; *Blechnum occidentale* U18622; *Botrychium lunaria* U18625; *Dicksonia antarctica* U18624; *Odontosoria chinensis* U18627; *Ophioglossum petiolatum* U18515; *Pteridium aquilinum* U18628; *Salvinia natans* X90413;

SEED PLANTS *Abies lasiocarpa* X79407; *Araucaria excelsa* D38240; *Cycas taitungensis* D85297; *Ephedra californica* U42492; *Ginkgo biloba* D16448; *Gnetum gnemon* U42416; *Welwitschia mirabilis* D85299; *Ceratophyllum demersum* D85300; *Chloranthus spicatus* D29787; *Citrus aurantium* U38312; *Glycine max* X02623; *Hedera helix* X16604; *Oryza sativa* X00755; *Zea mays* K02202.



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5. A molecular approach to bryophyte systematics

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5.1 SUMMARY

A comparison of Jungermanniidae and Marchantiidae as subclasses of the Hepaticopsida reveals problems with respect to the phylogenetic relations between both groups. Therefore, we used a molecular approach by sequencing the complete 18S rRNA gene (1,810–1,854 bp) of 40 bryophyte species to obtain numerical data. The sequences were aligned using the Alignment Editor 3.5a (Hepperle, 1995). Sequence data were used to construct evolutionary trees using the phylogeny programs PHYLIP 3.5c and MEGA 1.01. In this way the members of the ‘liverworts’ are clearly separated into two independent groups with a range of classes: Marchantiopsida (with Marchiales, Ricciales and Sphaerocarpales) and Jungermanniopsida (with the remaining orders). In all tree constructions we find that the Marchantiopsida are separated from the Jungermanniopsida by high bootstrap values. This class forms a clade together with Bryopsida, an indication that the Jungermanniopsida are more closely related to Bryopsida. Both of these are therefore monophyletic, going back to one common ancestor, perhaps a thallus-form like the extant Metzgeriales.

The evolutionary trees based on molecular data have been compared with several morphological and biochemical characters used in classical taxonomy.

KEYWORDS: Bryopsida, Jungermanniopsida, Marchantiopsida, molecular approach, bryophyte phylogeny, 18S rRNA gene.

5.2 INTRODUCTION

For about 200 years, the systematic classification of bryophytes has been based on morphological, anatomical, ontogenetic, biochemical, and developmental characters, and on the values assigned by taxonomists to these characters. Thus, a separation into three classes was established: Bryopsida, Anthocerotopsida and Hepaticopsida. The problem that arises from such a basis for classification is shown by the very late separation of the two classes, Anthocerotopsida and Hepaticopsida. As long as thallose growth versus leafy shoot growth remained the general character, Anthocerotopsida had to remain a part of the Hepaticae.

Then, also in recent classifications the growth form of the ‘thallus’ seems to have had an important influence on the taxonomic value. So the Hepaticopsida or

'liverworts' are bryophytes, in which at least partly 'thallus' growth forms are found, for example Metzgeriales, Marchantiales or Sphaerocarpales. However, a more detailed analysis of the above mentioned characters makes it clear that 'thallus' can mean very different structures in morphology, anatomy, ontogeny, and development (Vicktor & Bopp, 1992).

After a comparison of different characters of the hepaticas 'Jungermanniidae' and 'Marchantiidae', Schuster (1984) has shown that many randomly selected characters in both groups are quite different (Table 1). Therefore, some authors have proposed to separate the liverworts into two distinct classes, 'Jungermanniopsida' and 'Marchantiopsida', but the arguments for the two classes seem not to be strong enough to convince all taxonomists that this separation is necessary in order to

Table 1. Morphological characters of the two classes of 'liverworts' and 'mussci'. The characters of the 'liverworts' are adopted from Schuster (1984).

	Bryopsida (without Sphagnales)	Jungermanniopsida	Marchantiopsida
Sporophyte	Well preserved Seta elongating by cell division Complicated capsules, opening mechanism highly sophisticated (peristome)	Well preserved Seta elongation by cell elongation Capsule wall 2-10 stratose, complex 4-valved dehiscence mechanism	Reduced Seta hardly or not elongating Capsule wall 1-stratose, often indehiscent, not 4-valved
Sporogenesis	No elaters, only spores in sporangia Spores usually small (10-15 µm, except for Archidium 50-130 µm)	Sporogenesis preceded by a mitotic division for elater formation Spores usually small (19-24 µm)	Sporogenesis without prior mitotic division, elater sister cells to the sporeocyte Spores large (40-160 µm)
Gametophyte	Mostly mesophytic Tetrahedral apical cell 1-5-celled gametophytic diaspores abundant Leafy shoots, no thalli	Mesophytic Tetrahedral apical cell Many members with 1 - few-celled gametophytic diaspores Leafy shoots or simple thalli	Mostly xerophytic Never a tetrahedral apical cell Gametophytic diaspores absent; if present then highly differentiated Thalli mostly differentiated, with dimorphic cells
Sexual organs	Sterile filaments (paraphyses) between the gametangia Antheridia with early forming of 2 androgynial cells	Individual 'perianth' around the archegonia 4 primary androgynial cells	Archegonia never surrounded by an individual 'perianth' 1-2 primary androgynial cells
Germination and protonema (see NEHIRA, 1983)	With chloronema and rhizoids Mostly extended, filamentous (many exceptions)	Without germ rhizoids Small and 'globose' (few exceptions)	With germ rhizoids Fewer cells filamentous than thallose

answer open questions concerning the evolution of the bryophytes, and they prefer to speak of 'subclasses'.

To show the relation between different groups several evolutionary trees have been constructed (Mishler, 1994). The dilemma of all such evolutionary trees, based on structural characters, is, however, to avoid any subjective selection. These problems can be overcome with molecular approaches. The determination of base-exchanges in one or several genes of the relevant organisms provides a clear set of quantitative parameters, depending on the numeric accumulation of point mutations that can be used for the calculation of evolutionary relations between species, families and higher taxonomic categories. It does not mean that an exact knowledge of morphological data and the familiarity with many details is superfluous. They always have to remain the foundation of the discussion. In case of alternate possibilities the use of molecular markers delivers a last resort for making a decision.

5.3 MATERIALS AND METHODS

High molecular weight DNA was isolated by grinding 0.5–1 g of plant material in liquid nitrogen, following the method of Doyle & Doyle (1990). The plant material as far as it was not in the culture collection was deposited in the herbarium of the Botanical Institut. The 18S rDNA was amplified from the high molecular DNA, isolated using the forward and reverse amplification primers indicated in Fig. 1. The 18S rDNA was directly sequenced or ligated into the pUC 18 vector, with which *E. coli* DH-5 cells were transformed (Capesius, 1995).

The sequence of the coding and noncoding strand was determined with the sequencing method of Sanger, Nicklen & Coulson (1977) using the Sequenase 2.0 kit (USB) and $\alpha^{35}\text{S}$ dATP. The sequence was completely determined in both directions using the forward and reverse sequencing primers marked in Fig. 1, and deposited in the EMBL Database. The secondary structure was proved for all 40 species according to Huss & Sogin (1990) and Neefs & de Wachter (1990) by Dr V. A. R. Huss in order to optimize the alignment. For the alignment, the program Alignment Editor 3.5a (Hepperle, 1995) was used.

The neighbour-joining analysis was employed as a distance matrix method using the program package MEGA 1.01 (Kumar, Tamura & Nei, 1993). For maximum parsimony phylogeny the PHYLP version 3.5c was used (Felsenstein, 1995).

Several criteria have to be met for meaningful data, at least in bryophytes:

- 1) The selected gene or genes have to comprise a certain minimum number of base-pairs to allow base-exchanges.
- 2) The evolutionary rate of the selected gene should be appropriate for the question, as in the case of the 18S-rRNA gene.
- 3) Any contamination with foreign DNA-material must be avoided. Therefore, axenic cultures provide much more convincing results than collections from the field.
- 4) An exact determination of the species and the sequence data is a prerequisite.
- 5) For the calculation, not only the algorithm used but also the outgroups can be of significance. Therefore, different methods of calculation should be used and the base exchange should be related to different outgroups, if available.

Table 2. List of sequenced bryophyte species.

Taxonomic position	Species	No. of base-pairs	EMBL accession no.
I. Anthoceropsida			
Anthocerotales	<i>Anthoceros agrestis</i> PATON	1812	X80984
II. Marchantiopsida			
A. Sphaerocarpidae			
Sphaerocarpales	<i>Sphaerocarpos donnellii</i> AUST. <i>Riella helicophylla</i> MONT.	1815 1827	X85094 X89868
B. Marchantiidae			
Marchantiales	<i>Conocephalum conicum</i> (L.) LINDB. <i>Reboudia hemisphaerica</i> (L.) RADDI <i>Preissia quadrata</i> (SCOP.) NEES <i>Bucegia romanaica</i> RADIAN <i>Plagiochasma rupestre</i> (R. & G. FORST.) STEPH.	1854 1825 1817 1817 1829	X80987 X80981 X80211 X95476 X96500
Ricciales	<i>Riccia fluitans</i> L. <i>Ricciocarpos natans</i> (L.) CORDA	1827 1827	X78441 X89871
III. Jungermanniopsida			
Metzgeriales	<i>Fossumbronia pusilla</i> (L.) NEES. <i>Symploca brongniartii</i> AUST.	1810 1810	X78341 X91783
Jungermanniales	<i>Pellia epiphylla</i> (L.) CORDA <i>Scapania nemorea</i> (L.) GROLLE <i>Calypogeia arguta</i> NEES & MONT. <i>Lophocolea heterophylla</i> (SCHRAD.) DUM. <i>Trichocolea tomentella</i> (EHRH.) DUM. <i>Jungermannia leiantha</i> GROLLE <i>Jamesoniella autumnalis</i> (DC.) STEPH. <i>Plagiochila adiantoides</i> (Sw.) DUM.	1810 1810 1810 1810 1810 1810 1810 1810	X80210 X80983 X78439 X89872 X91782 X91784 X94261 X96499
IV. Bryopsida			
A. Sphagnidae			
Sphagnales	<i>Sphagnum fallax</i> HOFFM. em. WARNST. <i>Sphagnum cuspidatum</i> KLINGGR.	1816 1816	X78468 X80213
B. Bryidae			
Fissidentales	<i>Fissidens taxifolius</i> HEDW.	1842	X95934
Dicraeales	<i>Dicranum scoparium</i> HEDW.	1823	X89874
Pottiales	<i>Pottia truncata</i> (HEDW.) B.S.G.	1822	X95935
Bartramiales	<i>Bartramia pomiformis</i> HEDW.	1819	X96501
Funariales	<i>Funaria hygrometrica</i> HEDW. <i>Physcomitrella patens</i> (HEDW.) B.S.G.	1816 1818	X74114 X80986
Bryales	<i>Leptobryum pyriforme</i> (HEDW.) WILS. <i>Minium hornum</i> HEDW.	1819 1843	X80980 X80985
Neckerales	<i>Orthotrichum stramineum</i> HORNSCH. ex BRID. <i>Hedwigia ciliata</i> (HEDW.) P. BEAUV.	1827 1819	X89870 X91104
Hypnales	<i>Rhacocarpus purpurascens</i> LINDB. <i>Brachythecium rutabulum</i> (HEDW.) B.S.G. <i>Calliergonella cuspidata</i> (HEDW.) LOESKE <i>Hypnum cupressiforme</i> HEDW. <i>Plagiothecium undulatum</i> (HEDW.) B.S.G. <i>Rhytidadelphus squarrosus</i> (HEDW.) WARNST. <i>Hylocomium splendens</i> (HEDW.) B.S.G.	1819 1819 1819 1819 1819 1819	X89869 X94256 X94257 X94258 X94259 X94260 X95477

For our experimental work (performed by I. Capesius and M. Stech) the 18S-rRNA gene, which is present in all eukaryotes was used. It contains about 1810 base-pairs, some regions are conserved, others variable and partly informative. The variable regions comprise about 10% of the gene. We isolated the DNA from more

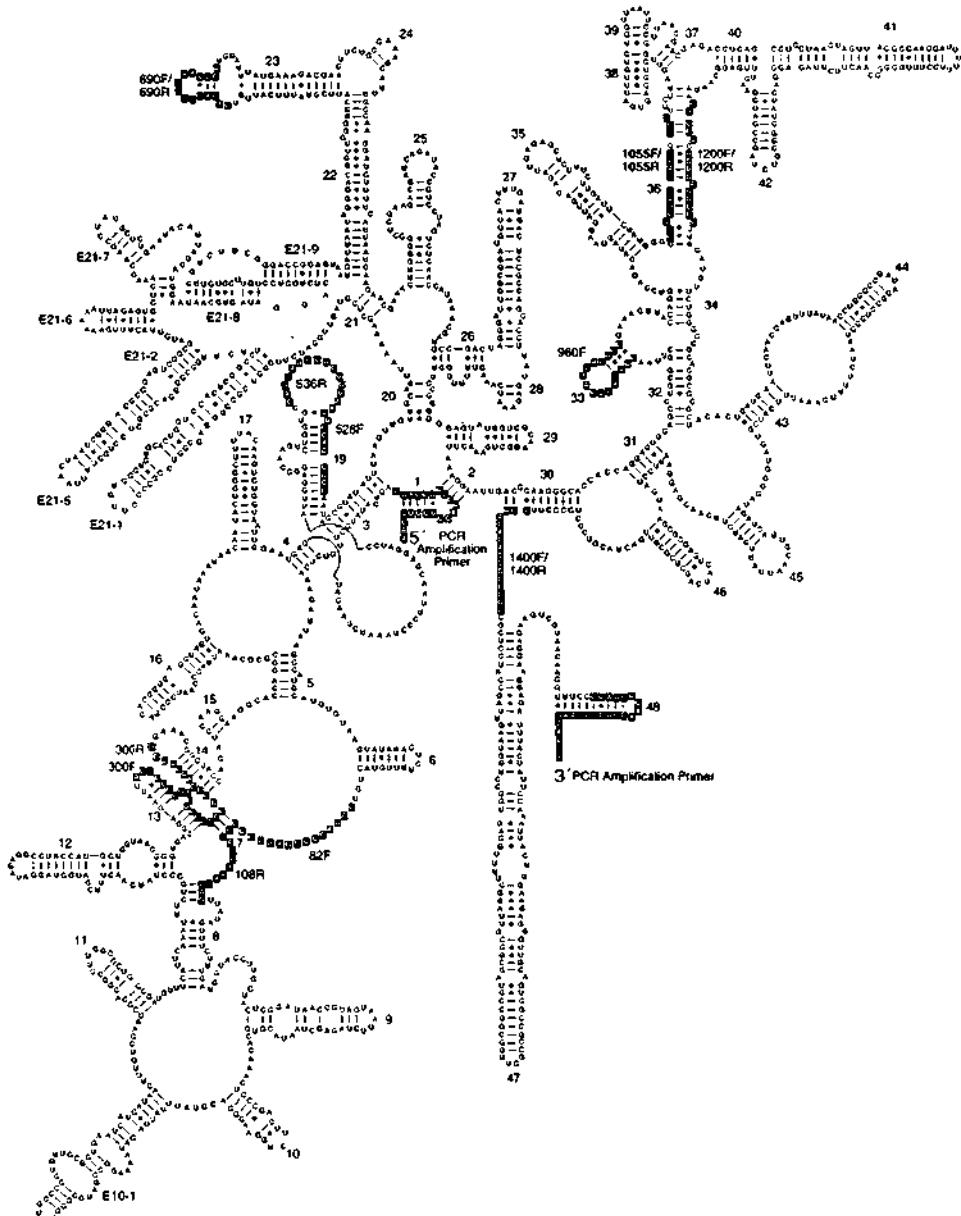


Figure 1. Secondary structure model of small subunit rRNA of *Funaria hygrometrica*. Shaded nucleotides represent the conserved annealing sites of the sequencing and PCR amplification primers used (Bopp & Capesius, 1996).

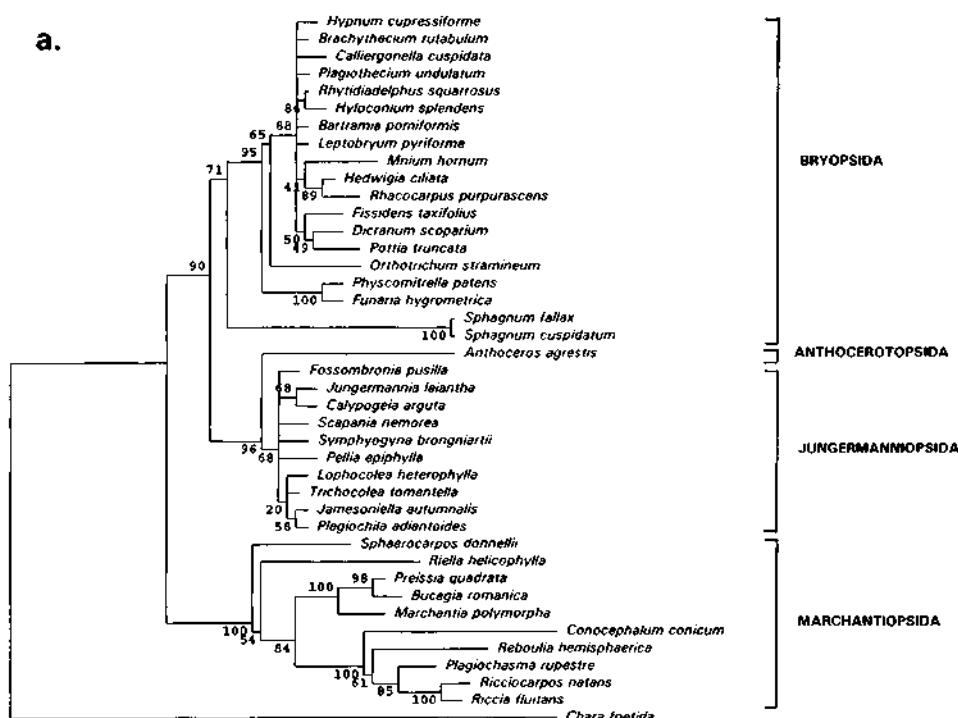


Figure 2. a. Phylogenetic tree based on the alignment of complete 18S rRNA sequences from 40 bryophyte species with the neighbour-joining method. Bootstrap analysis (500 resamplings) using the Jukes-Cantor algorithm of MEGA 1.01. *Chara foetida* was used as outgroup. The values above each node are confidence estimates (in %). The lengths of the branches are proportional to the distances between taxa.

than 40 bryophytes, most of them having long been cultivated in axenic (bacteria and algae free) culture.

5.4 RESULTS AND DISCUSSION

In Table 2 the sizes of the amplified 18S rRNA genes for all of the bryophytes that we tested are given. They vary between 1810 and 1854 base pairs. This difference results from insertions in two variable regions (E10 and E21) of the gene (Fig. 1). The data for *Marchentia polymorpha* and *Chara foetida* are taken from Kranz *et al.* (1995). The size of the gene in all Metzgeriales and Jungermanniales is identical and differs from all Marchantiales, Ricciales, and Sphaerocarpales, as well as from the Bryopsida.

This may be a first hint of a close relationship within the Jungermanniopsida, and a separation of them from the Marchantiopsida. A similar homogeneity in length among the Bryopsida was found for the Hypnales, in which all samples have 1819 base pairs.

Further discussion concerns the results of the two trees obtained by the neighbour-

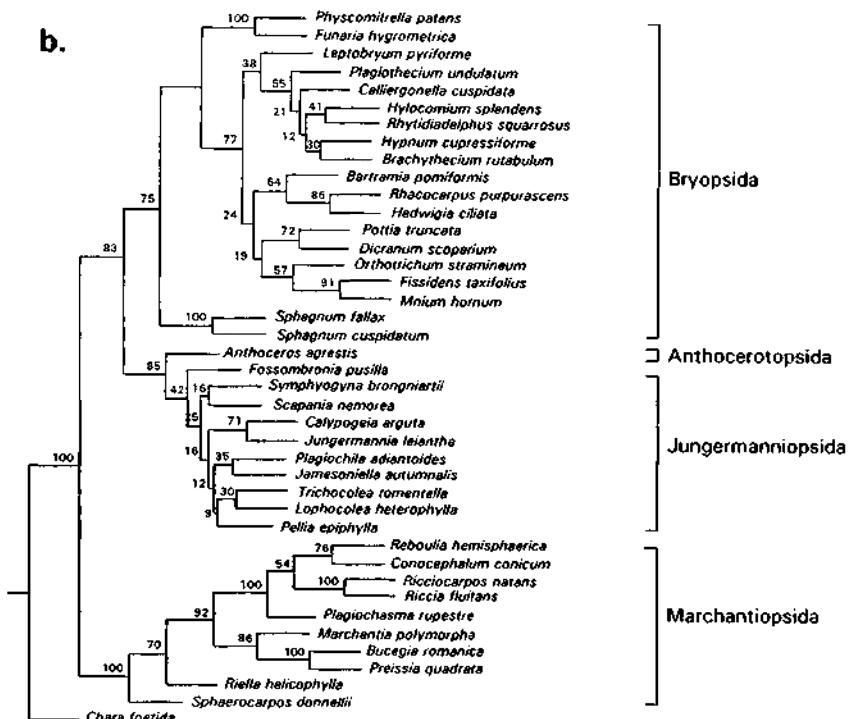


Figure 2. b. Maximum parsimony phylogeny using the PHYLIP version 3.5c. Cladogram of a bootstrap analysis (100 resamplings), for the same species as in Fig. 2a. The values above each node are confidence estimates (in %).

joining method (Fig. 2a) and the maximum parsimony phylogeny (Fig. 2b). The maximum likelihood analysis produced a similar tree.

All trees are highly congruent with respect to the main nodes, which means that the main statements of our conclusions that concern the higher taxonomic groups appear independent of the method used. In all trees (Fig. 2a, b) the Jungermanniopsida are separated from the Marchantiopsida by bootstrap values of 96–100%. This is in agreement with Kranz *et al.* (1995) and with previously published analyses of 5S rRNA genes (van de Peer *et al.*, 1990) as well as with partial sequences of some other genes (Chapman & Buchheim, 1992; Mishler *et al.*, 1994). The two groups are always separated; therefore, it is very well documented that the two clades have to be treated as two independent classes: Jungermanniopsida and Marchantiopsida.¹ Also the phylogenetic relationships based on biochemical analysis discriminate clearly between a Jungermanniales/Metzgeriales branch on one side and a Marchiales/Sphaerocarpales branch on the other side (Markham, 1990).

Whereas the Jungermanniopsida appear as a quite uniform group with very low pairwise distances from 0.3 to 1.7%, within the Marchantiopsida values of up to 5.2% are found (Capesius & Bopp, 1997). The higher distance is in agreement with

¹ In a personal note of 30 July 1996, B. Mishler informed us that based on the *rbcL* gene the two groups of liverworts are very divergent and the paraphyletic liverwort trees are not significantly worse.

the more pronounced morphological differences found within the Marchantiopsida, in which Marchantiaceae, Rebouliaceae and Ricciaceae are separated into well characterized 'types' (Crandall-Stotler, 1981). These types are confirmed by the molecular approach, where the tree families are separated by significant bootstrap values. It can also be seen that the Sphaerocarpales and *Riella* belong to the Marchantiopsida (Schuster, 1984), with an early separation from the main branch.

Metzgeriales and Jungermanniales, however, belong to one clade not separable by the bootstrap value. At an early stage Wilhelm Hofmeister (1851) recognized that the morphological as well as chemical characters (Huneck, 1983) within Metzgeriales and Jungermanniales form a continuum.

From an evolutionary point of view one may conclude that the Marchantiopsida are the older group, the Jungermanniopsida the younger one. However, before such a conclusion can be drawn we have to consider the position of the mosses in a evolutionary tree including tracheophytes. This determination is in preparation using the 25S rRNA gene.

In contrast to the traditional view of bryophyte taxonomy, the analysis of the extant bryophytes yields in all trees two sister groups that are *not* identical with the classification of liverworts and mosses. One group comprises only the Marchantiopsida, whereas the second comprises all other bryophyte species tested of the two classes Jungermanniopsida and Bryopsida.

In some trees a third clade, 'Sphagnopsida', appears (*cf.* Smith, 1978). However, these data from only two species are insufficient to make a final decision.

Examination of our evolutionary trees leads us to the conclusion that liverworts (or Hepaticopsida) consist only of the class 'Marchantiopsida'.

Anthocerotopsida — only one species, *Anthoceros agrestis*, was included hitherto — appear as a sister group to the Jungermanniopsida, separated by well established bootstrap values of 96% (Fig. 2a) or 85% (Fig. 2b).

We know that the conclusions of these results are contrary to established taxonomic tradition. However, Table 1 gives the impression that more similarities exist between Bryopsida and Jungermanniopsida than between Jungermanniopsida and Marchantiopsida. Because the characters used in this table by Schuster (1984) were chosen to show the differences between 'Jungermanniidae' and 'Marchantiidae', the characters are absolutely random for the mosses, which underlines the validity of this selection. We are aware, however, that a final discussion is only possible if a complete data set of morphological characters is examined cladistically in its own right (Mishler, 1994).

A few points in Table 1 illustrate the similarity between Bryopsida and Jungermanniopsida: in both classes the shoots grow from a three-sided apical cell, forming leaves in three lines that, as in mosses, can be equal in Jungermanniopsida after chemical treatment (Basile, 1990), diaspores have the same simple structure, and archegonia are surrounded by special elements. Characters such as spore germination and protonema development are insufficient for classification, because they are just as variable between the classes as within a class (Nehira, 1983).

We may conclude that the Bryopsida and the Jungermanniopsida are members of one clade with a common ancestor. Perhaps such an ancestor may have looked like extant Metzgeriales, which have been in existence for 350 million years (Frahm, 1994; Krassilov & Schuster, 1984). An early branch of this thalloid ancestor could

have resulted in the Bryopsida — a later branch in all extant Jungermanniales and Metzgeriales with great homogeneity in their molecular and structural characters.

We are aware that our results need the support of data from other genes and calculations with other outgroups, work which is in progress. However, there is no doubt that the molecular approach has given a completely new insight in the relations of the bryophytes.

5.5 ACKNOWLEDGEMENTS

We thank Michael Stech very much for his co-operation and the identification of a part of the bryopsida-sequences. We are also very grateful to Dr V. A. R. Huss for proving the secondary structure model of all species analysed and to Prof. Frahm for collection and determination of plant material.

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6. Molecular taxonomy of liverworts

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6.1 SUMMARY

Many taxonomic problems among closely related taxa cannot be solved with morphological analyses alone. Several molecular methods can be used to test whether morphological variation corresponds to genetic differences, or whether this variation is merely due to phenotypic plasticity. Among liverworts, three of these techniques are powerful tools for systematic investigations and for analysing polymorphism among and within taxa (isozymes, rDNA RFLPs and RAPDs). They prove the existence of three morphologically similar 'sibling' species in *Marchantia polymorpha* *sensu lato*. Conversely, isozyme and RAPD markers clearly show that morphological differences between *Lunularia cruciata* and *Lunularia thaxteri* are taxonomically not relevant and are induced by environmental conditions. Isozymes also permit the detection of hybrids. One is found in the *Porella platyphylla*—*P. cordaeana* species complex. It is shown to be a polyploid species corresponding to the previously described *Porella baueri*. The discovery of a new polyploid, hybrid liverwort species implies that the ploidy levels of many taxa of liverworts are not yet assessed and that reticulate evolution exists in these plants and can lead to speciation.

KEYWORDS: isozymes, RAPD, RFLP, sibling species, monomorphic species, allopolyploidy.

6.2 INTRODUCTION

Taxonomy is the description, naming and classification of organisms on the basis of morphology. Taxa have types that, in the case of bryophytes, are herbarium specimens. In this paper, we consider ways in which molecular techniques, that usually have to be used with living plant material, can improve taxonomy.

The diversity of molecular techniques available for the study of systematics is substantial. There are three main domains of application of these techniques in taxonomy: identification of species boundaries, studies of species structure and hybridization, and estimates of phylogenies.

Molecular data are independent data sets, less influenced by environmental conditions than morphology. Studies that combine both morphological and molecular data will provide much better interpretations of biological diversity than those that focus on just one approach, and open the possibility of investigating problems of evolutionary biology.

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6.3 METHODS

We used three molecular techniques to study speciation in liverworts. They will be described and illustrated with three examples. A more detailed description of the methods is provided in Bischler & Boisselier-Dubayle (1997).

Isozyme polymorphism is the technique most widely used for investigation of species boundaries and genetic structure of species. Detection of isozymes concerns post-translational expression of the genome. The combination of electrophoresis and staining specificity makes it possible to distinguish particular enzymes among hundreds that may be present in a crude tissue extract. Changes in the mobility of enzymes in an electric field are assumed to reflect changes in the encoding DNA. If the banding patterns of two individuals differ, these differences are supposed to be genetically based.

Restriction fragment length polymorphism (RFLPs) are sequence variations involving comparison of the number and size of fragments produced by digestion of DNA with restriction endonucleases: enzymes that cut DNA at a constant position within a specific recognition sequence, typically 4–6 base pairs (bp) long. The fragments are resolved according to their size by gel electrophoresis and are visualized after transfer and hybridization (Southern, 1975). This technique is suitable for molecular typing of taxa in liverworts.

Random amplified polymorphic DNA (RAPD) is a method based on the polymerase chain reaction amplifying DNA fragments with primers of arbitrary nucleotide sequences. This technique is particularly appropriate for haploid tissues, which are free of the dominant expression in heterozygotes of diploids. It allows the study of

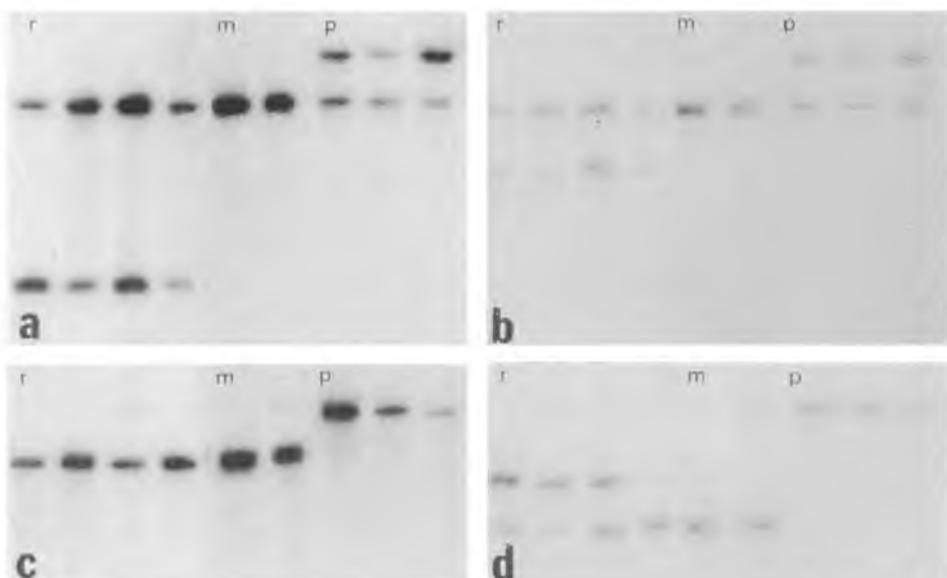


Figure 1. rDNA restriction fragment length polymorphisms for the three subspecies of *Marchantia polymorpha* (*r* = *ruderale*, *m* = *montivagans*, *p* = *polymorpha*). Autoradiograms of Barn HI (a, b) or Pst I (c, d) digests of DNA hybridized with 18S (a, c) or 26S (b, d) rDNA wheat probes.

species boundaries, investigation of population structure, and testing of hybridization hypotheses.

6.4 APPLICATIONS TO LIVERWORTS

6.4.1 *Marchantia polymorpha*

Marchantia polymorpha L. is a morphologically polymorphic species for which many varieties and forms have been described.

An enzyme polymorphism analysis of European colonies indicated the presence of three genetic entities, distinguished on the basis of esterase, peroxidase and acid phosphatase patterns (Boisselier-Dubayle & Bischler, 1989). Subspecific rank was attributed to these three entities because slight morphological differences characterize them (Bischler & Boisselier-Dubayle, 1991). In addition, the three subspecies have ecological preferences: subsp. *ruderale* Bischl. et Boisselier is found mainly in man-made habitats, subsp. *montivagans* Bischl. et Boisselier is distributed in the mountains, and subsp. *polymorpha* occurs in bogs.

The three subspecies are morphologically polymorphic and are difficult to distinguish. Their morphological differences are not constant during cultivation. Descriptions (Bischler & Boisselier-Dubayle, 1991) and tentative keys have been published (Bischler, 1993). The subspecies exhibit the same chromosome number, karyotype structure and flavonoid patterns, but their enzymatic patterns are distinct.

To confirm this genetic subdivision of an apparently single, morphologically polymorphic species, to assess genetic variability, and to infer genetic relationships between the three subspecies, DNA data were used in parallel.

Specific RFLP patterns were displayed with several restriction enzymes and hybridization with rDNA probes from wheat (Fig. 1). Restriction maps were drawn for each enzyme. Although these maps await completion, they are sufficiently informative for molecular typing of each subspecies. The RAPD technique was also used to confirm these results. Among the 19 random primers tested, eight provided scorable bands. The results clearly differentiate the three subspecies (Boisselier-Dubayle *et al.*, 1995b).

Isozyme data, rDNA hybridization and RAPD patterns gave the same results: the three approaches differentiated independently three distinct genetic entities within *M. polymorpha*. The genetic differences between them are greater than one would expect from their very similar morphological traits. In addition, our data invalidate the hybridization hypothesis forwarded by Burgeff (1943) and Schuster (1992), according to which subsp. *ruderale* is a hybrid between subsp. *montivagans* and subsp. *polymorpha*.

The three genetic entities within *Marchantia polymorpha* correspond to sibling taxa, i.e. genetically sharply differentiated taxa that do not interbreed but are difficult to separate on morphological evidence alone. Sibling species have been found in other liverworts of the orders Marchiales and Metzgeriales: *Conocephalum conicum* (L.) Dumort. (Odrzykoski & Szweykowski, 1991; Akiyama & Hiraoka, 1994), *Riccia dictyospora* M. Howe (Dewey, 1989), *Pellia epiphylla* (L.) Corda (Zielinski,

1987) and *Aneura pinguis* (L.) Dumort. (Szwejkowski & Odrzykoski, 1990). The discovery of sibling species has been one of the major findings in liverwort genetics.

6.4.2 *Lunularia cruciata*

Lunularia cruciata (L.) Dumort., a weedy species with nearly cosmopolitan range, was investigated with isozyme polymorphism and RAPDs. The genus *Lunularia* Adans. comprises only one additional taxon, *Lunularia thaxteri* A. Evans et Herzog (Herzog, 1938), characterized by a purplish thallus on the ventral side and purplish scales, and by strongly thickened epidermal cells.

The thalli of all colonies (19 from Europe, one from Madeira corresponding to *L. thaxteri*, and one from South Africa) displayed the same isozyme patterns. Few differences were observed with RAPDs (Fig. 2). Colonies from man-made habitats were found to be less variable than colonies from natural sites. Also, sexualised colonies were more distant from each other than sterile colonies, but the level of genetic variability was found to be very low compared to the wide range of habitats the species is able to colonize (Boisselier-Dubayle *et al.*, 1995a).

Morphological variation was shown to lack genetic basis and we concluded that the morphological differences displayed by *L. thaxteri*, probably due to habitat conditions, should not lead to taxonomic distinction.

6.4.3 *Porella platyphylla* — *P. cordaeana*

The *Porella platyphylla* (L.) Pfeiff. — *P. cordaeana* (Hübener) Moore species complex was investigated with isozyme polymorphism (Boisselier-Dubayle *et al.*, 1998). The genus *Porella* L. is known for its important phenotypic plasticity and for rather ill-defined species boundaries.

Glutamate oxaloacetate transaminase (GOT) is a dimeric enzyme with known sub-

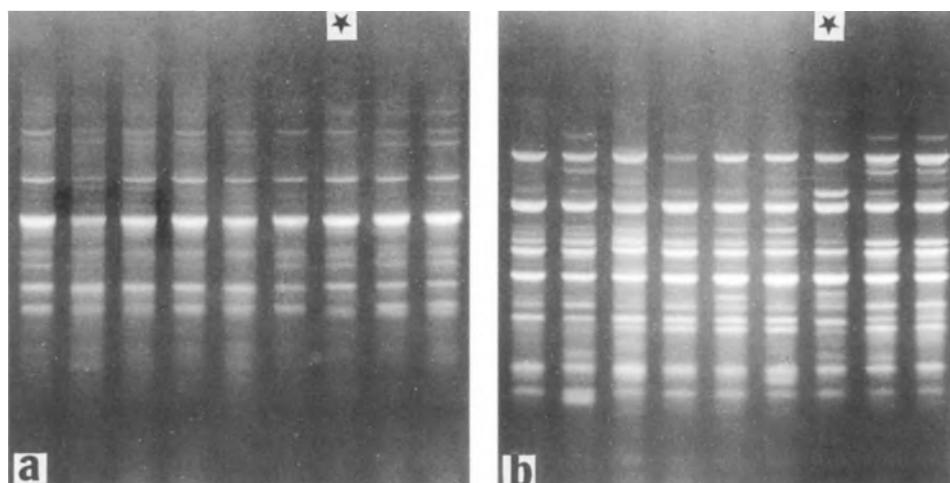


Figure 2. Random amplified polymorphic DNA patterns of *Lunularia cruciata* and *Lunularia thaxteri* (*) with OPG18 (a) and OPG02 (b) primers.

unit structure. It was detected in two zones in the species examined. The faster locus (*Got-1*) exhibited only one band, of different mobility in *P. platyphylla* and *P. cordaeana*, whereas three bands were always present in a third taxon (Fig. 3). This pattern conforms to a heterozygous status of the locus, suggesting that the locus is duplicated. The two homodimeric bands corresponded to the most frequent alleles seen respectively in *P. platyphylla* and *P. cordaeana*. Consequently, the duplication could be the result of hybridization between these two species and appeared to be a fixed change in the genome of the third taxon. The other *Got* locus (*Got-2*) exhibited variation.

The allelic distributions between the three genetic entities at the ten loci analysed strongly support the hypothesis of interspecific hybridization that was confirmed by chromosome counts. *Porella platyphylla* and *P. cordaeana* both had chromosome sets of $n=8$, whereas the third taxon had $n=2x=16$ chromosomes. Polyploidy has not been reported previously in *Porella*. However, the hybrid taxon exhibited three different genotypes, as seen with isozyme patterns: one found in eastern France and western Germany, closer to *P. platyphylla*, two in western France, closer to *P. cordaeana*.

The hybrid was suspected to correspond to *Porella baueri* (Schiffn.) C. E. O. Jens., a species described from Germany and known for its significant morphological intergradation with *P. platyphylla*. To test this hypothesis, we collected 17 colonies of *Porella* in the type locality of *P. baueri*. Ten belonged to *P. platyphylla*, seven to the hybrid species. It seems more than likely therefore that *P. baueri* corresponds to the allopolyploid *P. platyphylla* \times *P. cordaeana*.

The main purpose of our research is to define species boundaries, but it is also to identify morphological criteria that best distinguish the species and to propose improved keys. Consequently, morphological analyses including 39 variables (see Boisselier-Dubayle & Bischler, 1994) were computed for statistical treatments. Ten individuals per colony were analysed and close to 20 colonies were observed for each of the three species. The data were treated using a micro-computer version of SPAD.N (Cisia, Saint-Mandé, France).

The first treatment was a descriptive procedure allowing identification of the morphological variables that best characterized each of the three taxa. Only the leaf lobe cell size (fc) was relevant for the recognition of the hybrid species. A factorial correspondence analysis of the distinctive characters was then carried out to account for the relationships between morphology and the genetic characteristics of the taxa. A

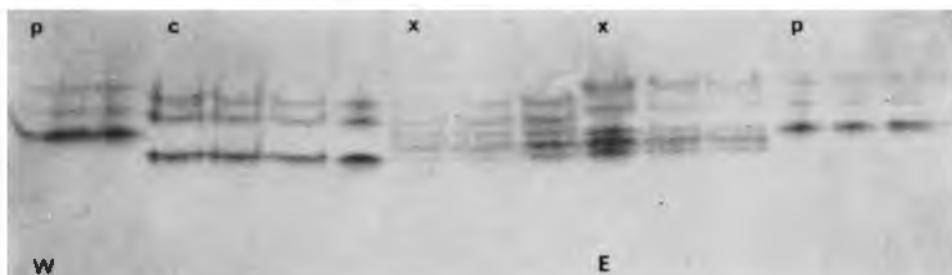


Figure 3. Isozyme patterns of glutamate oxaloacetate transaminase of *Porella platyphylla* (p), *Porella cordaeana* (c) and *Porella baueri* (x) collected in Western France (W) and Eastern France (E).

clear distinction between the colonies of *P. platyphylla* and *P. cordaeana* is seen on the first two axes (Fig. 4). The highest contributions to the positive side of the first factorial axis are provided by the morphological variables characterizing *P. cordaeana*. On the opposite, negative side of this axis, the variables are those characterizing *P. platyphylla*. The first together with the second axis subdivide the colonies of the polyploid taxon into two groups, one morphologically closer to *P. platyphylla*, the other with the main morphological characteristics of *P. cordaeana*. These two groups correspond to the genotypically different groups of the hybrid from the east and the west. Morphology and genetic variables appear to vary in concert. The third axis allowed the best differentiation of the hybrid species, with the variable leaf lobe cells

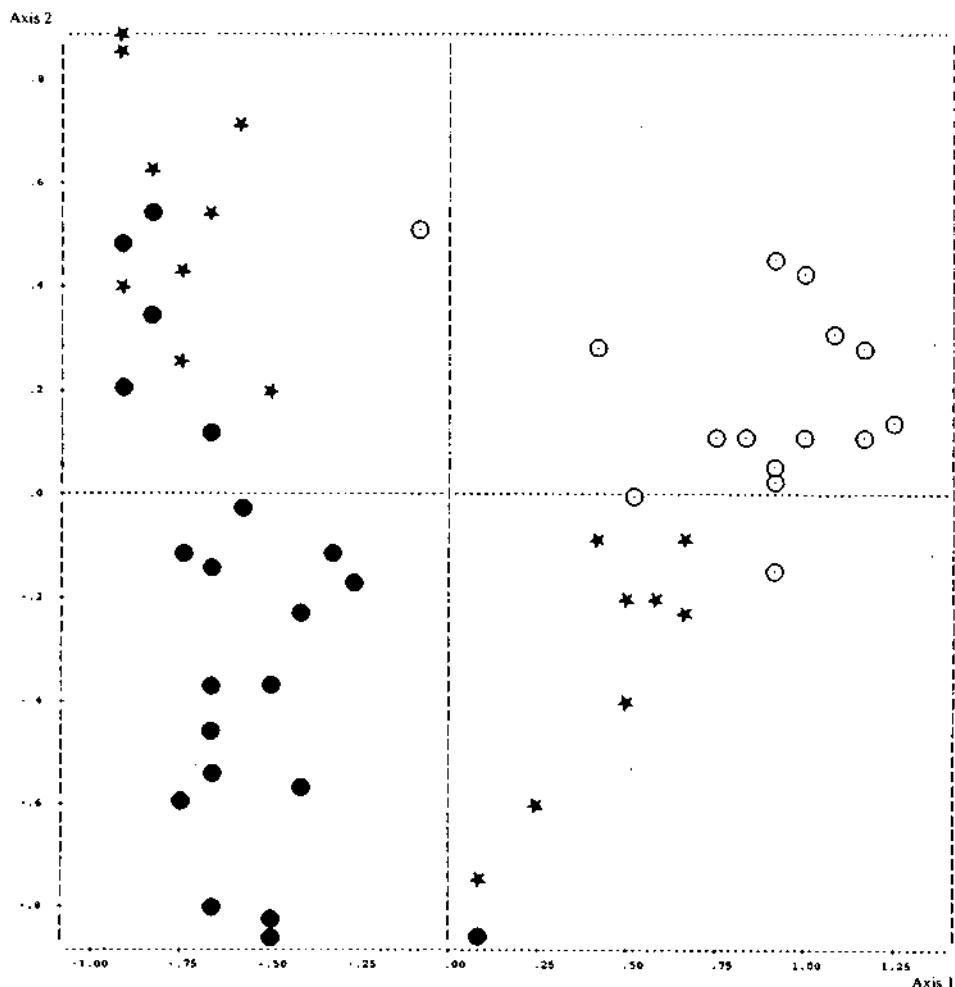


Figure 4. Projection of the colonies of *Porella* on the two first axes of the factorial correspondence analysis based on morphological characters. The coordinates of each colony are seen on the scales of the axes. *Porella platyphylla* (●), *Porella cordaeana* (○), and *Porella baueri* (★).

>37.5 providing the highest contribution. Female bracts and perianth structure, introduced as illustrative variables, also distinguish the hybrid.

The following key can be proposed to distinguish the three species (Fig. 5):

1. Lobule not twisted, obtuse or rounded apically. Lateral underleaf margins ventrally reflexed. Margin of perianth mouth toothed or ciliate.
 2. Leaf lobe cells 32–35 µm. Bract lobe and lobule with entire margins (occasionally with 1–2 teeth), rounded apically. Margin of perianth mouth with irregular, wide and spaced teeth *P. platyphylla*
 2. Leaf lobe cells 38–40 µm. Bract lobe and lobule toothed, acute apically. Margin of perianth mouth ciliate *P. baueri*
1. Lobule twisted, acute and tapering apically. Lateral underleaf margins flat. Leaf lobe cells 33–39 µm. Bract lobe and lobule with entire margins, lobule acute apically. Margin of perianth mouth entire to crenulate *P. cordaeana*

In conclusion, isozyme data and chromosome counts indicate an allopolyploid origin of *P. baueri*. The putative parental species are most likely *Porella platyphylla* and *P. cordaeana*. The hypothesis of a single event of interspecific hybridization is unlikely because the alleles detected in *P. platyphylla* and *P. cordaeana* have been detected separately in the polyploid taxon. If hybridization is assumed to have happened more than once, it does not fully explain the asymmetry in genetic constitution linked to the geographical distribution we observed in the polyploid. It could imply introgression (the transfer of genetic material between the hybrid and the parental species), with few hybridization events followed by gene flow across ploidy levels, from haploid to polyploid. The haploid allelic status of some loci in the hybrid, either with alleles from *P. platyphylla* or from *P. cordaeana*, could alternatively be explained by gene silencing. Polyploid genomes may evolve and, given sufficient time, the number of genes expressed in the polyploid could decline, reverting the polyploid to a level of expression similar to that of the parental species.

The discovery of a new polyploid, hybrid liverwort species could imply that interspecific hybridization might not be as rare as supposed and can lead to speciation, an evolutionary process not yet recorded in liverworts.

6.5 DISCUSSION

Molecular analyses should be used more widely for taxa delimitation in taxonomically difficult groups. Information on species boundaries from molecular data allows distinctions to be drawn between intraspecific morphological polymorphism and diagnostic characters. Once the species boundaries become clear, morphologically distinctive characters can be worked out, and improved keys can be proposed.

Studies in population biology in liverworts are still in their infancy. Additional data on population structure, polyploidy and hybridization are needed. Almost no data are available for Jungermanniales, the largest liverwort order, and none for the small orders Sphaerocarpales, Monocleales, Treubiales and Calobryales.

Future studies, combining morphological and molecular approaches, will certainly offer the potential for a better understanding of the processes that govern evolution

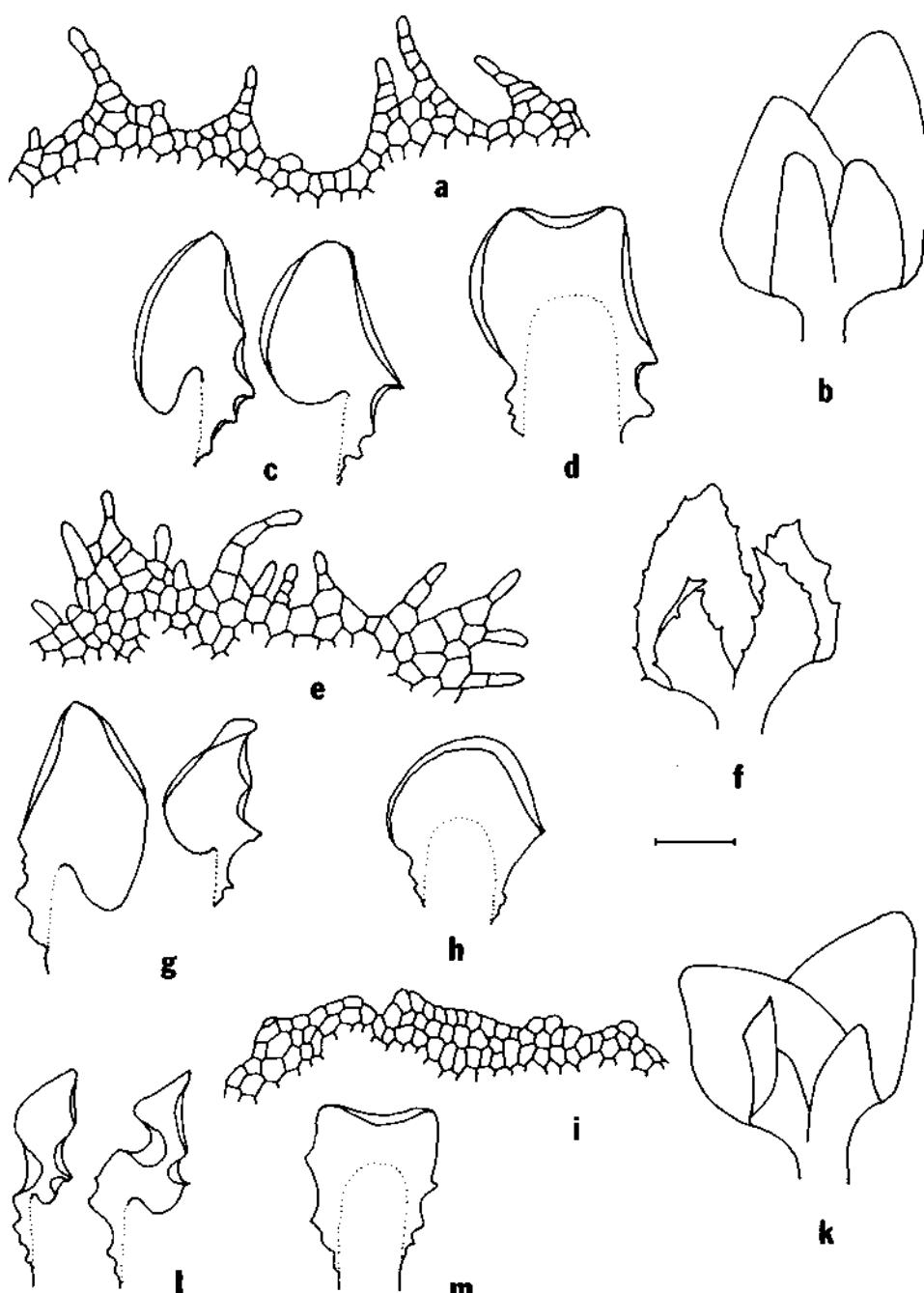


Figure 5. *Porella platyphylla* (a-d), *P. baueri* (e-h), and *P. cordaeana* (i-m). a, e, i: margin of perianth mouth, b, f, k: female bracts, c, g, l: lobules, d, h, m: underleaves (scale bars: a, e, i=100 µm, b-d, f-h, k-m=50 µm). France. a, c, d: Allier, Perreau 92511, b: Aude, Bischler, Boisselier & Pujos 92506, e-g: Vosges, Frahm & Siegel 925063, h: Morbihan, Bischler & Boisselier 92427, i, l, m: Morbihan, Bischler & Boisselier 92422, k: Tarn, Bischler, Boisselier & Pujos 92477.

in liverworts. Liverworts were shown to have low levels of genetic variability compared to mosses and other land plants (see Bischler & Boisselier-Dubayle, 1997 for review). Variation was found to be partitioned between rather than within populations. Gene exchange, even between colonies growing nearby, appears to be restricted. Nevertheless, interspecific hybridization exists and can lead to speciation. This finding has important evolutionary implications. Allopolyploidy, a dynamic process known in other land plants, is shown to occur also in liverworts and indicates that evolutionary potential exists and might be less reduced than believed previously.

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7. Spore colour and ornamentation in the taxonomy of *Asterella* (Marchantiales, Aytoniaceae)

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7.1 SUMMARY

The importance of spore characters in the taxonomy and classification of *Asterella* is briefly reviewed. Using mainly scanning electron microscopy (SEM), spores of seventeen species of the genus are illustrated and classified into eight 'Spore Types' based on colour, primary sculpturing of the distal surface, shape and similarity of proximal and distal sculpturing. Other spore characters are rejected from this primary classification though some of these are valuable in distinguishing species within the groups. Only minor variation in spore ornamentation is observed between different populations of individual species, and most of the species studied can be readily identified on spore characters. The value of SEM in solving problems of synonymy is demonstrated: the Japanese *Asterella crassa* is considered to be a likely synonym of the Indian *A. mussuriensis*, and the Philippine *A. levispora* is a likely synonym of the Indian *A. wallichiana* on the basis of spore ornamentation. The Malesian *A. blumeana* is shown to have unique spore morphology clearly distinct from that of the Indian *A. khasiana* with which it has long been confused. Some preliminary conclusions on infrageneric classification of *Asterella* are drawn; two of the three existing subgeneric divisions are supported.

KEYWORDS: Marchantiales, Aytoniaceae, *Asterella*, SEM, spores, classification.

7.2 INTRODUCTION

Spore characters have long been used in liverwort taxonomy. Stephani (1898–1900) in his *Species Hepaticarum*, in describing many thalloid liverworts, indicated spore colour and size for individual species. However, spore characters were scarcely used in classification of Hepaticae until quite recently, when the extent and significance of some of the available characters became more widely revealed by workers such as Udar (1964), Miyoshi (1966), Taylor, Hollingsworth & Bigelow (1974), Udar & Srivastava (1984) and Gupta & Udar (1986). Their studies have shown that within the bryophytes as a whole, the Marchantiales show some of the most diverse and striking elaborations of spore ornamentation. For this reason, SEM study of spores is now an essential requirement for any taxonomic study in Marchantiales.

A particularly useful review paper is that of Taylor *et al.* (1974) which describes

SEM techniques in detail, and provides spore micrographs and descriptions for many liverworts including four Marchantialean genera and species: *Targionia hypophylla* L., *Asterella tenera* (Mitt.) R. M. Schust., *Marchantia berteroana* Lehm & Lindenb. and *Neohodgsonia mirabilis* (H. Perss.) H. Perss. They also provided a practical terminology for the important characters they described, which has been largely followed here.

In other recent studies, SEM of spores has been put to the practical end of aiding taxonomy at the species level, and also in elucidating relationships of species within genera. Two workers have pioneered this approach in Marchantiales: H. Bischler-Causse in Paris and S. Perold in Pretoria. Bischler-Causse's monographic studies on *Plagiochasma* and *Marchantia* (Bischler, 1977, 1978, 1979a, b, 1984; Bischler-Causse, 1989) have used spore SEM as an important tool. Perold (1989) began her South African Marchantialean studies on *Riccia*, where spore SEM was emphasized. Later this approach was extended to other genera such as *Marchantia*, *Exormotheca*, *Asterella*, *Cryptomitrium* and *Plagiochasma* (Perold, 1994a, b, c, d, 1995). Many of the differences observed in these genera at high resolution under SEM can be seen under light microscope in sufficient detail to be of practical taxonomic value.

Within *Asterella*, the papers by Miyoshi (1966), Grolle (1989), Frey, Grolle & Kürschner (1991), Nath & Asthana (1992) and Perold (1994b) have already demonstrated the great diversity of colour and sculpturing even within a limited range of species of the genus. The present study represents part of an ongoing monograph of *Asterella*, initially of the Eurasian species, but with study of spores from a worldwide range of taxa. No classification of spore-types in *Asterella*, nor any assessment of the value of spore characters in infrageneric classification, have previously been made.

The aims of this study were to investigate the extent to which spore characters (primarily as observed under SEM) are of value in taxonomic revision and classification of the genus *Asterella*, in particular: 1) to establish the constancy of selected spore characters within individual species; 2) to look for taxonomic differences between species; 3) to investigate type specimens of three poorly-known taxa to help resolve their identity; 4) to look for relationships between species or groups of species.

7.3 MATERIALS, METHODS AND CHARACTER SELECTION

7.3.1 Materials

Preliminary study of spores of over 80 named taxa of *Asterella* and its synonym *Fimbriaria* was made under the light microscope and SEM as part of a world revision of the genus. From these a representative sample of 17 species was selected for this study, in order to include a broad range of spore types, including some species known from previous studies to be closely related to each other, and including the type species of *Asterella*, the North American *A. tenella* (L.) P. Beauv. The 17 taxa are listed in Table 1 along with the sources of the material studied.

Of these, more than one sample of several species was included to study constancy of characters. Three poorly-known taxa, *A. blumeana*, *A. crassa* and *A. levipora*

Table 1. Species of *Asterella* investigated.

Species	Country	Collector & no.	Herbarium
<i>A. abyssinica</i> (Gott.) Vanden Berghen	Rwanda	Frahm 8256	E
<i>A. blumeana</i> (Nees) Pandé & K. P. Srivast.	Java	Reinwardt s.n. (TYPE)	STR
	Celebes	Kjellberg 6h	S
<i>A. bolanderi</i> (Austin) Underw.	U.S.A.	Stern s.n.,	E
<i>A. crassa</i> Shimizu & S. Hatt.	Japan	Shimizu s.n. (TYPE)	NICH
<i>A. gracilis</i> (F. Weber) Underw.	Russia	Nilsson-Ehle s.n.	LD
	Japan	Shimizu s.n.	G
	U.S.A.	Gradstein 2256	U
<i>A. khasiana</i> (Griff.) Pandé et al.	Nepal	Long 16635	E
	Bhutan	Long 10653	E
<i>A. levipora</i> (Steph.) H. A. Mill.	Philippines	Curran 6533 (TYPE)	G
<i>A. macropoda</i> (Spruce) A. Evans	Bolivia	Lewis 84-990	F
<i>A. multiflora</i> (Steph.) Kachroo	Nepal	Townsend 92-155	E
<i>A. muscicola</i> (Steph.) S. W. Arnell	Lesotho	Duckett & Matcham 5	E
	Lesotho	Duckett & Matcham 11	E
<i>A. mussuriensis</i> (Kashyap) Verd.	Nepal	Long 16618	E
	China	Long 18580	E
	China	Long 19199	E
<i>A. palmeri</i> (Austin) Underw.	U.S.A.	Doyle 2245	U
<i>A. persica</i> (Steph.) M. Howe	Iran	Stapf s.n.	BM
<i>A. saccata</i> (Wahlenb.) A. Evans	Austria	Baumgartner H.E.1201a	E
<i>A. tenella</i> (L.) P. Beauv.	U.S.A.	Schuster M40	U
<i>A. wallichiana</i> (Lehm. & Lindenb.) Grolle	China	Long 18882	E
	Burma	Wallich s.n. (TYPE)	S
<i>A. wilmsii</i> (Nees) S. W. Arnell	South Africa	Perold & Koekemoer 2921	E

Herbarium abbreviations: BM, Natural History Museum, London; E, Royal Botanic Garden, Edinburgh; F, Field Museum of Natural History, Chicago; G, Conservatoire et Jardin botaniques, Geneva; LD, Botanical Museum, Lund; NICH, Hattori Botanical Laboratory, Nichinan; S, Swedish Museum of Natural History, Stockholm; STR, Jardin Botanique, Strasbourg; U, Institute of Systematic Botany, Utrecht.

were selected to investigate their possible synonymy with better-known species: the Malesian *A. blumeana* has often been reported as occurring in India, e.g. by Stephani (1898-1900) and Kashyap (1929-1934), yet its differences with the Indian *A. khasiana* have never been indicated; the Japanese *A. crassa* has been regarded as a close relative of the Indian *A. mussuriensis*, and the Philippine 'endemic' *A. levipora* has never been properly investigated to establish its status as a distinct taxon or synonym of some other species.

7.3.2 Methods

Light microscope observation of spore colour was made using a Wild Stereomicroscope, using where possible a recently-dehisced mature dry capsule from a herbarium specimen.

The methodology for preparation of spores for SEM follows that of Taylor *et al.* (1974). Herbarium specimens were searched for spores using a Stereomicroscope and a dehisced or semi-dehisced sporogonium was selected to ensure that the spores were ripe. A few spores were removed from the capsule using a fine moistened brush and attached to an aluminium SEM stub using double-sided adhesive tape or a carbon disc. For some old but important type specimens (e.g. *A. blumeana*), no intact sporophytes remained, but a few spores were found mixed with debris in the specimen packet. These were carefully removed for study.

Stubs were then coated with gold/palladium in a sputter-coater prior to study. Acetolysis, a technique for cleaning pollen grains and spores of extraneous material (Clarke, 1979) was found to be unnecessary and was not undertaken. Spores were studied using both a JEOL JSM T200 Scanning Electron Microscope and a Ziess DSM 962 Digital Scanning Electron Microscope. The scanning voltage was normally 5kV or 10kV. Images were photographed on Ilford Technical Pan Film.

7.3.3 Character selection

The spore characters used by past workers on *Asterella* (a good example is Evans, 1920) have primarily been colour, diameter, development of wing and sculpturing of proximal and distal surfaces, all as observed under light microscope. More recent studies using SEM have revealed further useful spore characters in *Asterella*, particularly by Taylor *et al.* (1974), Frey *et al.* (1991) and Perold (1994c), for example development of primary and secondary areolae, presence of fine reticulation or pores within areolae and on walls (muri) of the areolae; presence of a central dome or bulge on the proximal facets and similarity of proximal and distal sculpturing.

Some of the above characters have been excluded from the present study, in particular spore diameter, which has been found to vary greatly within individual species, and provides no qualitative differences between species or groups of species (although individual species pairs may in some cases be clearly distinguished by spore diameter). Also excluded is wing development as in many taxa the wing is not clearly demarcated from one or both surfaces of the spore and cannot be accurately measured. Rejected too are all characters relating to structure of areolae as they are absent from non-areolate taxa.

The characters selected for this study are therefore those considered to be available in all taxa and clearly distinguishable: primary ornamentation of distal surface, spore shape, similarity of proximal and distal sculpturing and spore colour. These were scored as follows for each taxon:

Primary distal ornamentation. Spores were described as ridged (Fig. 1a-h), regularly areolate (Fig. 2a-d) or irregularly areolate (Fig. 3c, d).

Shape. Spores were of two types only, trilete (Fig. 5b, d, f, h) or alete (Fig. 3a, b).

Proximal/distal sculpturing similarity. Spores were classed as having similar (Figs 2a, b; 3e, f) or dissimilar (Fig. 5e-h) sculpturing.

Colour. Spores were classed as yellow (sometimes orange-yellow), brown/red-brown or black.

7.4 OBSERVATIONS

The results are presented in Table 2 and Figs 1 to 5.

7.4.1 Constancy of selected spore characters within individual species

For those species where more than one sample was studied, the species were constant in all the characters tabulated in Table 2. However, as can be seen from the micrographs, there is some variation in other characters in two of the species. In *A. missouriensis* (Fig. 2a-e), there is variation in the thickness of the muri between the areolae, with one population (Fig. 2c, d) showing more strongly thickened muri than the other two. In *A. wallichiana* there is variation in the depth of the primary ridges of the distal face, and in the degree of striation of the surface of these ridges. Some variation was found in spore colour within individual species, but this was within the broad colour categories defined above.

7.4.2 Taxonomic differences between species

The four main characters used in the study are not sufficient to distinguish all species studied, but enable eight different groups of species to be defined (Table 2). Some

Table 2. Summary of spore characters in Asterella.

Spore type	Primary sculpturing (distal)	Spore shape	Proximal/distal sculpturing similarity	Spore colour	Species studied	Figs
I	Ridged	Trilete	Similar	Black	<i>A. palmeri</i>	1a, b
II	Ridged	Trilete	Similar	Brown, red-brown	<i>A. wallichiana</i>	1c, d, e
III	Ridged	Trilete	Similar	Yellow-brown	<i>A. levigata</i>	1f
IV	Irregularly areolate	Trilete	Similar	Black	<i>A. saccata</i> <i>A. muscicola</i>	1g, h 3c, d
V	Regularly areolate	Trilete	Similar	Brown, red-brown	<i>A. crassa</i> <i>A. macropoda</i> <i>A. multiflora</i> <i>A. missouriensis</i> <i>A. persica</i>	2f, g 4c, d 3e, f 2a, b, c, d, e 3g, h
VI	Regularly Areolate	Trilete	Similar	Yellow	<i>A. bolanderi</i> <i>A. wilmsii</i>	4a, b 4e, f
VII	Regularly Areolate	Trilete	Dissimilar	Yellow, orange-yellow	<i>A. abyssinica</i> <i>A. blumeana</i> <i>A. khasiana</i> <i>A. tenella</i>	4g, h 5a, b, c 5d, e, f 5g, h
VIII	Irregularly Areolate	Alete	Similar	Yellow	<i>A. gracilis</i>	2h, 3a, b

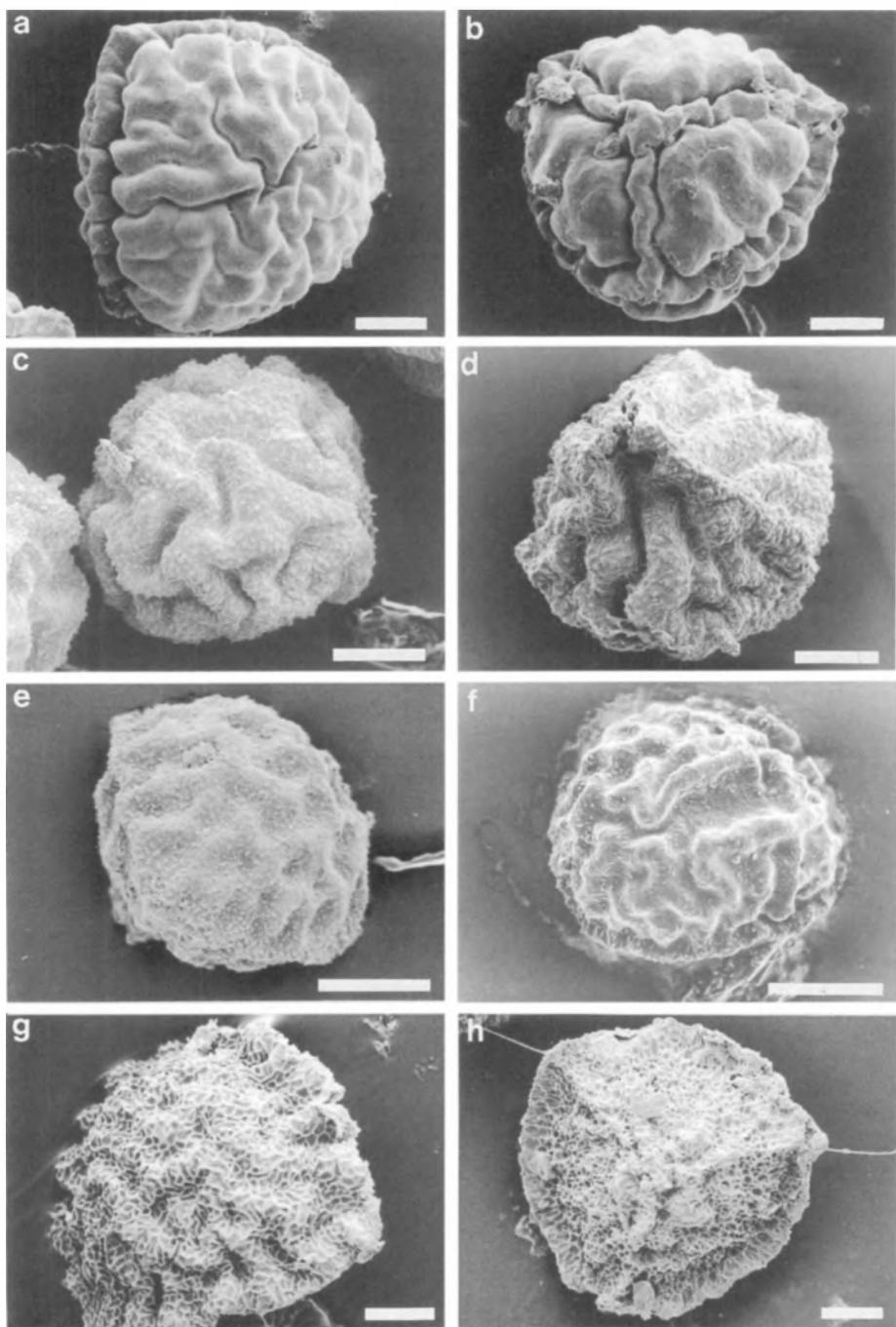


Figure 1. *Asterella* spores. a, b, *A. palmeri* (U.S.A., Doyle 2245); c, d, *A. wallichiana* (China, Long 18882); e, *A. wallichiana* (type, Burma, Wallich s.n.); f, *A. levispora* (type, Philippines, Curran 6533); g, h, *A. saccata* (Austria, Baumgartner 1201a). a, c, e, f, g, distal views; b, d, h, proximal views. Scale bar = 20 μm .

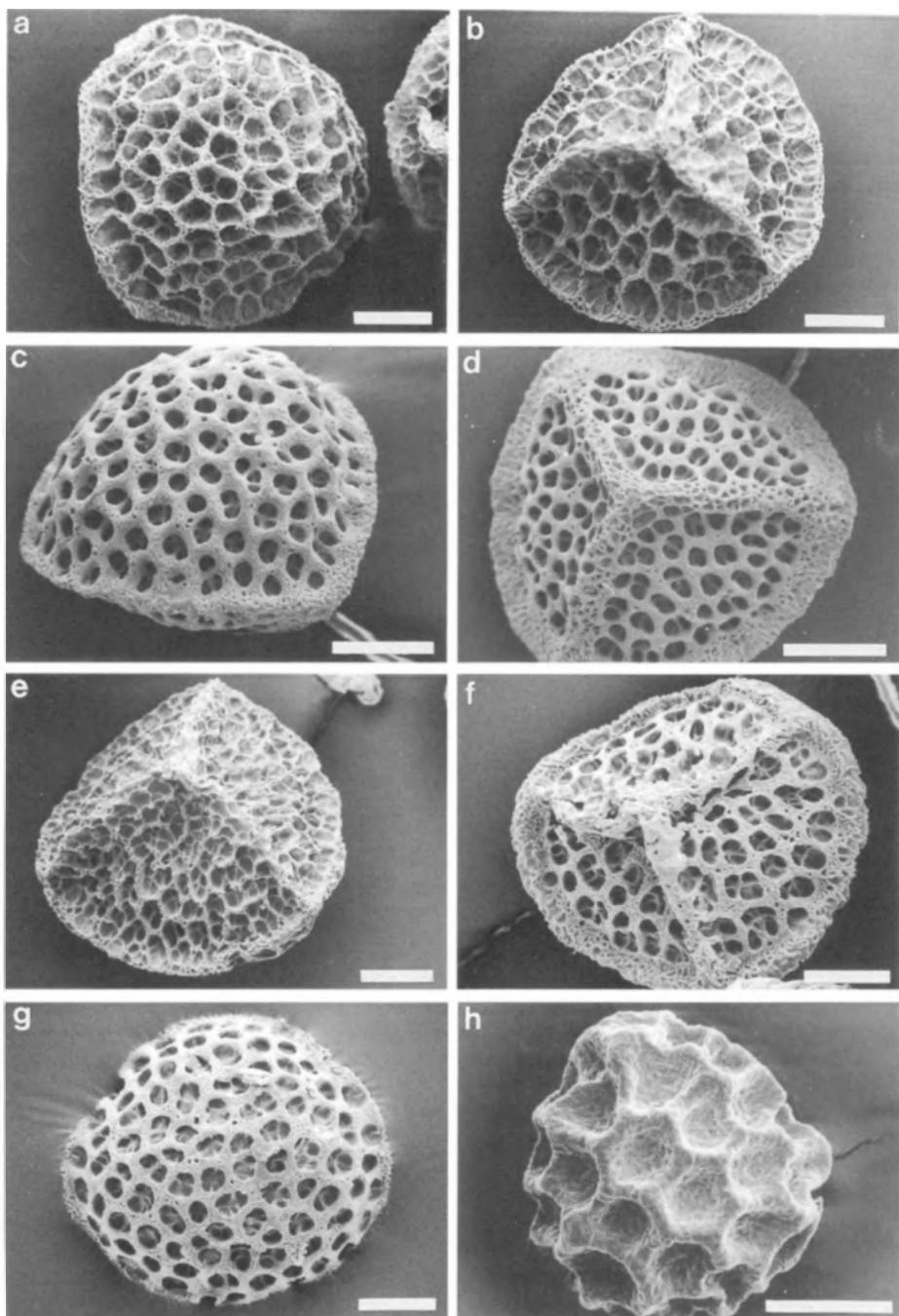


Figure 2. *Asterella* spores (contd.). a, b, *A. missouriensis* (China, Long 19199); c, d, *A. missouriensis* (Nepal, Long 16618); e, *A. missouriensis* (China, Long 18580); f, g, *A. crassa* (type, Japan, Shimizu s.n.); h, *A. gracilis* (U.S.A., Gradstein 2256). a, c, g, h, distal views; b, d, e, f, proximal views. Scale bar = 20 μm .

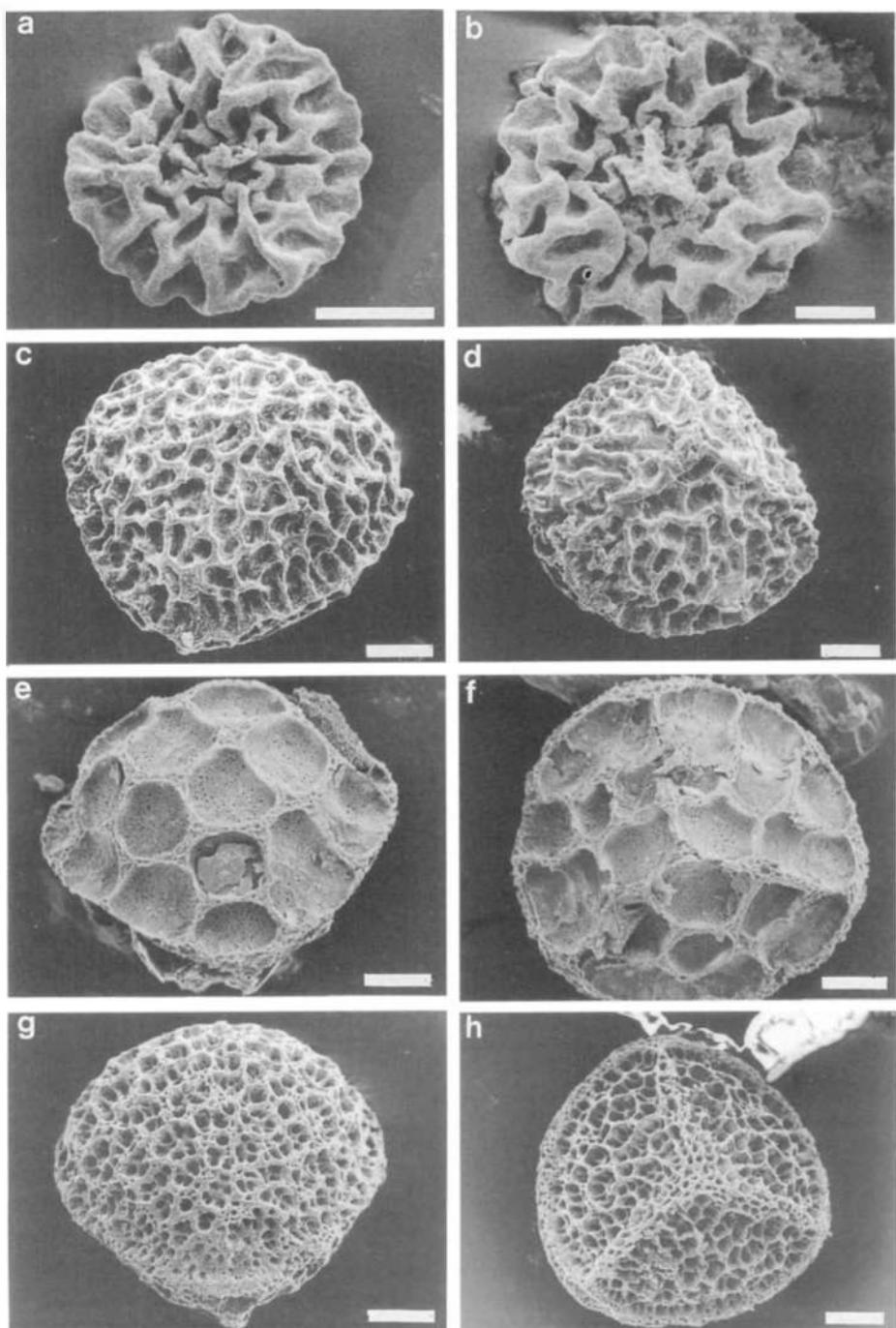


Figure 3. *Asterella* spores (contd). a, *A. gracilis* (Russia, Nilsson-Ehle s.n.); b, *A. gracilis* (Japan, Shimizu s.n.); c, *A. muscicola* (Lesotho, Duckett & Matcham 5); d, *A. muscicola* (Lesotho, Duckett & Matcham 11); e, f, *A. multiflora* (Nepal, Townsend 92-155); g, h, *A. persica* (Iran, Staps s.n.). c, e, g, distal views; a, b, d, f, h, proximal views. Scale bar = 20 μm .

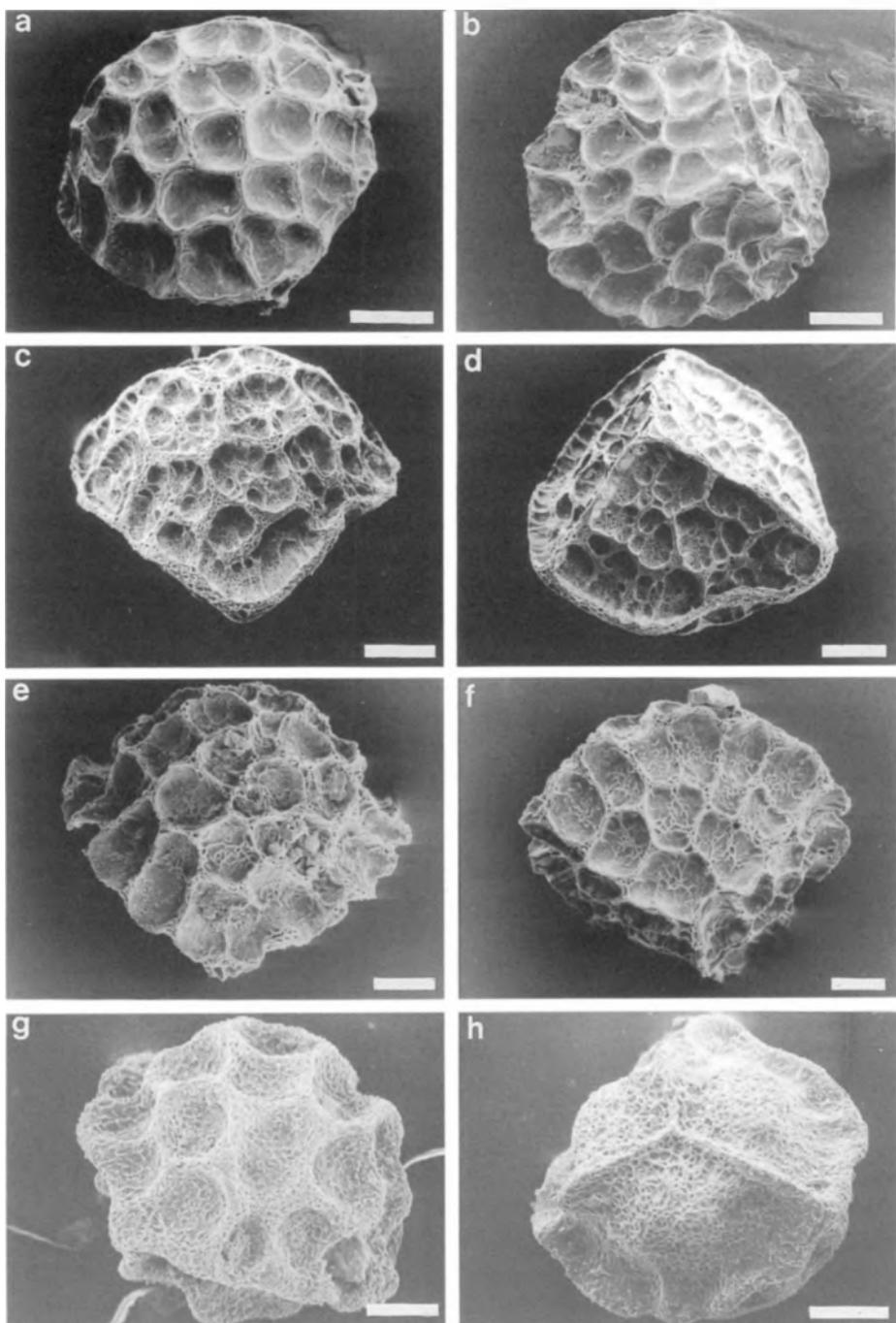


Figure 4. *Asterella* spores (contd). a, b, *A. bolanderi* (U.S.A., Stern s.n.); c, d, *A. macropoda* (Bolivia, Lewis 84-990); e, f, *A. wilmsii* (S. Africa, Perold & Koekemoer 2921); g, h, *A. abyssinica* (Rwanda, Frahm 8256). a, c, e, g, distal views; b, d, f, h, proximal views. Scale bar = 20 μm .

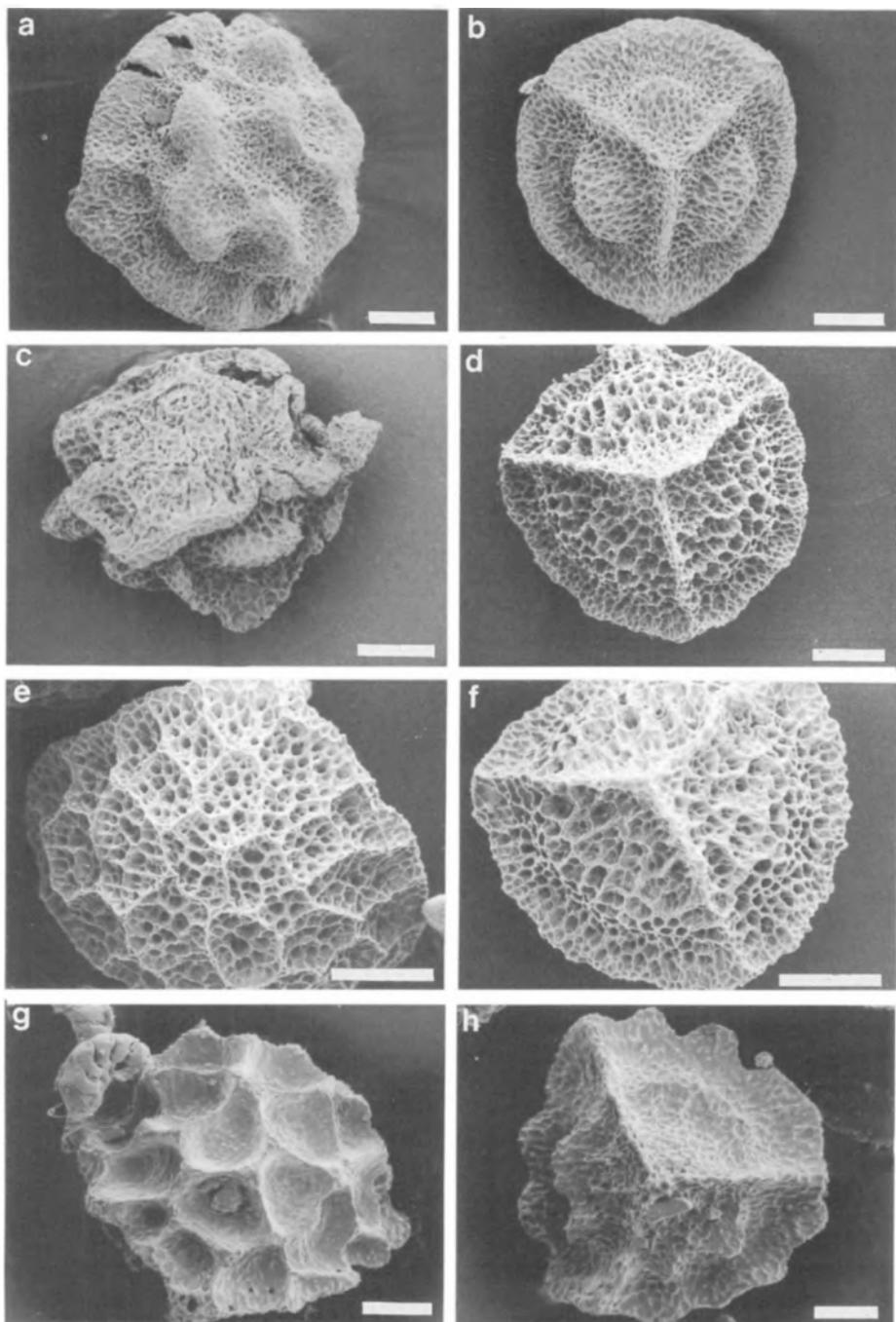


Figure 5. *Asterella* spores (contd.). a, b, *A. blumeana* (Celebes, Kjellberg 6h); c, *A. blumeana* (type, Java, Reinwardt s.n.); d, *A. khasyana* (Bhutan, Long 10633); e, f, *A. khasyana* (Nepal, Long 16635); g, h, *A. tenella* (U.S.A., Schuster M40). a, e, g, distal views; b, d, f, h, proximal views; c, lateral view. Scale bar = 20 μ m.

species (e.g. *A. palmeri*, *A. saccata*, *A. gracilis*) have unique spores and are readily distinguishable from all other taxa studied purely on spore characters. Within individual groups some taxa appear to be indistinguishable on spore characters (e.g. *A. crassa* and *A. mussuriensis*), while others (e.g. *A. mussuriensis*, *A. multiflora* and *A. persica*) can be distinguished by use of additional characters such as areole diameter and the presence/absence of secondary areolae. These differences are clearly visible in the micrographs.

7.4.3 Investigation of type specimens of three poorly-known taxa

a. *A. levispora*. The spore illustrated of the type specimen of this species (Fig. 1f) shows that it falls within Type II and is similar in all important respects to the samples of *A. wallichiana* studied (including the type in Fig. 1e). This provides strong evidence that *A. levispora* is a synonym of *A. wallichiana*.

b. *A. crassa*. The spore illustrated of the Japanese type specimen of this species (Fig. 2f, g) shows that it falls within Group V, and is indistinguishable from one of the populations of *A. mussuriensis* from Nepal (Fig. 2c, d). Given the variation discussed above for *A. mussuriensis*, *A. crassa* falls well within its range on spore characters. Spore morphology therefore provides compelling evidence that the taxa are synonymous.

c. *A. blumeana*. This species falls within Group VII in Table 2. The type material of this taxon (from Java) is very scanty with very few spores available for study. However, the somewhat damaged spore of the type illustrated (Fig. 5c) is clearly a very good match for that of the excellent more recent specimen from Celebes (Fig. 5a, b). These spores are strikingly distinct from all the others in Group VII (particularly *A. khasyana*, see Fig. 5d-f) in the distal surface forming a bulging 'crown' with very obscure primary areolae, and in the proximal facets each with a very striking bulge. They demonstrate that *A. blumeana* is a species with very distinctive spore morphology quite unlike *A. khasyana*. No specimens with the spores of *A. blumeana* have been seen from the Indian region.

7.5 DISCUSSION

7.5.1 Relationships between species or groups of species

Table 2 presents a classification of the 17 taxa studied, based on spore characters. However, translating these similarities and differences into a formal infrageneric classification cannot be undertaken without (a) studying a wider range of taxa worldwide and (b) taking all other important characters, particularly of the gametophyte, into consideration. These reservations apart, a number of interesting relationships are suggested by Table 2.

Grolle (1976, 1989) has provided the only recent infrageneric classification of *Asterella*, based primarily on gametophytic characters, especially those of the carpoccephalum and pseudoperianth. He divided the genus into three subgenera: Subg. *Asterella* containing only two species, *A. tenella* and *A. gracilis*, Subg. *Brachyblepharis* containing around ten species, and Subg. *Phragmoblepharis* containing many species.

The last subgenus was divided into three Sections: *Phragmoblepharis*, *Pappiae* and *Lindenbergianae*. Spore characters were scarcely used in the subgeneric classification, except that Subg. *Brachyblepharis* was described as having yellow coarsely reticulate spores.

It is not proposed to classify *Asterella* species purely on the basis of spores, as that would ignore some important gametophytic characters which must be considered in defining subgenera. However, based on the present study and other published and unpublished data on spores, the following observations can be made on Grolle's classification:

1. Subgenus *Brachyblepharis*. All members of this subgenus have Type VII spores, which adds weight to its distinctness. Type VII is unique in being the only spore type with dissimilar proximal/distal sculpturing.

2. Subgenus *Asterella*. The type species of *Asterella*, *A. tenella*, has Type VII spores remarkably similar to those of Subg. *Brachyblepharis*. The other species placed by Grolle in Subg. *Asterella*, *A. gracilis*, has Type VIII spores quite different from those of *A. tenella*, in that they have similar proximal/distal sculpturing and are alete (lacking a trilete mark). This suggests that Subg. *Asterella* may be an unnatural grouping, and that the relationship of its two constituent species with each other and with Subg. *Brachyblepharis* should be reassessed.

3. Subgenus *Phragmoblepharis*. Into this subgenus fall all the species studied having spore types I, II, III, IV, V and VI. The fact that Grolle subdivided this subgenus into three sections confirms that he considered it heterogeneous. If the observed spore differences have any significance in classification, they would suggest that Subgenus *Phragmoblepharis* requires further subdivision to recognise the very striking spore patterns, especially those with spore Types I and II (*A. palmeri* and *A. wallichiana*).

7.5.2 *Constancy of selected spore characters within individual species*

The present, albeit limited, study of 17 taxa has demonstrated that the qualitative spore characters selected are consistent and reveal stable differences between different species of *Asterella*. To some extent this conflicts with the view of Evans (1920, p. 258) who suggested that spore ornamentation could be environmentally modified and should be used in taxonomy with caution. It is likely that Evans' remarks were due partly to the limitations of light microscopy in observing spore ornamentation differences, and possibly also to the use of unripe spores where sculpturing has not fully developed. However, Evans himself used spore characters throughout his key to North American species.

7.5.3 *Taxonomic differences between species*

Many of the observed differences (Figs 1–5) between spores of different *Asterella* species are very striking indeed and provide excellent taxonomic characters. In many other liverwort genera the range of spore types is much narrower, even in other Marchantiales such as *Marchantia* and *Plagiochasma* (Bischler, 1978, 1979a, b, 1984;

Bischler-Causse, 1989). *Asterella* may therefore be considered as outstanding within the Bryophyta in the taxonomic value of the spores.

7.5.4 Investigation of type specimens of three poorly-known taxa to help resolve their identities

Because many old type specimens of *Asterella* and other Marchantiales are often small and fragmentary, the fact that spores remain intact for up to 200 years means that they are very valuable in reliably ascertaining the correct identity of these old types. In the three cases investigated, the spore micrographs obtained from the types of *A. blumeana*, *A. crassa* and *A. levipora*, have clarified their taxonomic affinities: on spore characters *A. blumeana* is clearly distinct from *A. khasiana*, *A. crassa* is indistinguishable from *A. missouriensis* and *A. levipora* is indistinguishable from *A. wallichiana*.

7.5.5 Relationships between species or groups of species

Spore characters as observed in this study appear to support to some extent the infrageneric classification of Grolle (1976, 1989), in that Subg. *Brachyblepharis* consistently has Type VII spores; Subg. *Phragmoblepharis* is heterogeneous (as recognised by Grolle) encompassing six different spore types none of which is found in other subgenera; Subg. *Asterella*, however, is problematic in that one of its species (the generitype *A. tenella*) has Type VII spores very like those of Subg. *Brachyblepharis*. The relationships of its two species, *A. gracilis* and *A. tenella*, require further study, to ascertain if Subg. *Asterella* and Subg. *Brachyblepharis* should be united.

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8. The ordinal classification of the mosses: questions and answers for the 1990s

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8.1 SUMMARY

Peristomial characters provide a solid basis for the recognition of four primary clades of arthrodontous mosses, but carry little phylogenetic information regarding their relationships. Molecular data support the recognition of these peristome based clades but also are informative in resolving the evolutionary history of these four groups. A 'next generation' classification of the mosses recognizes clades with orders in four subclasses of Arthrodontous mosses. The mosses (Bryophyta) are divided into four classes. Both molecular and morphological data are significant in providing new information for the development of phylogenies of mosses. The resulting classifications should be constructed from phylogenies based on molecular and morphological approaches.

KEYWORDS: *rbcL*, phylogeny, mosses, Bryopsida, peristome, molecular systematics, 18-S, classification.

8.2 INTRODUCTION

As early as the 1820s, Bridel (1826–1827) argued that certain characteristics were more important than others in ordering moss species into a system that portrayed relationships. He proposed that these relationships were best revealed by the position of the sex organs, especially whether the perichaetium was produced terminally at the end of the main shoot (acrocarypy) or produced laterally along the main shoot (pleurocarypy). However in the late 1850s, Mitten (1859) suggested that the characters found in peristomial features held the most important information for ordering moss taxa into a system that reflected natural relationships. This view was further developed by Philibert (translated by Taylor, 1962) in the next 20 years, and in the early 1900s Fleischer (1904 in 1904–1923) formulated a classification of mosses within which the higher ranks were ordered based on peristomial features. This peristome-based classification was largely followed by most bryologists of the early part of the 20th century, including Cavers (1910–1911), Brotherus (1924–1925a, b), Dixon (1932), and Reimers (1954), all of whom wrote important, reasoned classifications of the mosses. All of these authors considered moss peristomes to be essentially of two types (nematodontous and arthrodontous) with the latter divided into two types

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(haplolepidous and diplolepidous). Classifications followed this ordering, wherein the arthrodontous mosses were always placed firstly into the haplolepidous groups and secondly into the diplolepidous groups.

Crosby (1980) proposed that the haplolepidous peristome may in fact be derived from the diplolepidous type, and suggested that in classifications of mosses, the haplolepidous groups should come second, rather than first, indicating their more advanced nature. Crosby's idea was important in that it encouraged bryologists to think further about how peristomial features could be utilized in classifying mosses. Similarly, Edwards' (1979, 1984) careful analyses of mature peristome forms allowed more detailed comparisons of peristomes to be made, and clarified the often misunderstood concepts of haplo- and diplolepidae. Edwards (1979) utilized formulae for describing the mature form of the peristome wherein the number of cells comprising the three fundamental development layers [outer (OPL); primary (PPL); and inner (IPL)] are given for a two-cell segment of the PPL; the first number being for the OPL and the last number for the IPL.

Vitt (1981a) suggested that arthrodontous peristomes consisted not of two primary types, but of four; and proposed that both the haplolepidous (0:2:3 — *Dicranum*-type) and two diplolepidous (4:2:8 {4} — *Bryum*-type and 4:2:4 {2} — *Orthotrichum*-type) peristomes were all derived from an ancestral diplolepidous peristome (4:2:4 — *Funaria*-type). Vitt indicated that several key character changes had taken place in the evolution of these four peristomial types, particularly: 1) thickening of the outer peristomial layer, 2) additional division yielding IPL endostomial cilia, 3) OPL/primary peristomial layer [=exostome] loss or reduction, 4) alternate positioning of the endostome segments, and 5) asymmetric division of the inner peristomial layer. Shaw *et al.* (1987, 1989a, b) and Schwartz (1994) emphasized the developmental features of the IPL as critical to our understanding of the mature form of the arthrodontous peristome.

In 1982, and revised in 1984, Vitt proposed an ordinal classification of the mosses that utilized these four peristomial types as the key characters, with species having the *Funaria*-type (diplolepidous opposite) placed first, followed by those with the *Orthotrichum*-type (diplolepidous thickened OPL), then those with the *Bryum*-type (diplolepidous alternate and with additional IPL divisions producing cilia), and finally those with a *Dicranum*-type (haplolepidous) peristome.

The various classifications presented during the past 200 years have surely changed over time, and these changes have focused our attention on important and often new questions that need answering. Here we attempt a discussion of several of these critical questions, in particular: 1) Can peristomial features resolve the relationships of the arthrodontous peristome types? 2) What contribution can be made by molecular data to resolving the higher (order and above) relationships within mosses? 3) Can a 'next generation' classification at the ordinal rank be developed at this time for our use in current major floristic treatments?

8.3 RELATIONSHIPS BASED ON PERISTOME FEATURES

What follows is our view of the critical characters of the arthrodontous peristome from which phylogenetically significant information can be derived. Seven character complexes are evident (Fig. 1).

1. THICKENED OPL: The *Orthotrichum*-type peristome has a much thickened OPL when compared to the PPL. Most other mosses have the primary peristomial layer component of the exostome thicker than the OPL. The thickened OPL is here considered a synapomorphic feature of the Orthotrichales.

2. CILIA: Cilia are unique to the *Bryum*-type peristome wherein the basic IPL pattern has eight cells of very different sizes for each two of the PPL (but note that there is variation from 4–14 IPL cells). Cilia develop from secondary divisions of IPL cells that lie opposite to the exostome teeth. This secondary developmental pattern is unique to the *Bryum*-type peristome.

3. EXOSTOME LOSS: Exostome loss occurs commonly in nearly all lineages of mosses in association with habitat specialization (Vitt, 1981a) and endostomial reduction is also often apparent. These cases of peristome reduction are clearly homoplasic to the consistent and uniform lack of development of the OPL/PPL of all haplolepideous species where the endostome (PPL/IPL) forms the basic peristome. Here the lack of development of the OPL/PPL is considered a synapomorphy for the *Dicranum*-type peristome, with exostome reduction in all other groups homoplastic.

4–5. CELL DIVISIONS: In the *Polytrichum*-type (Shaw, pers. comm.) as well as the *Funaria*-type (Shaw *et al.*, 1989a; Schwartz, 1994) and *Orthotrichum*-type (Lewinsky, 1993; Goffinet, 1996) peristomes all cell divisions are symmetric. In both the *Bryum*-type and *Dicranum*-type peristome development, the first division in the IPL after completion of the fundamental square cell pattern is asymmetric. Shaw *et al.* (1989a, b) show that this asymmetric division takes place when the amphithecum is in three layers in the *Dicranum*-type peristome, whereas it occurs when the amphithecum is in four layers in the *Bryum*-type. A key question is whether this first asymmetric division in these two peristome types is a) homologous, b) derived one from the other, or c) evolved independently? The developmental evidence appears to support independent evolution of asymmetries due to a different timing of divisions, however, other interpretations are possible. If in fact, these asymmetric divisions are considered homologous, then this peristome feature supplies a much needed synapomorphy for these two peristome groups.

6–7. SEGMENTS/TEETH ARRANGEMENT: In the *Funaria*-type peristome, the endostome segments lie opposite to the exostome teeth; the single peristome (endostome) of the *Dicranum*-type lies opposite to where the exostome teeth would be positioned if they developed. Most Orthotrichaceae taxa have an alternate arrangement of segments (Vitt, 1981b) while in the *Bryum*-type peristome, segments are always alternate to teeth (with cilia in the opposite position). Thus two groups have alternate arrangements of segments and teeth; the question is whether these are homologous. Currently, evidence suggests that they are not. In the *Bryum*-type the alternate arrangements are developed due to additional cell divisions after an asymmetric IPL division, whereas in the *Orthotrichum*-type alternate arrangements are developed from differences in wall thickening following a symmetric IPL division (Vitt, 1981b). Thus the alternate position does not yield a synapomorphy for any two of the four peristome groups.

In summary, we argue that three peristome types independently evolved from a *Funaria*-type peristome which is characterized by a 4:2:4 cell pattern, a thin OPL,

no cilia, an IPL with all symmetric divisions, the presence of an exostome (at least primitively), and an opposite arrangement of segments. Currently no morphological synapomorphies link these four peristome types (Fig. 1) and the cladogram derived from the analysis of the seven key features remains unresolved (Fig. 2).

8.4 RELATIONSHIPS BASED ON MOLECULAR DATA

It appears that peristome features can be utilized to derive and characterize the major groups of arthrodontous mosses, however, data from peristomes do not yield informative synapomorphic features that can be used to show relationships between these four groups. Molecular data from both chloroplast (*rbcL*) and nuclear (ribosomal 18S) genes are now available and these data can contribute significantly to our understanding of higher moss relationships.

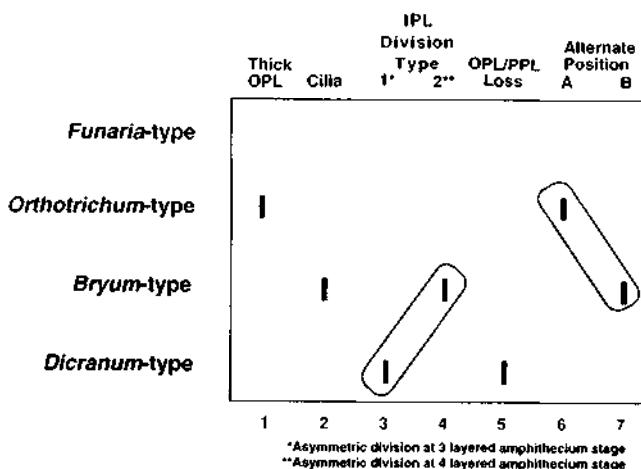


Figure 1. Seven critical synapomorphic features of arthrodontous peristomes and their occurrence among the four major peristome-derived moss groups. OPL — Outer peristomial layer; IPL — Inner peristomial layer; PPL — Primary peristomial layer; Alternate position (see text for details).

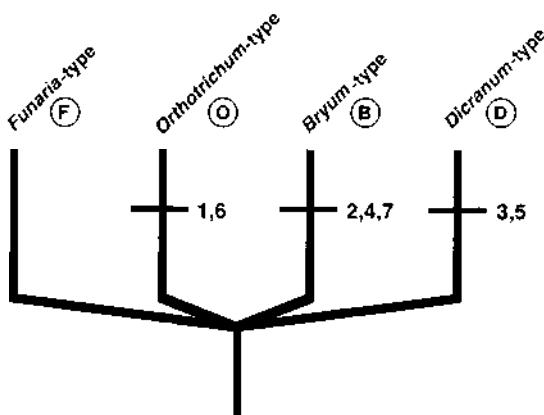


Figure 2. Unresolved cladogram of four major moss groups based on arthrodontous peristome features. Numbers refer to synapomorphic features in Fig. 2.

Summaries of molecular data sets from Goffinet (1996 — Fig. 3) and Hedderson *et al.* (1997 — Fig. 4) indicate all or in part the following patterns:

1. The Nematodontous mosses form the sister group to the Arthrodontous mosses.
2. *Buxbaumia*, *Diphyscium*, and *Tetraphis* should be placed in the Nematodontous clade.
3. *Andreaea* and *Takakia* are basal to the Nematodontae.
4. The Funariales (with opposite diplolepideous peristome type) is sister to (and basal to) all other Arthrodontous mosses.
5. The diplolepideae are paraphyletic.
6. The Splachnales form a sister group to the Orthotrichales-ciliate clade.
7. The Orthotrichales are sister to the ciliate mosses (however the ciliate mosses may or may not be monophyletic).
8. The patristically most derived mosses are those with a ciliate peristome.

Cladograms derived from molecular data are largely congruent and indicate similar ordering of these four major peristome groups (Figs 3–4). Using the molecular data to reconstruct basic principles inherent in peristome data reveals the following set of conclusions.

- a) The *Funaria*-type peristome with its opposite pattern and symmetric cell divisions is ancestral to other arthrodontous peristome types.
- b) The haplolepideae are monophyletic and derived from a diplolepideous ancestor; based on molecular data, the asymmetric IPL division appears to have arisen twice.
- c) The Orthotrichales do not belong within the ciliate mosses and the alternate position of segments is most likely to be independently derived in this group.

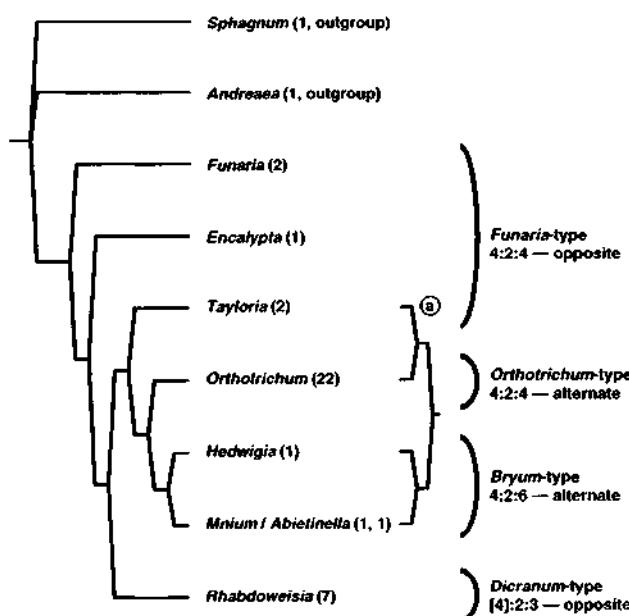


Figure 3. Phylogenetic relationships among major lineages of arthrodontous mosses based on *rbcL* sequence data (modified from Goffinet, 1996). Tree is one step longer than most parsimonious topology, represented in (a). Numbers in parentheses indicate number of taxa representing clade in phylogenetic analysis. Peristome formula OPL:PPL:IPL ([]) = cells typically missing in mature peristome.

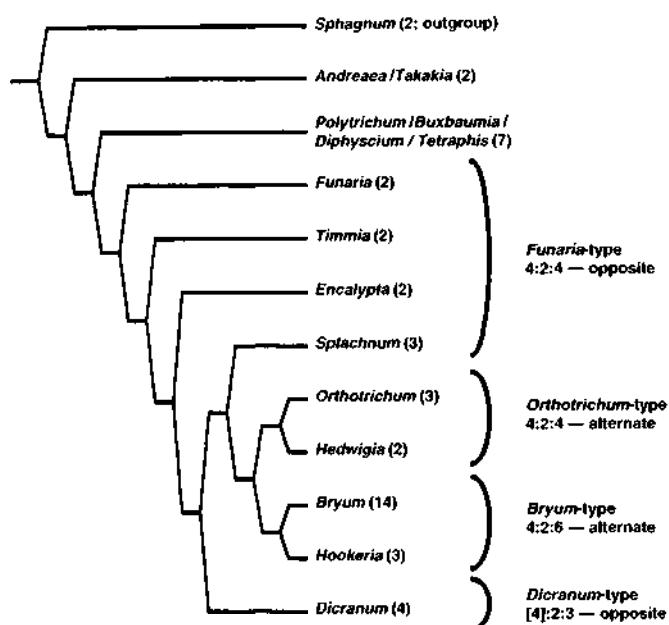


Figure 4. Phylogenetic relationships among major lineages of mosses based on 18S nrDNA sequence data (summarized from Hedderson *et al.*, 1997). Numbers in parentheses indicate number of taxa representing clade in phylogenetic analysis. Peristome formula OPL:PPL:IPL [] = cells typically missing in mature peristome.

d) Ciliate mosses appear to be monophyletic, but there is a lack of agreement of relationships within the group (T. A. Hedderson, unpublished results).

e) Despite the important information yielded by molecular techniques and peristome patterns, some problems remain unresolved (*i.e.* relationships of the nematodontous taxa; the Splachnales and the Encalyptales), and additional morphological data other than those from the peristome need to be critically examined.

8.5 A 'NEXT-GENERATION' CLASSIFICATION

Fifteen orders (or suborders if we are consistent with hepatic classifications in 1984; Schuster, 1984) were recognized by Vitt (1982). True mosses were recognized as the subclass Bryidae separated from the Sphagnidae and Andreaeidae (but without *Takakia*).

Significant recent research, in addition to the molecular data presented above, includes a better understanding of *Takakia* (Smith & Davison, 1993), more details of peristome architecture and development (Shaw, 1985; Shaw & Crum, 1984; Shaw & Rohrer, 1984; Shaw *et al.*, 1989a, b; Schwartz, 1994), and rethinking of the relationships of the pleurocarpic taxa (Buck & Vitt, 1986; and more recently, Hedenäs, 1994, 1995). Furthermore, current molecular data on the Orthotrichales (Goffinet, 1996) and the acrocarpous Bryales (Hedderson *et al.*, 1997) have greatly increased our knowledge of these groups.

The ordinal classification presented here is our interpretation of our current state of knowledge. Annotations (following section) are included to guide an understand-

ing of the current problems, and questions are posed to guide and stimulate future research.

Bryophyta (Mosses)¹

Sphagnopsida ²	Bryopsida
Sphagnidae ³	Funaridae ⁵
Sphagnales	Funariales
	Timmiales ⁶
	Encalyptidae ¹¹
	Encalyptales
	Splachnidae
	Splachnales ⁷
Andreaopsida	Orthotrichidae ⁸
Andreaidae	Orthotrichales
Takakiales	Hedwigiales ⁹
Andreaobryales	Bryidae
Andreaeales	Bryales ¹⁰
	Leucodontales
	Hypnales
Polytrichopsida	Hookeriales
Polytrichidae	Dicranidae
Tetraphidales	Pottiales
Polytrichales	Dicranales (includes Fissidentales)
Buxbaumiales	Seligeriales
Diphysciales ⁴	Grimmiales

8.6 COMMENTS AND QUESTIONS

¹ Currently it is debatable whether hepatics and mosses form a monophyletic clade (e.g. Hedderon *et al.*, 1996) or whether bryophytes are paraphyletic as argued by Mishler & Churchill (1984). Many developmental data suggest a lack of relationship between hepatics and mosses (Crandall-Stotler, 1980), and this problem is not resolvable at present. A monophyletic interpretation requires recognition of the mosses at the phylum rank (Bryophyta), whereas a paraphyletic interpretation should recognize mosses at the subphylum rank (Bryophytina). Either classification can be contained within the current presentation.

² Most parsimonious with molecular data, and consistent with morphological data, is a classification that recognizes four classes of mosses — Sphagnopsida with only *Sphagnum* (excluding *Protosphaignum* whose position is uncertain); Andreaopsida containing *Takakia*, *Andreaobryum*, and *Andreaea* (defined by capsules with slits); Polytrichopsida for all Nematodontous mosses (based largely on molecular data both *Buxbaumia* and *Diphystrum*, as well as *Tetraphis*, are placed here, each in their own orders); and Bryopsida for all Arthrodontous mosses.

3. The relationships of *Sphagnum* and *Andreaea* are not yet fully resolved. Based on sequence data of the 16S chloroplast rDNA gene (Mishler *et al.*, 1992), *Andreaea* is sister to a clade composed of mosses (including *Sphagnum*), thalloid liverworts, and vascular plants, whereas in a combined data set of both chloroplast rDNA genes, 16S and 23S, *Sphagnum* is sister to a clade including *Andreaea*, *Polytrichum*, and *Tetraphis*. *Andreaea* and *Sphagnum* were also included in a phylogenetic reconstruction of green plants using *rbcL* sequence data (Manhart, 1994). Here too the relationships are ambiguous. *Andreaea* was generally found sister to a clade including *Sphagnum*, but the sister group to *Sphagnum* itself, was not resolved. Clearly these results indicate that the phylogenetic affinities of both *Andreaea* and *Sphagnum* need to be further addressed.

4. Shaw *et al.* (1987) showed that *Diphyscium* has a haplolepidous peristome. Gametophytically, the plants show much similarity to the Encalyptaceae and some members of the Pottiaceae. Comparison of 18S gene sequences reveal however, that *Diphyscium* shares several signature sequences with *Buxbaumia* and the Nematodontaceae, suggesting a close relationship between these taxa. *Diphyscium* clearly needs further work.

5. We argue that much evidence supports the proposal that the four peristome types represent the four fundamental clades of Arthrodontous mosses; this is supported by both peristome and molecular characters. We think these four clades should be recognized as subclasses: 1) the Funaridae defined by 4:2:4, unfused, symmetric, opposite peristomes; 2) the Dicranidae defined by [4]:2:3 asymmetric, opposite peristomes with no OPL/PPL development; 3) the Orthotrichidae defined by 4:2:4, symmetric, mostly alternate peristomes with the OPL thickened or fused; and 4) the Bryidae defined by 4:2:8, asymmetric, ciliate peristomes. However, it is presently unclear how some of the more derived lineages (*i.e.* Timmiales, Encalyptales, Splachnales) should fit within these four clades. Also, presently it is not entirely clear that the ciliate mosses are monophyletic; rather the ciliate condition could just as well have evolved several times and instead represent a highly derived grade either independent from the Orthotrichidae (thus polyphyletic) or including the Orthotrichidae (thus paraphyletic). A thorough study of the ciliate lineage may indeed propose multiple origins of a number of groups that each require recognition at a higher rank.

6. 18S molecular data indicate the surprising placement of *Timmia* in a clade near *Funaria*. Interestingly, Zander (1993) suggested the primitiveness of *Timmia* when he was examining relationships of the Pottiaceae. Murphy (1988) found that the development of the peristome of *Timmia* clearly has a 4:2:8 pattern with symmetric IPL divisions. There is no evidence in these data that excludes *Timmia* from a relationship with *Funaria*. This interesting problem needs to be further examined; but for now we choose to include *Timmia* within the Funaridae; however, it is possible that the taxon requires a separate subclass.

7. Both *rbcL* and 18S data place members of the Splachnaceae sister to the Orthotrichalean clade, rather than the Funarialean clade. We accept the molecular data for now; but this placement needs to be further resolved.

8. The Orthotrichidae may include only one or two orders. Here we follow Goffinet (1996) who excluded from this clade *Amphidium* and *Drummondia*, as well as the

Erpodiaceae, the Rhachiteciaceae (all Dicranidae), and the Microtheciellaceae (Bryidae).

⁹ Molecular data currently do not resolve the position of the Hedwigiaceae. Cladistic results from *rbcL* and 18S molecular data either place *Hedwigia* within the ciliate clade or the *Orthotrichum* clade. De Luna's (1995) morphological analysis indicates relationships with the Orthotrichales. This is a problem needing further work; but for now we include the Hedwigiales as a distinct order placed in the Orthotrichidae. [Note: the presence of pseudoparaphyllia in Hedwigiales is difficult to rationalize within the Orthotrichidae, however as Hedenäs (Chapter 9) points out, pseudoparaphyllia are difficult to define.]

¹⁰ The Bryales as presented here represent all ciliate acrocarpous/ cladocarpous mosses, while the pleurocarps are dispersed over the remaining three orders. The Bryalean group from which these are derived is not apparent at present. Since the monophyly of the Bryales is not sure, it is not clear whether the pleurocarps are monophyletic. Central to this problem is definition of the Leucodontales (see LaFarge-England, 1996). As noted above (5), the monophyly of the ciliate clade needs further study and is being addressed separately by T. A. Hedderson and C. Cox.

¹¹ The affinities of the Encalyptales remain unresolved. Comparisons of 18S data (Hedderson *et al.*, 1996) suggest that the Encalyptales are sister to all arthrodontous mosses, except the Funariales, a placement congruent with Edwards (1979). *RbcL* data (Goffinet, 1996) partly support such relationships but equally parsimonious is a sister group relationship with the haplolepideae only, a hypothesis that cannot be dismissed on peristome features alone (Edwards, 1984). Until further resolved, we place *Encalypta* in its own subclass, sister to the Dicranidae. Further work is badly needed, particularly studies addressing developmental aspects of the peristome in order to better resolve the position of this important genus.

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9. Cladistic studies on pleurocarpous mosses: Research needs, and use of results

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9.1 SUMMARY

To investigate the phylogeny of the entire group of pleurocarpous mosses, a stratified sampling procedure is described. In this, selected representatives of the entire group are first studied, and their phylogenetic relationships evaluated. Clades identified in the first overview are then sampled more thoroughly, and the procedure is repeated until one reaches the taxonomic level of particular interest. This method copes efficiently with limitations in time and computer capacity that are otherwise present in large-scale phylogenetic studies, and makes it possible to formulate phylogenetic hypotheses that can be tested and elaborated with new information. There is a major problem in our interpretation of many morphological characters. Uncertainties concern continuously variable quantitative characters, the frequent transformations of organs, and the frequent occurrence of parallel evolution of similar character states. Using examples from leaf lamina cell size and shape, several organs of the stem, perichaetial branches, and specialized sporophytes, the need for continued basic research on characters and their relevance for the function of the plants is emphasized. Cladistic analyses of pleurocarpous mosses based on morphological characters give slightly lower consistency indexes than in other organisms. This may be due to higher frequencies of similar, non-homologous character states, of character state reversals, or of quantitative characters in pleurocarps than in organisms in general. It is stressed that despite uncertainties and the lack of knowledge in many fields, we must still analyse all the available data in a rational way, to produce testable hypotheses about phylogeny. Only in this way can we hope to separate historical factors from more recent adaptations and derive a rational basis for decisions concerning both our use and protection of the pleurocarpous mosses.

KEYWORDS: Characters, consistency index, evolution of adaptations, phylogeny, pleurocarpous mosses.

9.2 INTRODUCTION

Pleurocarpous mosses have their female sexual organs, and thus also their sporophytes, on shortened and specialized lateral branches (*cf.* La Farge-England, 1996). Most diplolepidous pleurocarpous mosses seem to belong to one monophyletic

group, that is, they have only one ancestor. In addition several of the diplolepidous families which do not belong to this group, but where a pleurocarpous organization occurs, seem to have evolved among the close ancestors of this main group (Buck & Vitt, 1986; Hedenäs, 1994). According to Walther (1983), approximately 6500 species of pleurocarpous mosses exist. This is likely to be an overestimate if we consider the taxa described at present, because of numerous taxonomic synonyms, but may be true if we include yet undescribed species. The classification of the pleurocarps we find in floras is still similar to the one introduced by Fleischer (1900–1922) and Brotherus (1924, 1925). These authors based their system on relatively few ‘key characters’, such as the appearance of the nerve of the vegetative leaves, the shape of the median lamina cells, the presence or absence of leaf cell papillae, the presence or absence of paraphyllia, and whether the shoots are flattened or not, but also features such as the occurrence in drier or wetter habitats were included. Emphasis was also placed on whether the peristomes are perfect or specialized, but not on all differences between the different types of perfect peristomes that exist. Buck (1991) reviewed the different kinds of characters that have been used in the familial classification of pleurocarpous mosses through history, and concluded that gametophytic characters have probably been undervalued in many classifications. Several modern investigations have shown that many of Fleischer’s and Brotherus’ ideas on the relationships between different groups of pleurocarpous mosses are incorrect (*e.g.* Buck & Crum, 1978; Buck, 1980a, b, 1981, 1994; Buck & Ireland, 1985; Buck & Vitt, 1986; Hedenäs, 1987, 1989, 1994, 1995).

Despite these new studies and the rapid development of phylogenetic studies during recent years, we still know relatively little about the large-scale phylogeny of the pleurocarpous mosses. Cladistic studies concerning this group have also been few: Rohrer (1985), Granzow-de la Cerda (1992), Zomlefer (1993), Hedenäs (1994, 1995, 1997a, b), Hyvönen & Enroth (1994), De Luna (1995), and Hedenäs & Kooijman (1996). Buck & Vitt (1986) made a semi-cladistic overview of the pleurocarpous mosses, but included symplesiomorphies, that is, shared ancestral character states to define their taxa. Because such character states are uninformative as regards relationships between taxa, their classification is not reliable in all parts. However, their paper as well as my own studies (Hedenäs, 1994, 1995, 1997a, b) show that there is still a need for much work in this field. I will here discuss some of the more urgent problems remaining, as well as the advantages of a better knowledge of the phylogeny of the pleurocarps.

9.3 METHODOLOGY OF LARGE-SCALE PHYLOGENETIC STUDIES

Due to the many pleurocarpous moss species, no one can achieve an intimate knowledge of the entire group within a reasonable period of time. Computer programs available for cladistic analyses, such as HENNIG86 and PAUP, also put limits on how many taxa can be analysed simultaneously. The number of taxa should preferably not exceed 70–80. Thus, any attempt at understanding the large-scale relationships among the pleurocarps must be based on a well planned sampling of representative taxa in a successive and hierarchical way. This means that the first study or studies should aim at building a rather coarse skeleton, where the different

parts are later studied in more and more detail. First, all taxa of interest, here all the pleurocarpous mosses (e.g. Hedenäs, 1995), are considered. As the study proceeds, one clade of the first phylogenetic tree is studied in more detail (e.g. Hedenäs, 1997a), and at the next level an even less inclusive monophyletic group. The process continues until the lowest level of interest is reached.

At each stage of this 'top-down' analysis we produce a framework to which we can add further taxa or elaborate the details of parts. Starting the other way around, with a single larger genus or a single family, we are less likely to achieve the overview necessary for an understanding of the entire pleurocarp group. In addition, for each genus or family, deviating taxa are often left just outside the group of interest, or they have to be tentatively placed somewhere else by the investigator without a true understanding of their relationships. To approach the large-scale phylogenetic relationships of the pleurocarpous mosses from 'below' is thus a much less efficient and much more time-consuming method than the approach suggested here. With the limited time that is available for research projects nowadays, any study starting from below is almost certainly doomed to fail.

9.4 MORPHOLOGICAL CHARACTERS IN NEED OF FURTHER INVESTIGATION

All methods aiming at an understanding of the phylogenetic relationships between organisms, including the cladistic ones, depend on the quality of the data that are available, and on their interpretation. Here I will point to a few examples of problems that are possibly more evident for pleurocarpous mosses than for vascular plants or many animal groups. The character discussion is partly based on a database with 267 species (occasionally subspecies or varieties; list of included taxa deposited at S), with variable completeness as regards the registered characters. For example, sporophytes were not seen in all species.

One of the main problems in bryophytes concerns the many continuously variable, quantitative characters. There is no universal agreement on how to treat these types of characters in cladistic analyses (Chappill, 1989; Stevens, 1991), but it is clear that in bryophytes they constitute a relatively large proportion of the information we use for classification. Examples of quantitative characters used in higher level classifications of pleurocarpous mosses are cell shape in vegetative leaf lamina, leaf margin denticulation, leaf nerve development, capsule orientation and degree of peristome specialization (Fleischer, 1900–1922; Brotherus, 1924, 1925; Buck & Vitt, 1986). Width and elongation in the median leaf lamina cells both show variation between different species and between higher taxa (Fig. 1). Species belonging to the Neckeraceae, Brachytheciaceae, Plagiotheciaceae and Sematophyllaceae have significantly narrower leaf cells than species of the Hookeriales (Table 1). However, some members of the Hookeriales possess as narrow cells as most members of the other families (Fig. 1). Members of the Neckeraceae and the Hookeriales have significantly less elongate cells than the other taxa (Table 1), but the two categories overlap (Fig. 1). Thus, while these characters are informative for these relatively well circumscribed taxa, the overlap present complicates their use as distinguishing features. A similar character is the number of layers in the upper portion of the calyptra (the part which is not filled by the theca, in cucullate calyptas in the part

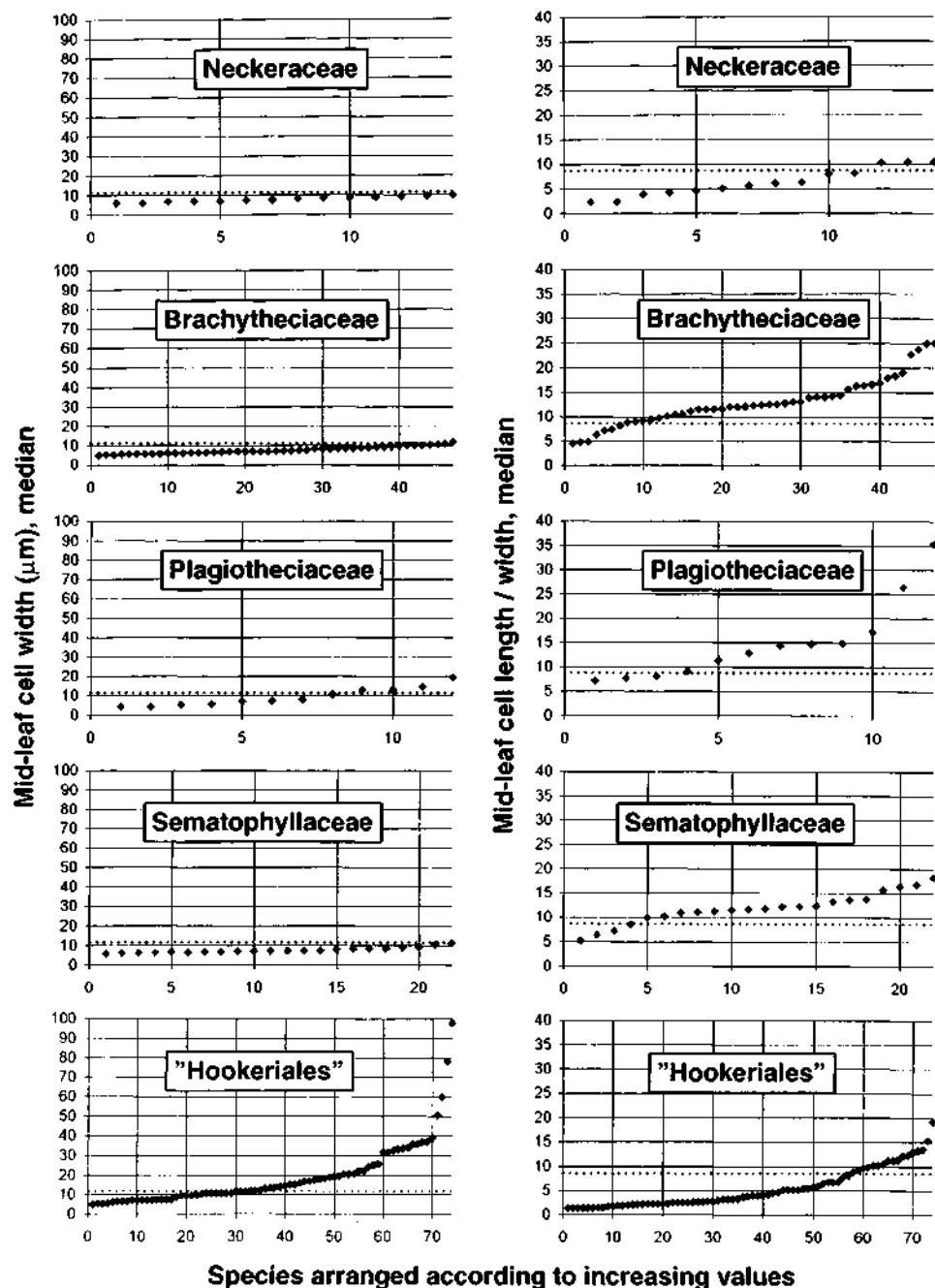


Figure 1. The mid-leaf cell widths and the quotients mid-leaf cell length/width (median values of the measurements for each species) in species of Neckeraceae ($n=14$), Brachytheciaceae (47), Plagiotheciaceae (12), Sematophyllaceae (22) and Hookeriales (74) studied. The mean cell width, 10.8 (s.d. 10.1) μm , and the mean value of the quotient, 8.7 (s.d. 5.7), for the total set of species studied ($n=265$) are indicated by dotted lines; the means for the respective taxa are given in Table 1.

Table 1. Comparison of (a, b) two mid-cell characteristics in the species of the Neckeraceae, Brachytheciaceae, Plagiotheciaceae, Sematophyllaceae and Hookeriales studied, (c) the number of cell layers in the upper calyptra in species of the Brachytheciaceae, Sematophyllaceae and Hookeriales, and (d) spore size in relation to capsule orientation (relative to seta). Means and ranges of the median values for the measurements of each species are given. The results of pairwise Anova for the families/capsule orientation categories are indicated by letters appended on the mean values, where different letters mean that significant differences exist ($p < 0.05$ -0.001) between two groups. In addition, the Brachytheciaceae and the Plagiotheciaceae differ significantly ($p < 0.05$) for cell width in mid-leaf.

a) Cell width in mid-leaf					
Taxon	Neckeraceae	Brachytheciaceae	Plagiotheciaceae	Sematophyllaceae	Hookeriales
n =	14	47	12	22	74
mean (s.d.), μm	7.8 (1.2) ^a	7.5 (1.7) ^a	9.3 (4.5) ^a	7.6 (1.4) ^a	19.0 (16.2) ^b
range, μm	6.0-9.8	4.8-11.6	4.2-19.5	5.8-11.2	4.8-97.6

b) Quotient cell length/width in mid-leaf					
Taxon	Neckeraceae	Brachytheciaceae	Plagiotheciaceae	Sematophyllaceae	Hookeriales
n =	14	47	12	22	74
mean (s.d.), μm	6.2 (2.7) ^a	12.9 (4.8) ^b	14.9 (8.0) ^b	11.9 (3.2) ^b	5.4 (4.0) ^a
range, μm	2.2-10.4	4.4-25.0	7.1-35.2	5.1-18.4	1.2-19.1

c) Number of cell layers in upper calyptra					
Taxon	Brachytheciaceae	Sematophyllaceae	Hookeriales		
n =	17	22	64		
mean (s.d.), μm	4.3 (1.0)	4.4 (1.0)	4.7 (1.6)		
range, μm	2.5-7.0	3.5-7.0	2.5-9.5		

d) Spore size in relation to capsule orientation					
Capsule orientation	horizontal	inclined	erect		
n =	121	21	51		
mean (s.d.), μm	14.9 (4.0) ^a	15.1 (3.2) ^a	21.1 (9.5) ^b		
range, μm	8.4-34.6	10.5-22.6	9.8-51.4		

above the split), but in this case no significant differences were found between some of the taxa where the leaf cell measurements were informative (Table 1).

Due to the difficulties with delimiting clear-cut states for quantitative characters, several taxa included in an analysis can be expected to be impossible to refer to either of two states. If larger numbers of specimens are studied for each taxon, statistical methods can be employed to separate states, and taxa (e.g. Vitt, 1980; Hedenäs & Kooijman, 1996 — habitat characters). However, this is often not possible if large numbers of species where little material is available have to be included, as is often the case in large-scale phylogenetic studies. Another approach is to use the maximum and minimum measurements for each included character (e.g. cell length), and to specify state-limiting values for both. Only taxa with both their maximum and minimum measurements exceeding or below the limiting values are referred to either state, whereas those having one value above and one below the respective limits cannot be referred to either category (Hedenäs, 1995). A weakness of this method is that the limiting values have to be set in a subjective way. The important thing is to recognize the uncertainties regarding the character state of many taxa, at the same time allowing the inclusion of information present in quantitative characters for other taxa.

Another large problem in pleurocarps is our still insufficient knowledge about what different organs actually are, or how they should be defined. Pseudoparaphyllia have received considerable attention in several modern papers (e.g. Ireland, 1971; Akiyama, 1990a, b; Nishimura & Matsui, 1990a, b; Akiyama & Nishimura, 1993). Although the differentiation between these and paraphyllia seems to cause few problems, paraphyllia being spread all over the stems whereas pseudoparaphyllia are located around branch primordia or branch bases, the delimitation between pseudoparaphyllia and proximal branch leaves has yet to be clarified in a satisfying way. Paraphyllia have most likely evolved several times among the pleurocarpous mosses, for example, in the Hylocomiaceae and the Thuidiaceae (cf. Hedenäs, 1995). Indeed, Goebel (1915) and Rohrer (1985) pointed out that there are differences in the ontogeny of paraphyllia in these two families. The use of axillary hairs in pleurocarp taxonomy was reviewed by Hedenäs (1990), who described the structure of these hairs in 200, mainly European species. Most axillary hairs are uniserial, but in *Meteoriumpolytrichum* Dozy & Molk.) one may find 7–22-celled, branched or partly biserrate axillary hairs. Although axillary hairs are usually inserted in the leaf axils, in many species, for example in the Hookeriales, they are inserted on the stem $\frac{1}{2}$ – $1\frac{1}{2}$ cells above the axil. In scattered specimens of some species (e.g. *Eurhynchiumasperipes* (Mitt.) Dix., *Vesiculariopsisspirifolium* (Dus.) Broth.) single axillary hairs may grow from the stem, seemingly lacking any connection with leaf axils, and in *Helodiumblandowii* (Web. & Mohr) Warnst., and sometimes in *Abietinellaabietina* (Hedw.) Fleisch. and *Palustriella decipiens* (De Not.) Ochyra, paraphyllia frequently have axillary hairs. The latter suggests that the paraphyllia are in some way homologous with leaves in these species. It thus seems that some stem-borne structures in pleurocarpous mosses may not always be strictly fixed in their origin, position, and perhaps function.

The potential for transformation of organs is perhaps best illustrated by the perichaetal branches. In addition to archegonia and calyptrae, all structures that can occur in normal vegetative stems can be found also in the perichaetal branches (also

with the strict definition of pleurocarpy of La Farge-England, 1996), although some organs are rare or strongly modified. Thus, perichaetal branches are almost certainly homologous with normal vegetative branches. In species having paraphyllia, these are often found also in the basal portions of the perichaetal branches (e.g. *Helodium blandowii*, *Pleuroziopsis rutenica* (Weinm.) Britt.), and the same is the case with rhizoids. Strongly modified paraphyllia can in addition sometimes be found intermixed with the paraphyses in *Thuidium tamariscinum* (Hedw.) B., S. & G., and rhizoids are sometimes found high in the perichaetal branches, often only in the uppermost part, for example in *Crossomitrium patrisiae* (Brid.) C. Müll., *Distichophyllum pulchellum* (C. Müll.) Mitt., *Helicoblepharum fuscidulum* (Mitt.) Broth. and *Racopilum spectabile* Reinw. & Hornsch. Sometimes the last species, and species such as *Conardia compacta* (Drumm.) Robins. have rhizoids also on the perichaetal leaves. Even branch primordia may occur in the basal parts of perichaetal branches, although this is probably relatively rare. I have seen it only in one perichaetal branch of *Anomodon longifolius* (Brid.) Hartm. and in one of *Warnstorffia exannulata* (B., S. & G.) Loeske (cf. La Farge-England, 1996). Axillary hairs of perichaetal leaves are usually rather similar to those of vegetative leaves, but are often more numerous per axil and/or consist of more cells. Above the uppermost perichaetal leaves, on the vaginula and sometimes on the lower calyptra, most species have similarly built hairs that usually consist of more cells, called paraphyses. The similar structure makes it likely that axillary hairs and paraphyses are homologous organs. Paraphyllia-like outgrowths that are unlikely to be homologous with paraphyllia in other species occur on the vaginula in some species, for example in the genus *Callicosta*. The perichaetal leaves are developed in a way that supposedly protects the archegonia, enhances their fertilization and/or protects the young sporophyte from desiccation and other adversities. Obviously this can be achieved in different ways, because the inner perichaetal leaves are shaped and oriented according to a few distinct patterns (Hedenäs, 1989). For example, the inner perichaetal leaves of members of the Brachytheciaceae and Hylocomiaceae species with horizontal capsules and perfect peristomes have more or less spreading upper parts which give the perichaeta a large exposed adaxial leaf surface. The inner perichaetal leaves of Amblystegiaceae species are mostly entirely erect, and those of the Hookeriales are small (markedly smaller than the vegetative leaves) and erect or from their base slightly directed outwards. Species growing in more exposed habitats, such as tree stems or rock surfaces, have erect inner perichaetal leaves, in this case usually correlated with so called 'erect' capsules (longitudinal axis of spore capsule parallel with seta) and specialized peristomes. That adaptation to the habitat is involved in the latter case can be seen when genera of relatively exposed habitats, such as *Neckera* and *Homalothecium* have quite differently shaped and oriented inner perichaetal leaves (and spore capsules) than the more plesiomorphic taxa of less exposed habitats in the same families (e.g. *Homalia* and *Brachythecium*, respectively). Thus, the appearance of the perichaetal leaves may be related either to the phylogenetic history of the taxa or to the habitat in which the species grow. Further insights into this area could be achieved by a development of models to explain the advantages or disadvantages for fertilization and desiccation protection with differently shaped and oriented perichaetal leaves, in relation to different habitat conditions.

A third great problem as regards character interpretation in the pleurocarps con-

cerns correlated adaptations in several characters. The perichaetal leaves and capsule orientation were already mentioned, but the most impressive examples are found in the peristomes. This has been discussed repeatedly in the literature (e.g. Grout, 1908; Vitt, 1981; Crum, 1972; Proctor, 1984; Shaw & Robinson, 1984; Buck, 1991; Hedenäs, 1995, 1997a), and most authors seem to agree that parallel adaptations to certain habitat conditions have caused the similar appearances of erect sporophytes. However, taxa above the species level are still frequently being circumscribed mainly based on their specialized peristomes (e.g. Florschütz-de Waard, 1992), or taxa are grouped mainly on general differences between specialized and unspecialized peristomes even if gametophyte characters do not always support this (e.g. Crosby, 1974; Buck & Vitt, 1986). Thus, for a better understanding of the relationships of taxa with specialized sporophytes there is still a need for research on many of these correlated character states and how they may have evolved.

Starting with spore size, we find an impressive variation among the pleurocarpous mosses, with median sizes between just under 10 μm and just above 50 μm among 200 studied species. Species with erect capsules have, on the average, significantly larger spores than those with inclined or horizontal capsules (Fig. 2, Table 1). The

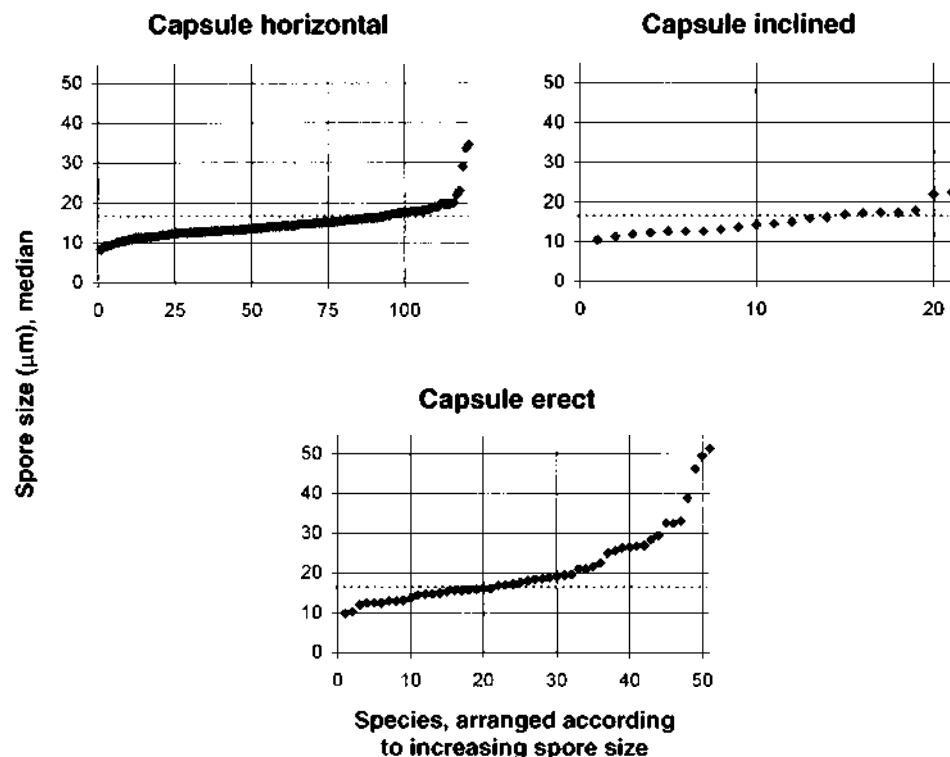


Figure 2. The spore sizes (median value for each species) in the species studied with horizontal (n = 121), inclined (21) and erect (longitudinal axis of capsule parallel with that of the seta) (51) capsules, respectively. The mean spore size, 16.5 (s.d. 6.4) μm , for the total set of studied species (n = 201, including species with cernuous to pendulous capsules) is indicated by dotted lines; the means for the respective categories are given in Table 1.

variation within each capsule orientation category is relatively large, but the average spore diameter is as much as 50% larger in the studied species with erect capsules than in those with other capsule orientations. In peristomes several specializations are correlated with capsule orientation, and we will here look at three relatively unambiguous features. The endostome cilia and basal membrane, and the trabeculae of the upper inside of the exostome teeth, tend to be reduced in species with 'erect' capsules (Fig. 3). Null hypotheses that there are no associations between capsule orientation and these peristome specializations can be rejected (Chi-square test, $p < 0.001$ for all three peristome characters). More interesting is the degree to which specializations in the characters occur together, and in which categories of capsule orientation this occurs. When species are grouped according to the states of the three peristome characters just discussed, none of those with long cilia, a high basal membrane and normal exostome trabeculae has erect capsules (Fig. 4). On the other hand, all species with short (or absent) cilia, a low or absent basal membrane and reduced exostome trabeculae have erect or, more rarely, inclined capsules. Eighteen of these species belong to the Hookeriales or the Sematophyllaceae, the others to taxa as taxonomically diverse as *Aerobryopsis*, *Entodon*, *Eumyurium*, *Garovaglia*, *Habrodon*, *Isodrepanium*, *Leptodon*, *Leucodon*, *Mesonodon*, *Oedipodium*, *Prionodon*,

Capsule orientation

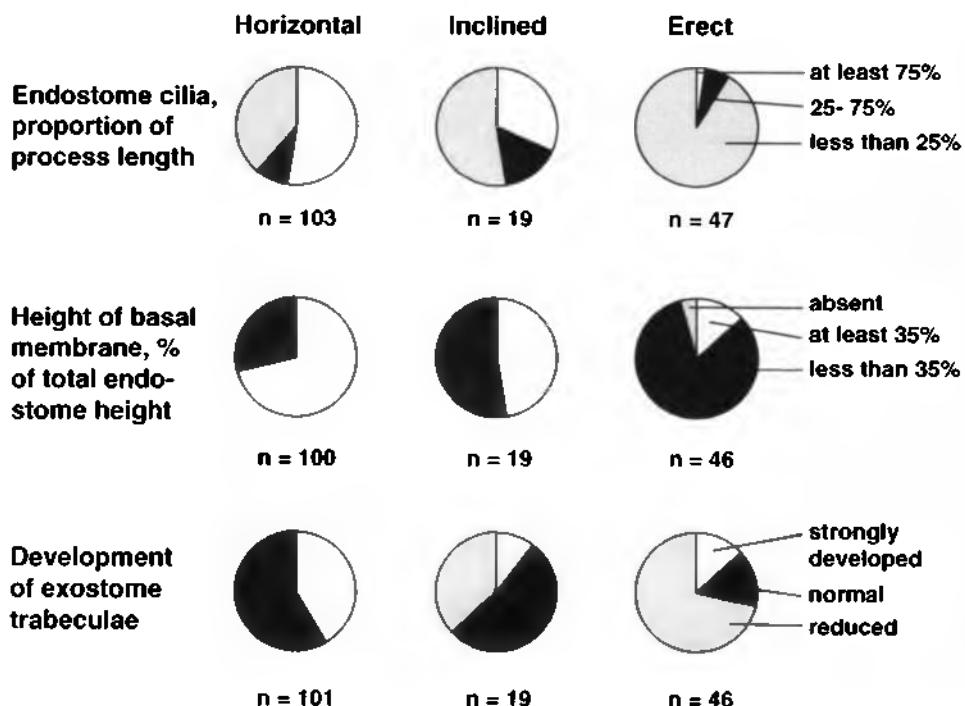


Figure 3. Endostome cilia length (proportion of process length), height of endostome basal membrane (proportion of total endostome height), and development of exostome trabeculae (see text) in relation to capsule orientation ('erect' = longitudinal axis of capsule parallel with that of the seta).

Pterogonium, *Rhynchostegium* (*R. cylindricheca* Dix.), *Rutenbergia*, *Scorpiurium*, *Struckia* and *Symphyodon*. This strongly supports the hypothesis that in a large number of cases these sporophyte character states and several other ones that are usually found together with them, such as 'peristomes inserted below the capsule mouth', lack of cross-striolate or reticulate ornamentation on the lower exostome outside, and narrow exostome teeth and endostome processes, have evolved together and in parallel in many taxa that are not closely related.

Explanations for the correlated specializations in several sporophytic and perichaetal features have been discussed by Grout (1908), Patterson (1953), Pais (1966), Vitt (1971, 1981), Crum (1972), Shaw & Robinson (1984), Mueller & Neumann (1988) and Buck (1991). Many species with 'erect' capsules are found as epiphytes or on more or less vertical rocks, but also on the ground along sea shores (e.g. *Sanionia orthothecoides* (Lindb.) Loeske) and in alpine or arctic regions (e.g. *S. nivalis* Hedenäs) similar adaptations are found. Of course, only species growing on the ground have truly erect capsules, whereas those of species growing on more or less vertical surfaces are horizontal, because the sporophytes are directed outwards from the substrate. Evidently, capsule orientation in itself cannot explain the observed differences between the peristomes and perichaeta. Instead, whether the species grow in habitats that are exposed to stronger winds or not and whether it is favourable to spread the spores under moist or dry conditions are more likely to be the decisive factors for the sporophyte adaptations discussed here. Ingold (1959) and Shaw & Robinson (1984) discussed possible mechanisms of spore dispersal in relation to the influence of wind, including the shaking of the sporophyte and the creation of vortices that may suck out the spores from the capsule, but further experimental studies of the kind performed by Delgadillo & Péres-Bandin (1982) are needed to evaluate this better. For a fuller understanding of different sporophyte adaptations, there is a clear need for experimental studies of species with differently developed sporophytes. In addition, mathematical calculations of the influence of winds of different strengths on differently developed sporophytes, using simplified models of the latter, would probably cast much light on which are the optimal structures under different conditions. Co-operation between bryologists and technical institutions dealing with air currents in relation to small structures would probably be most rewarding in this context. The relationship between hygrocastique (open during moist periods) and xerocastique peristomes and the habitat has been better evaluated (Patterson, 1953; Pais, 1966; Mueller & Neumann, 1988). Species with hygrocastique peristomes are mostly corticolous, meaning that the spores are likely to need moisture on the bark of the potential host trees to germinate. Thus, it should be advantageous to spread the spores under moist conditions. Pais (1966) showed that in arid areas of Africa no species with hygrocastique peristomes occur, whereas they seem to dominate in humid regions of tropical Africa. The structural background for the different peristome movements in relation to humidity is relatively well understood (Mueller & Neumann, 1988). However, for the interpretation of which peristome structures are homologous and which are analogous, structural differences between the peristomes in xerocastique and hygrocastique mosses that are not directly related to the peristome movement (but possibly still a result of the adaptation to a certain kind of habitat) should be further studied.

If all the individual sporophyte characters of the kind discussed above are included

in a phylogenetic analysis, without further evaluation of their ontogeny, detailed microstructure and function, they are likely to unite taxa that are unlikely to be closely related. This effect is due to the large number of sporophyte characters that obviously evolve together in a parallel way in quite unrelated taxa, and that thus show much congruence in the analyses. Even if the sporophyte specializations are summarized in one or a few characters, they occasionally tend to join taxa that seem to be unrelated for other reasons, such as *Aerobryopsis*, *Floribundaria*, *Prionodon*, *Pterogonium* and *Rutenbergia* placed in one clade in the study by Hedenäs (1995).

As a contrast to the above correlated peristome specializations, strongly developed exostome trabeculae (Fig. 4; Hedenäs 1994: Fig. 4), as well as a strongly furrowed or split outer exostome layer, occur mainly in the Hookeriales and the Sematophyllaceae. In these two taxa, short endostome cilia are also common, without any of the other states that accompany general peristome specializations in species with 'erect' capsules. All species with 'erect' capsules, and 40 out of 42 species with horizontal capsules that have strongly developed trabeculae, belong to these two taxa.

Despite many recent contributions on characters in pleurocarpous mosses, there are obviously many problems remaining. There is especially a need for large-scale

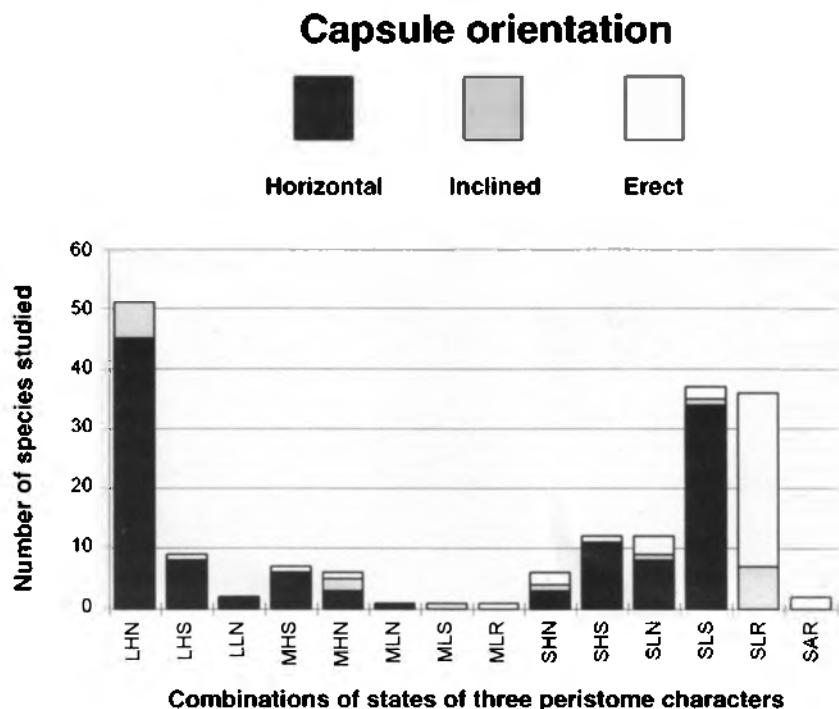


Figure 4. Combined states of three peristome characters (cf. Fig. 3) and their relation to capsule orientation. Under each bar the three letters indicate the states of the three characters as follows: Lower letter = 'endostome cilia length', L: long (at least 75% as long as processes), M: medium (25–75%), S: short (<25% as long as processes). Middle letter = 'endostome basal membrane height', H: high (at least 35% of endostome height), L: low (<35% of endostome height), A: absent. Upper letter = 'development of exostome trabeculae', N: normal, S: strongly developed, R: reduced (see text for explanations of the states of the last character).

comparative studies of the morphology, anatomy and ontogeny of many organs. Likewise, it is apparent that a better knowledge of the functional aspect of several characters would aid our understanding. These deficiencies in our knowledge should not prevent us from attempts to understand the relationships among the pleurocarpous mosses, but the hypotheses we generate today will be replaced by new ones once we have a better understanding of the characters on which they are based.

9.5 LARGE-SCALE CLADISTIC STUDIES, AND FUTURE EXPECTATIONS

At present, there exists only a handful of published cladistic analyses of pleurocarpous mosses (see legend of Fig. 5). Most were restricted to single genera, and it is not possible to draw general conclusions from these. To evaluate whether the degree of homoplasy in analyses of pleurocarpous mosses, as measured by the consistency index, differs from that in other organisms studied (Sanderson & Donoghue, 1989), I evaluated 16 analyses based on morphological data, some not yet published (Hedenäs, unpublished data; Hedenäs & Kooijman, unpublished data). The consistency indices for pleurocarpous mosses are in the lower part of the range that could be expected from the study of Sanderson & Donoghue (1989) (Fig. 5). Also, Hyvönen & Enroth (1994) remarked that the consistency index for their analysis of *Pinnatella* was slightly lower than what could be expected from Sanderson & Donoghue's (1989) figures. However, Sanderson & Donoghue (1989) included few

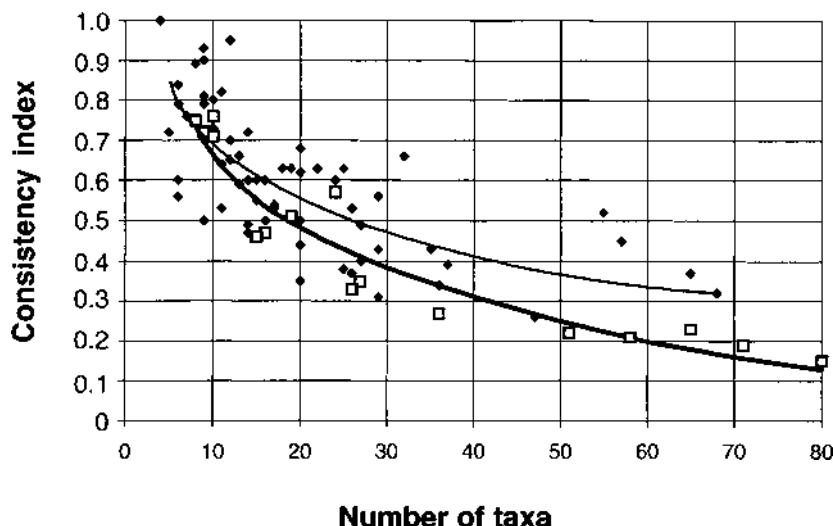


Figure 5. The relationship between the consistency index and the number of taxa in cladistic analyses. ◆ and thin curve = data from Sanderson & Donoghue (1989). □ and thick curve = data from 16 analyses of pleurocarpous mosses (Granzow-de la Cerda, 1992 (reanalysed by me with HENNIG86); Hyvönen & Enroth, 1994; Zomlefer, 1993 (best and worst Cl cited); Rohrer, 1985 (reanalysed for large and small sets of taxa by Hedenäs, 1995); Hedenäs, 1994, 1995, 1997a, b, and an unpublished analysis of the Hookeriaceae, one of the Callichistaceae, Hedenäs & Kooijman, 1996 (with 8 and 9 taxa), and an unpublished analysis at the species level in the same monophyletic Amblystegiaceae group).

analyses involving numerous taxa, so it is uncertain whether the low indices for the pleurocarp analyses are unusual, or a consequence of including large numbers of taxa. The analyses of the Pottiaceae by Zander (1993), which included between 70–80 terminal taxa, yielded consistency indices similar to those of the larger pleurocarp analyses evaluated here, so if the amount of homoplasy is really greater than for other organisms, this pattern may be valid for all mosses. If real, this difference could indicate several things. First, very similar, non-homologous states of characters could be more frequent in mosses than in other organisms. Alternatively we may not yet have reached a level of understanding of many structures in mosses comparable to that in, for instance, vascular plants. Second, reversals in, or oscillations between, states of characters could be relatively frequent in mosses. Third, due to the relative scarceness of unambiguous qualitative characters in mosses, bryologists use more quantitative characters than is common for students of other organisms. It is again evident that we need to spend more effort on clarifying which character states are truly homologous and which are the results of parallel evolution. On the other hand, if there are numerous homoplastic characters, and we cannot decrease their number by more intense character studies, they should not be excluded from the studies because we must base our evolutionary hypotheses on all available information. In addition, even if homoplastic characters cannot be used as strict criteria for membership of a group they are still often useful in group characterization (cf. Bremer & Struve, 1992).

The first results of large-scale analyses indicate that most of the diplolepidous pleurocarpous mosses form a monophyletic group (Hedenäs, 1994, 1995), united by their lack of differentiation into epidermis, guide cells and stereids in their leaf nerves. In other groups just outside this main group, a pleurocarpous organization may have evolved independently (Hedenäs, 1994). Within the large main group, the study by Hedenäs (1995) identified one clade comprising the Hookeriales, the Sematophyllaceae and a few representatives of other taxa. This supports the suggestions of Robinson (1971, 1975, 1986) of a closer relationship between these, rather than traditional ideas (cf. Buck & Vitt, 1986). Another clade identified in Hedenäs' (1995) study consists of the Plagiotheciaceae in the sense of Hedenäs (1987, 1989), rather than in the traditional sense or with the narrow circumscription of Buck & Ireland (1985). It is also suggested that families such as the Amblystegiaceae, the Rhytidaceae, some members of the Thuidiaceae, and temperate zone members of the Hypnaceae are closely related, something which was indicated also by Hedenäs (1989). *Brachythecium*-like sporophytes and perichaetia (in Brachytheciaceae, Hylocomiaceae, Hyocomiaceae, Ctenidiaceae, the relatives of *Heterocladium* in the Thuidiaceae) evolved at most a few times. Of the traditional orders, only the Hookeriales seems to be monophyletic, but a continued recognition of this order would necessitate raising the Sematophyllaceae and other less inclusive taxa of the Hypnales to the order level. The Isobryales apparently represents a basal grade among the pleurocarps, and is a paraphyletic group. These results may be a bit difficult to digest at first, but as indicated above, some reclassifications had been suggested by earlier authors. However, the earlier suggested changes were not based on all characters, but on relatively few characters that were either 'new' at that time, or for some reason considered more important than other ones ('key characters').

As taxonomic entities, the old Fleischer-Brotherus orders are not applicable in a

modern phylogenetic context, and they should thus be abandoned. However, it is still premature to suggest a new classification at the order level. Hypotheses about relationships generated by an overview study, such as the one described here, should be tested by studying other taxa and other, independent character sets, such as developmental, ultrastructural, chemical, and molecular data. Hopefully, in the end a consensus will be reached by comparing and combining the different data sets as achieved by Bremer & Jansen (1991) and Bremer & Struwe (1992) for vascular plants, by Mishler *et al.* (1994) for green algae and land plants, and by Tehler (1995) for a group of lichenized fungi and for fungi in general.

One of the main aims of our efforts to better understand the phylogenies of organisms, is to provide a basis for a more rational approach to many biological problems. With cladistic methods we can potentially generate hypotheses and classifications that are more predictive and more generally valid in biological theories than both intuitive classifications and classifications based on more restricted sets of features of the organisms, such as ecological guilds or life-history types. Thus, cladistically based classifications should also be the most generally meaningful ones for other areas of biology than taxonomy. For example, they are the most useful ones for those interested in exploiting and protecting bryophytes (*cf.* Mishler, 1995; Hedenäs, 1996). Phylogenetic knowledge is essential for a sound interpretation of different ecological and biological traits of species, such as habitat adaptations (e.g. Hedenäs & Kooijman, 1996), and life history traits (*cf.* Hedderson & Longton, 1995). Only with a good hypothesis regarding the evolutionary history of a group can we trace the evolution of character states, including non-morphological adaptations of different kinds, and hence understand which features are explained by the evolutionary history of the group, and which are newly evolved. Knowledge about the evolutionary history of a group helps explain some phytogeographic patterns, and may even contribute to the understanding of the geological history of the earth (*cf.* Humphries & Parenti, 1986; Forey *et al.*, 1992).

There is still a long way to go before we have enough knowledge about the pleurocarpous mosses to feel very sure about the detailed relationships of most taxa. There is especially a need for large-scale comparative studies of the morphology, anatomy and ontogeny of many organs. Likewise, it is apparent that a better knowledge of the functional aspects of several characters would aid our understanding. This is, however, no argument against analysing the data we already have in a rational way. Only in this way can we generate truly testable hypotheses regarding relationships, and reach the best possible hypotheses regarding how different characteristics of taxa have evolved, irrespective of whether these characteristics refer to morphology, anatomy, chemistry, habitat, life history or interactions with other species.

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10. Revised generic classification of the Orthotrichaceae based on a molecular phylogeny and comparative morphology

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10.1 SUMMARY

The Orthotrichaceae with nearly 600 species that are distributed among 27 genera, are one of the largest families of mosses. The current generic circumscription of the family has recently been the focus of morphological as well as molecular studies. The systematic implications of these investigations are summarized here and a new, annotated classification of the Orthotrichaceae based on the molecular phylogeny as well as on results from comparative morphology is presented. The Orthotrichaceae are divided into two subfamilies each composed of two tribes: the Zygodontae and Orthotrichae (Orthotrichoideae), and the Schlotheimiae and Macromitrieae (Macromitrioideae). Molecular or morphology-based phylogenies suggested that several genera of the Orthotrichaceae represent para- or polyphyletic assemblages. Consequently, *Macrocoma* subg. *Trachyphyllum* is here raised to the generic level as *Matteria gen. nov.*, *Bryodixonia* is synonymized with *Ulota*, and *Zygodon* is divided into three genera, including *Codonoblepharon* Schwaegr. and *Bryomalaea gen. nov.* A key to all genera of the Orthotrichaceae is presented.

KEYWORDS: Phylogeny, classification, key, Orthotrichaceae, systematics, mosses.

10.2 INTRODUCTION

One goal of phylogenetic studies is to elucidate trends in character evolution, in order to better understand the relationships among taxa sharing particular character-states. Since characters, morphological or otherwise, form the basis for arranging taxa in groups of higher ranks, classification should reflect the evolutionary history of the organisms under consideration (Donoghue & Cantino, 1988). Classification of the Bryopsida has historically relied on comparative morphological studies (see review in Vitt, 1984). With the advent of molecular techniques (see Hillis & Moritz, 1990), new sets of characters, *a priori* thought to occur in large numbers (but see Doyle, 1992), and for which homology is more easily established (see Wägele, 1995), have become available. Molecular studies on bryophytes, have used DNA nucleotide sequences primarily for addressing relationships of mosses to other green plants (e.g. Mishler *et al.*, 1992, 1994; Waters *et al.*, 1992; Manhart, 1994; Capesius, 1995; Krantz *et al.*, 1995; Bopp & Capesius, 1996; Hedderon, Chapman, and Rootes, 1996). The

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phylogeny of the Bryopsida (*sensu* Vitt, 1984) or the monophyly of orders or families of mosses has, however, drawn virtually no attention. Two studies have recently addressed specific systematic questions using nucleotide sequences of the chloroplast gene *rbcL* as well as data from comparative morphology: Withey (1996) investigated the phylogenetic affinities of the Spiridentaceae, whereas Goffinet (1997a) examined the circumscription and phylogeny of the Orthotrichales. A summary of morphological and molecular investigations on the systematics of the Orthotrichaceae (Goffinet, 1997a) is presented here and used as a framework for a revised generic classification of the family.

The Orthotrichaceae are a cosmopolitan family that with nearly 600 species (Vitt, 1982) are one of the most diverse families of mosses. Vitt (1984) included 21 genera in the family, which has since been expanded to 27 genera following the transfer of *Kleioweisiopsis* and *Uleastrum* from the Pottiaceae (Zander, 1993) as well as the establishment of *Stoneobryum* (Norris & Robinson, 1981), *Ceuthotheca* (Lewinsky-Haapasaari, 1994) and *Orthomitrium* (Lewinsky-Haapasaari & Crosby, 1996). Finally the genus *Amphidium*, a genus of gymnostomous taxa initially excluded from the Orthotrichaceae by Vitt (1973) has now been tentatively retained (Vitt, 1993) in the family based on similarities in the anatomy of the capsule between *Amphidium* and diplolepideous mosses (Lewinsky, 1976). Vitt (1972) divided the Orthotrichaceae into four subfamilies that are defined by the shape of the calyptrae, the direction of growth of the stem and the position of the perichaetia. Vitt (1982) hypothesized that the Zygodontioideae (Schimp.) Broth., defined by their cucullate calyptrae, were sister to all other subfamilies, including the Drummondioideae Vitt from which they differ by their orthotropic growth and their perichaetia that are terminal on the stem rather than on the branch. The Orthotrichoideae and the Macromitrioideae Broth. share mitrate and typically plicate calyptrae, but differ in the position of the female gametangia and the direction of caudine growth: the Orthotrichoideae are composed almost exclusively of acrocarpous orthotropic taxa, whereas the Macromitrioideae have been defined historically by creeping stems with lateral branches terminated by a perichaetium (Brotherus, 1925; Vitt, 1972). Unlike Brotherus (1925), Walther (1983) and Crum (1987), Vitt (1990) included *Desmotricha* Lindb. in the Macromitrioideae, rather than in a distinct subfamily, arguing that *Desmotricha* was clearly related to *Macromitrium*, albeit only distantly so in patristic terms.

The current circumscription of the Orthotrichaceae yields a family that *a priori* cannot be defined by a single synapomorphy due to its broad morphological variation (e.g. perichaetia terminal on the stem or on the branch, stems orthotropic or plagiotropic, caudine central strand differentiated or not, costal guide cells present or absent, basal laminal cells with straight or wavy walls, seta straight or twisted). This morphological heterogeneity may be best explained by the polyphyletic circumscription of the family. Critical examination of morphological characters led Goffinet (1997b, 1998) indeed to exclude the following genera from the Orthotrichaceae: *Kleioweisiopsis* (transferred to the Ditrichaceae), *Trigonodictyon* (Grimmiaceae), *Octogonella* and *Uleastrum* (Rhachitheciaeae). The Orthotrichaceae, now reduced to 22 genera (*Pleurozygodontopsis* being synonymized with *Zygodon*; Goffinet, 1998), still included two elements of controversy, namely *Amphidium* and *Drummondia*. Both genera are anomalous within the Orthotrichaceae by their median guide cells in the costa or the presence of a differentiated caudine central strand (Lewinsky,

1977, also reported a central strand in *Schlotheimia*, an observation that could not be confirmed based on the study of nearly 30 taxa, Goffinet, 1997a). Most gametophytic features are not conserved enough to be informative at higher levels (see Hedenäs, 1995) and the ordinal classification of mosses is therefore based primarily on peristomial features (Vitt, 1984; Vitt, Goffinet, & Hedderson, 1998). Within such a system, addressing the ordinal affinities of taxa with no peristome (e.g. *Amphidium*) or with a peristome that is reduced to a low membrane (e.g. *Drummondia*) or with atypical patterns of divisions (e.g. Rhachitheciaeae, Goffinet 1997b) is therefore virtually impossible unless unique gametophytic characters strongly support alternative affinities (e.g. *Uleastrum*, Goffinet, 1997b).

DNA sequences offer sets of characters that are independent from morphology and thus allow for an unbiased evaluation of morphology based systematic hypotheses. Their use in phylogenetic reconstructions often yields phylogenies conflicting with those obtained from morphology (Hillis, 1987), but these discrepancies are often just as striking as the incongruencies between different morphology-based phylogenies or different gene trees (Patterson, Williams, & Humphries, 1993). The first bryological application of gene sequence data to a systematic problem was presented by Waters *et al.* (1996) who used molecular characters derived from the nuclear gene 18S encoding for the small subunit rRNA to discriminate between two hypotheses regarding the ordinal affinities of the gymnostomous genus *Calommion* (see Vitt, 1995). Molecular characters thus appeared appropriate for addressing the relationship of *Amphidium* and *Drummondia* to the Orthotrichaceae. A cladistic analysis of sequences of the chloroplast gene *rbcL* from both taxa together with 35 representatives of the Orthotrichaceae and other major lineages of arthrodontous mosses, revealed that both *Amphidium* and *Drummondia*, are more closely related to haplolepideous mosses than to the Orthotrichaceae or other diplolepideous taxa (Goffinet, Bayer & Vitt, 1998). DNA sequence comparisons thus support Vitt's (1973) and Shaw's (1985) respective hypotheses that *Amphidium* and *Drummondia* should be excluded from the Orthotrichaceae.

The Orthotrichaceae are thus restricted to 20 genera that are now distributed among three subfamilies: the Orthotrichoideae, Zygodontioideae, and Macromitrioideae. Unlike Vitt (1982) who suggested that within a monophyletic Orthotrichaceae, the Orthotrichoideae are sister to the Macromitrioideae, Churchill & Linares (1995) felt that the Orthotrichoideae had strong affinities to the Zygodontioideae and did not share a common evolutionary history with the Macromitrioideae. They therefore proposed recognizing the Macromitrioideae as a distinct family, the Macromitriaceae S.P. Churchill. De Luna (1995), too, considered the Orthotrichaceae to represent an evolutionary grade. He suggested that the Orthotrichoideae and the Zygodontioideae form a monophyletic group with the Rhachitheciaeae, whereas the Macromitrioideae are more closely related to other cladocarpous taxa of the Orthotrichales (e.g. Erpodiaceae) with which they form a sister group to the Leucodontales. A cladistic analysis of *rbcL* sequences of 20 representatives of all three subfamilies of the Orthotrichaceae together with other arthrodontous mosses (Goffinet *et al.*, 1998), yields a phylogeny congruent with both Churchill & Linares' (1995) and De Luna's (1995) hypotheses regarding the monophyly of a clade including both the Orthotrichoideae and the Zygodontioideae. This analysis did not however support a polyphyletic origin of the Orthotrichaceae, as

explicitly suggested by De Luna (1995). The monophyly of the Orthotrichaceae was well supported in the neighbour-joining analysis, whereas only 15 out of 39 trees resulting from the parsimony analysis retained the Orthotrichaceae as monophyletic. Alternative most parsimonious trees suggested that the combined Orthotrichoideae and Zygodontioideae are sister to the Splachnales, but this hypothesis was rejected based on morphology (see Goffinet *et al.*, 1998).

The molecular phylogeny included representatives of ten genera of the Orthotrichaceae; for the remaining ten genera material was either not available or did not yield good quality DNA for the amplification of the *rbcL* gene. The affinities of these taxa, most of which are mono- or bispecific, were examined by comparative morphology. Although a formal cladistic analysis of characters was presented by Goffinet (1997a), many uncertainties remain with regards to the homology of the character-states used. The following annotated classification represents the first attempt in mosses to integrate results from a phylogenetic reconstruction using molecular data with those obtained from comparative morphology. The topology obtained from the cladistic analysis of the *rbcL* sequence data is taken as support for recognizing two major lineages within the Orthotrichaceae, whereas the generic circumscription is based mostly on morphological character-state distributions. The ancestor to the Orthotrichaceae is hypothesized to have had 1) thick-walled and smooth laminal cells differentiated into lower rectangular cells, and upper isodiametric cells, 2) terminal caudine perichaetia, and 3) a diplolepidous peristome with an exostome composed of 16 free teeth that were more heavily thickened on their OPL than on their PPL, and an endostome composed of 16 segments resulting from symmetric divisions, and laying alternate to the teeth. These hypotheses imply that the fusion of the exostome teeth into eight pairs arose independently in the Orthotrichoideae (including the Zygodontioideae *sensu* Brotherus 1925) and the Macromitrioideae, and that the development of papillae is homoplastic between taxa of the Zygodontae, Orthotrichae, and Macromitrieae.

10.3 CLASSIFICATION

ORTHOTRICHACEAE Arnott, Disp. Méth. esp. mousses: 13. 1825 (1826?, *fide* Margadant, 1968)

Zygodontaceae Schimp., Coroll. Bryol. Eur. 39. 1856; Type: *Zygodon* Hook. & Tayl.

Macromitriaceae S.P. Churchill, Bibl. J.J. Triana 12: 588. 1995, *syn. nov.* Type: *Macromitrium* Brid.

Plants pale or dark green to rusty brown, 1 to 10 cm tall. Stems erect or plagiotropic, branched sympodially or monopodially; outer cortical cells typically with very thick and coloured walls, parenchyma cells thick-walled and hyaline to yellowish, to rarely very thick-walled and orange-red in colour, central strand lacking. Rhizoids red, thick-walled, smooth to papillose, sparsely to abundantly branched. Axillary hairs with one or two differentiated basal brown cells, and one to several, hyaline, rectangular, thick-walled upper cells. Leaves erect, appressed or variously flexuose when dry, spreading when moist, spirally inserted or in five ranks, plane,

rugose to undulate, flat or keeled, ovate, oblong, lanceolate, to linear, apex acute, acuminate or obtuse, rarely retuse. *Costae* always present, single, reaching apex to excurrent, composed of two ventral guide cells, dorsal cells mostly differentiated into substereids, rarely undifferentiated and chlorophyllose; chlorophyllose cells covering the adaxial surface of the guide cells generally absent. *Basal laminal cells* differentiated, rectangular and hyaline to isodiametric and chlorophyllose, thick-walled, rarely nodose or porose, flat, smooth to papillose or tuberculate. *Upper laminal cells* thick-walled, isodiametric to short rectangular, rarely long rectangular, flat to bulging, smooth, unipapillose to pluripapillose, papillae clavate to conical, simple or bifid. Dioicous, monoicous (autoicous, rarely synoicous or paroicous), or phyllodiocous. *Perichaetia* terminal on stem or branches, paraphyses always present, long, linear, cells rectangular, apical cell pointed. Perichaetal leaves weakly to strongly differentiated, oblong-lanceolate to linear-lanceolate; costa percurrent to strongly excurrent and forming arista; basal cells differentiated and often extending far up the leaf, rectangular to rhombic, hyaline, smooth, tuberculate or prorate, upper cells chlorophyllose, isodiametric, smooth to papillose. *Perigonia* axillary or terminal, bud-like. Perigonial leaves strongly differentiated, short, concave; costa often weakly differentiated, ending well below the apex; basal cells rhombic, rather thin to moderately thick-walled, hyaline to yellowish; chlorophyllose cells restricted to apex. Monosetous, *setae* short or long, sinistrorse to dextrorse at the apex, outer cortical cells thick-walled, parenchyma cells thick-walled, central strand present to rarely lacking. *Vaginulae* glabrous to hairy, ochrea well developed or absent. *Capsules* immersed, emergent, to exserted, cylindric to globose, mouth wide or constricted with neck well differentiated to almost lacking. *Stomata* with two guard cells, in neck, lower or rarely upper portion of the capsule, pore elongate, phaneroporic to cryptoporic, and subsidiary cells weakly to strongly covering guard cells. *Exothecial cells*, rectangular, anticlinal and outer periclinal walls moderately to very thick, rarely strongly collenchymateous, inner periclinal wall moderately thick, cells often differentiated into longitudinal bands, forming ribs, cells at mouth short, isodiametric to oblate. *Annuli* typically absent or poorly differentiated. *Peristomes* double, simple or lacking. Prostomes of 64 cells present or absent, reduced to base of tooth, or if well developed caducous and peeling. Exostome teeth 16, free or fused into eight pairs, or forming continuous membrane, long acuminate to truncate, or even absent, erect, reflexed or recurved; OPL of 32 symmetric cells, heavily thickened, papillose, striate, reticulate or lamellate; PPL of 16 symmetric cells, moderately thickened on the exostome, here smooth, papillose, striate, rarely reticulate. Endostome segments, 16, 8 or lacking, inflexed, flat or keeled, lanceolate to linear, or truncate, free or fused into continuous membrane, connecting membrane one to three cells high, or lacking, cilia absent; PPL smooth, thin to very thin; IPL of 16 to 32 symmetric to strongly asymmetric cells, papillose to striate papillose, moderately thick. *Calyptrae* cucullate or mitrate, narrowly cylindric, broadly campanulate, to conic, covering the capsule completely or only the operculum, smooth to plicate, entire, lobate or moderately to deeply lacerate; outer tissue unistratose to tristratose, composed of thick-walled isodiametric to rectangular cells, outer cells smooth, papillose or prorate; hairs lacking or present, uniseriate to foliose, composed of thick-walled, smooth, papillose or prorate cells. *Operculi* flat to mostly conic, rostrum short to long, rarely absent, erect

or curved, straight or oblique. Exosporic or endosporic, spores granulose, reticulate or pitted, isomorphic to strongly dimorphic.

I. Orthotrichoideae

Zygodontoideae (Schimp.) Broth., Nat. Pfl. 11(2): 11. 1925. **syn. nov.**

Plants pale to deep green. *Stems* typically orthotropic, and sympodially branched, rarely plagiotropic and monopodially branched. *Costae* with or without chlorophyllose cells covering adaxial surface. Dioicus, monoicous (autoicous, rarely synoicous or paroicous), never phyllodioicus, acrocarpus, rarely cladocarpous. *Setae* dextrorse or sinistrorse. *Exostome* teeth free or fused, never reduced to membrane. *Endostome* segments free, connecting membrane present or absent, never reduced to a continuous membrane. *Calyptrae* cucullate or mitrate.

A. *Zygontaeae* (Schimp.) Goffinet¹ (see annotations below)

Basal laminal cells mono- or dimorphic, evenly thick-walled, rarely nodose, or porose. *Chlorophyllose laminal cells* flat, rarely bulging, papillae one to six, small clavate to coarse, simple to bifid at base, never bifid from a stalk. *Setae* dextrorse or sinistrorse. *Calyptrae* cucullate, entire, glabrous rarely hairy, smooth, covering upper half of capsule, with outer tissue bistratose, composed of thick-walled cells with oblate to rectangular lumens in transverse section, upper cells smooth or prorate.

1. *Codonoblepharon* Schwaegr.², Spec. Musc. Suppl. 2(1): 142. 1824.
2. *Bryomalaea* Goffinet³
3. *Leratia* Broth.⁴, Öfvers. Finska Vetensk.-Soc. Förh. 51A(17): 14. 1909.
4. *Zygodon* Hook. & Tayl., Musc. Brit. 70. 1818.
5. *Leptodontiopsis* Broth.⁵, Wiss. Ergebn. Deutsch. Zentr. Afr. Exp. 2: 146. 1910.
6. *Pleurorthotrichum* Broth.⁶, Öfvers. Finska Vetensk.-Akad. Handl. 47: 1. 1905.
7. *Stenomitrium* (Mitt.) Broth.⁷, Nat. Pfl. 1(3) 464. 1902.

B. *Orthotrichaeae*

Basal laminal cells monomorphic, evenly thick-walled to nodose, never porose. *Chlorophyllose laminal cells* flat, never bulging, papillae one or two, rarely more, conical, rarely small and clavate, simple to branched from a stalk. *Setae* sinistrorse. *Calyptrae* mitrate, entire to lobate, hairy, rarely glabrous, plicate, rarely smooth, covering capsule completely or only the upper half, or even only operculum, with outer tissue unistratose, composed of thick-walled cells with round lumens in transverse section.

1. *Orthotrichum* Hedw., Spec. Musc.: 162, 1801.
2. *Muelleriella* Dusén, Bot. Not. 1905: 304. 1905.
3. *Orthomitrium* Lewinsky-Haabasaari & Crosby, Novon 6: 2, 1996.
4. *Stoneobryum* Norris & Rob., Bryologist 84: 96. 1981.
5. *Ulota* Mohr⁸, Ann. Bot. 2: 540. 1806.

Bryodixonia Sainsb., Trans. Roy. Soc. New Zealand 75: 177. 1945. **syn. nov.**

II. Macromitrioideae Broth., Nat. Pfl. 11(2): 25. 1925.

Desmothecoideae Broth. ex Crum, Mem. New York Bot. Gard. 45: 603. 1987.
syn. nov. Pseudo-Macromitrioideae Broth., Nat. Pfl. 11(2): 49. 1925 (*nom. inv. art. 19.1*); Desmothecoideae Walther in Gerloff and Poelt, Engler's Syllabus der Pflanzenfamilien, vol. 2: 65, 1983. (*nom. inv. art. 32.1*)

Plants pale to deep green, to rusty brown. *Stems* plagiotropic, creeping to rarely pendulous, monopodially, rarely also sympodially branched. Lateral branches erect, sympodially branched. *Costae* without chlorophyllose cells covering adaxial surface. Phyllodioicous, rarely dioicous or monoicous (never synoicous or paroicous), cladocarpous, rarely also acrocarpous. *Setae* dextrorse. *Exostome* teeth free, fused into pairs, or teeth reduced to continuous membrane. *Endostome* free, connecting membrane present or absent, well developed to truncate or reduced to a continuous membrane. *Calyptae* mitrate, rarely cucullate.

A. Schlotheimiae Goffinet⁹

Plants deep green to rusty brown. *Basal laminal cells*, smooth or prorate, not tuberculate. *Upper laminal cells* always flat and smooth. Cladocarpous. *Calyptae* mitrate, smooth, cylindric to conic, base lobate, outer tissue bi- to tristratose composed of thick-walled cells with oblate to rectangular lumens in transverse section. *Peristomes* always well developed; exostome of 16 unfused teeth; endostome segments opposite or alternate to the exostome teeth.

1. *Schlotheimia* Brid., Sp. Musc. 2: 16. 1812.

B. Macromitrieae

Plants green to deep green. *Basal laminal cells*, smooth or tuberculate, not prorate. *Upper laminal cells* flat or bulging, smooth, uni- to pluripapillose, papillae simple or bifid at base. Cladocarpous, rarely also acrocarpous. *Calyptae* mitrate, rarely cucullate, plicate, rarely smooth, campanulate to conic, entire to deeply lacerate, outer tissue unistratose composed of thick-walled cells with round lumens in transverse section. *Peristomes* well developed or reduced, often reduced to membranes, exostome of 16 fused, rarely unfused, teeth; endostome segments alternate to the exostome teeth.

1. *Macromitrium* Brid., Mant. Musc.: 132. 1819.
2. *Desmotheca* Lindb., J. Lin. Soc. Bot. 13: 184. 1872.
3. *Ceuthotheca* Lewinsky-Haabasaari, Lindbergia, 19: 18, 1994.
4. *Leiomitrium* Mitt., Phil. Trans. Roy. Soc. Lond. 168: 390, 1879.
5. *Cardotiella* Vitt, J. Hattori Bot. Lab. 49: 101, 1981.
6. *Groutiella* Steere, Bryologist 53: 145. 1950.
7. *Macrocoma* (C. Müll.) Grout, Bryologist 47: 4, 1944.
8. *Matteria* Goffinet.¹⁰
9. *Florschuetziella* Vitt, Bryologist 82: 16, 1979.

10.4 ANNOTATIONS AND NOMENCLATURAL CHANGES

1. **Zygodontae** (Schimp.) Goffinet comb. nov.

Zygodontaceae Schimp., Coroll. Bryol. Eur. 39, 1856. Type: *Zygodon* Hook. & Tayl.

2. **Codonoblepharon** Schwaegr. Spec. Musc. Suppl. 2(1): 142, 1824.

Type: *C. menziesii* Schwaegr. Spec. Mus. Suppl. 2(1): 142, 1824. (the type material of *C. menziesii* has been lost in the mail, P. Geissler, pers. comm., but it is suspected that a duplicate of the original material was distributed, J. Lewinsky-Haapasaari, pers. comm.).

Zygodon Hook. & Tayl. sect. *Codonoblepharum* (Schwaegr.) C. Müll. Linnea 18: 669, 1845.

Thyridium Mitt. sect. *Codonoblepharum* (Schwaegr.) C. Müll. in Jaeg & Sauerb. Ber. S. Gall. Naturw. Ges. 1877–1878: 414, 1880 (Ad. 2: 678).

Zygodon sect. *Bryoides* Malta, Acta Univers. Latv. 6: 281, 1923. syn. nov.

Schwaegrichen (1824) included a single species in *Codonoblepharon* and defined the genus by the double peristome, composed of 16 paired, reflexed exostome teeth, and erect arcuate segments, unisexual plants with terminal capitulate gametangia, and cucullate calyptrae. Müller (1849, 1851) and Jaeger (in Jaeger & Sauerbeck, 1874) accepted Schwaegrichen's concept and included more double peristomate species in *Codonoblepharon* (as *Zygodon* sect. *Codonoblepharum* in Müller, 1849, 1851). Malta (1926) divided the genus *Zygodon* into four sections based on gametophytic characters (e.g. ornamentation of laminal cells). Section *Bryoides* Malta was defined by smooth laminal cells, and included species such as *Z. menziesii*. Although the latter is the type of the genus *Codonoblepharon*, and thus of *Zygodon* sect. *Codonoblepharum*, Malta refrained from using the name *Codonoblepharon* because Schwaegrichen (1824) defined this taxon by features of the peristome rather than gametophytic characters. Though the name *Codonoblepharon* was first used by Schwaegrichen (1824), the concept used here for the genus follows that of Malta (1926) for *Zygodon* sect. *Bryoides*. *Codonoblepharon* thus includes only species with smooth laminal cells; and of the eleven species included by Jaeger (in Jaeger & Sauerbeck, 1874) in *Codonoblepharon*, only two are retained here: *C. menziesii*; and *C. pungens* (C. Müll.) Jaeg. (Ber. St. Gall. Naturw. Ges. 1872–1873: 119, 1874). The type material of the following taxa has not been studied but it is presumed that these too, belong to *Codonoblepharon*:

Zygodon corralensis Lorentz, Bot. Zeit. 24: 187, 1866.

Z. gracillimus Fleisch., Musci Flo. Buitz. 2: 392, 1902–1904.

Z. humilis Thw. & Mitt., J. Lin. Soc. 13: 304, 1873.

Z. menziesii var. *angustifolius* Malta, Acta Univers. Latv. 10: 317, 1924.

Z. microtheca Dixon ex Malta, Acta Univers. Latv. 10: 315, 1924.

Z. minutus C. Müll. & Hampe, Linnea 28: 209, 1856.

Z. parvulus Geheeb & Hampe, Enumer. Muscorum brasiliens. 23, 1897.

Zygodon forsteri (Dicks.) Mitt. (Ann. Mag. of natur. hist. 2. ser. 8: 321, 1851) included by Malta (1926) in sect. *Bryoides*, may however not belong to *Codonoblepharon*. *Zygodon forsteri* is the only species of this group to occur in the northern temperate zone, whereas all other species are mostly distributed in subtropical to tropical regions or in the southern temperate zone (Malta, 1926). Thus,

Z. forsteri could have evolved from an ancestor belonging to *Zygodon sensu stricto*, and might have lost the papillae.

3. *Bryomaltaea* Goffinet gen. nov.

Zygodonti Hook. et Tayl. affinis. Plantae epiphytiae acrocarpae autoicae. Folia ligulata ovato-lanceolata obtusa erecto-appressifolia. Cellulae superiores subquadrate papillosae. Calyptrae cucullatae laeves glabrae. Peristomium duplex.

Type: *Bryomaltaea obtusifolia* (Hook.) Goffinet comb. nov.

Zygodon obtusifolius Hook. Musci Exo.: 159. 1820. Type: New Zealand; *Knight* (lectotype: BM!; isotype: H)

Zygodon sect. *Obtusifolii* Malta, Acta Univers. Latv. 6: 282. 1923.

Plants small, to 1.5 cm tall. Stems orthotropic, sympodially branched. Leaves erect-appressed, ovate-oblong, obtuse. Costa ending below apex, with two ventral guide cells, covered on the abaxial surface by rather short to isodiametric chlorophyllose cells. Basal cells weakly or not differentiated from upper laminal cells. Upper laminal cells isodiametric, thick-walled, strongly bulging, coarsely papillose, papillae two or three, coarse, bifid (or in pairs). Acrocarpous, autoicous, or dioicous. Perichaetal leaves weakly differentiated. Capsule exserted, sporogonium ribbed, stomata phaneroporic, in neck. Peristome double, alternate. Exostome of 16 teeth fused into eight pairs. Endostome of eight segments. Annulus none. Operculum conic, with a short oblique, rostrum. Calyptrae cucullate, smooth, with prorate cells at apex.

This new genus is named in honour of N. Malta for his monographic treatment of the genus *Zygodon* (Malta, 1926).

Bryomaltaea is easily distinguished from *Zygodon* by its erect appressed and obtuse leaves. One specimen from Thailand (Touw 8473, ALTA) differs from typical *B. obtusifolia* by being dioicous. Whether *Bryomaltaea* is indeed monospecific as suggested by Malta (1926, as *Zygodon* sect. *obtusifolius*) needs to be critically reexamined (see Lewinsky, 1990).

4. *Leratia* Broth.

Traditionally *Leratia* has been placed in the Macromitrioideae (Brotherus, 1925; as *Leratiella* Broth. & Syd.; Crum, 1987; Vitt, 1982) a relationship based on a 'Macromitrium-like peristome' with deep-set endostome and a short, and irregularly dissected membrane (Crum, 1987). While a relationship with the Orthotrichaceae has been argued against by Crum (1987; as Orthotrichoideae), affinities to the Zygodontaceae have never been hypothesized, even though *Leratia* has non-plicate cucullate calyptrae, reminiscent of *Zygodon* sensu lato.. *Leratia* may indeed be better considered closely related to *Bryomaltaea obtusifolia*. Both taxa share bulging laminal cells, that are ornamented with 'bifid to c-shaped' papillae (see Malta, 1926; Crum, 1987), chlorophyllose cells covering most of the abaxial surface of the costa, and prorate cells at the apex of the calyptrae (also seen in *Z. pungens*). A preperistome, as observed in *Leratia*, is also sometimes present in *B. obtusifolia* (see Lewinsky, 1990). *Leratia* differs from *Bryomaltaea* in the anatomy of the costa (the cells are strongly incrassate throughout), and by the smooth capsule (Goffinet, 1997a). *Bryomaltaea obtusifolia* is a widespread species known from Asia, Africa, South America, and Australasia (Lewinsky, 1990; Vitt, 1993). In Australasia, it is known from several localities in New Zealand, but only from one in Australia (Tasmania;

Lewinsky, 1990). *Bryomaltaea* is currently considered monospecific (Malta, 1926, as *Z. sect. Obtusifoliae*), but examination of populations from Thailand, Mexico, and Australasia reveals variation in peristome ornamentation (Goffinet unpubl.) that may be indicative of cladogenesis. The Australasian populations are considered conspecific (Lewinsky, 1990), and their disjunct distribution between New Zealand and Tasmania as well as their overall scattered distribution in New Zealand may suggest poor dispersal capabilities. Within such a scenario and considering that *Leratia* is endemic to New Caledonia, from where *B. obtusifolius* has not been reported yet (Pursell & Reese, 1982), it is easily conceivable that *Leratia* was derived from *B. obtusifolia* through long distance dispersal or represents a vicariant of the latter.

5. *Leptodontiopsis* Broth.

Leptodontiopsis was described by Brotherus for *L. fragilifolia* Broth., a species endemic to the high elevations in East Africa (Brotherus, 1910). *Leptodontiopsis* differs from *Zygodon* *sensu stricto* mainly by the smooth capsule, and dimorphic basal cells. *Leptodontiopsis* shares the latter character, referring to the presence of strongly incrassate cells forming distinct longitudinal bands alternating with hyaline, moderately thick-walled cells, with *Stenomitrium* and *Pleurorthotrichum*. Whether these three genera share a common ancestor needs to be addressed further; for now, *Leptodontiopsis* is better retained as a genus distinct from *Zygodon*, and with affinities to *Stenomitrium* and *Pleurorthotrichum*. Malta (1926) described *Zygodon fragilifolius* based on two collections from Mount Kilimanjaro and attributed the species to Brotherus. His description fits that of the type of *Leptodontiopsis fragilifolia*: both have fragile leaf apices, and dimorphic basal cells. I have not yet seen the type of *Z. fragilifolius* Broth. ex Malta, but if this specimen is identical to *Leptodontiopsis fragilifolia* Broth., the latter name would have priority, if *Leptodontiopsis* should be synonymized with *Zygodon*.

6. *Pleurorthotrichum* Broth.

Pleurorthotrichum chilense, easily distinguished within the Orthotrichaceae by the narrowly lanceolate and long acuminate perichaetial leaves (Lewinsky-Haapasaari, 1994), actually shares several character-states (including plesiomorphic ones) with the Zygodontioideae, and particularly *Stenomitrium*. The perichaetae are terminal on the stem, and not on short lateral branches as suggested by Lewinsky-Haapasaari (1994). When a perichaetium is formed, the stem resumes growth through a single lateral innovation immediately below the apex or up to a centimetre further down. This axis is clearly identifiable as a branch by the presence of juvenile leaves at its base (see La Farge-England, 1996). Not all branches are developed following a perichaetium formation on the main axis, but these appear to be basitonus, rather than acrotonous as in the Macromitrioideae. Male gametangia were unknown for *Pleurorthotrichum* and phylloidioicy (a character state hitherto known only from the Macromitrioideae) was suspected (Lewinsky-Haapasaari, 1994). Perigonia were found, however, in at least two collections (*Carl & Inga Skottsberg 211 — H-Br.*; *Mahu 10119 — H*), on plants monomorphic with the perichaetium bearing plants. The calyptra is cucullate and in transverse section reveals a similar cell outline as in other species of the Zygodontae. The hairs on the calyptrae, reminiscent of the Orthotrichae or Macromitrioideae, are not incompatible with the Zygodontae,

since they occur also in the African species, *Z. trichomitrius* Hook. & Wils., a species which also has differentiated perichaetial leaves (Malta, 1926). The chlorophyllose cells are distinctly papillose, and whereas the upper laminal cells bear only one or two papillae (see Lewinsky-Haapasaari, 1994) the proximal chlorophyllose cells are ornamented by up to six, albeit small, papillae, similar to most species of section *Zygodon*. The transfer of *Pleurorthotrichum* from the Orthotrichaceae to the Zygodontae is thus well justified based on morphological characters. *Pleurorthotrichum* differs from *Zygodon* by the long acuminate leaves, dimorphic basal cells, strongly differentiated perichaetial leaves, differentiated parenchyma cells surrounding the central strand in the setae, and the reduced endostome (see Goffinet, 1996).

7. *Stenomitrium* (Mitt.) Broth.

Stenomitrium shares with *Pleurorthotrichum* and *Leptodontiopsis* dimorphic basal cells: the proximal portion of the leaf is composed of alternating bands of clear, evenly thick-walled cells and cells with strongly incrassate, nodose to porose, yellow walls. Malta (1926) reports yellow-coloured basal cell walls from various Andean species of *Zygodon* (e.g. *Z. nivalis* Hampe), and nodose walls from a southern South American species, *Z. bartramoides* (Dusén) Malta. *Zygodon bartramoides* had been included in (section) *Stenomitrium* by Brotherus (1925), but excluded by Malta (1926). Both authors may have disagreed with regard to the affinities of *Z. bartramoides*, but both rejected recognizing *Stenomitrium* at the generic level because *Z. bartramoides* was seen as the obvious link between *Stenomitrium* and *Zygodon*, since it shared five-ranked leaves with the former and acrocarpy with the latter (Brotherus, 1925; Malta, 1926). Griffin (1990), also placed *Stenomitrium pentastichum* in *Zygodon*, but unlike previous authors, described *Z. bartramoides* as having creeping stems with erect branches. Material of *Z. bartramoides* was not available for study, but may be crucial in understanding the affinities of *Stenomitrium*. *Leptodontiopsis*, *Pleurorthotrichum*, and *Stenomitrium* each exhibit at least one unique character state that is *a priori* difficult to reconcile with the genus *Zygodon* or even with section *Zygodon* only. *Stenomitrium* also differs from *Zygodon* by the distal portion of the seta that is twisted to the left, a character *Stenomitrium* shares with *Pleurorthotrichum*.

8. *Ulota* Mohr

Ulota perichaetialis (Sainsb.) Goffinet comb. nov.

Bryodixonia perichaetialis Sainsb., Trans. Roy. Soc. New Zealand 75: 177. 1945.
Type: New Zealand, 'on bark of subalpine scrub, Mount Egmont, ca 4,000 feet; coll. G.O.K. Sainsbury, January 1945, no. 6005' (holotype: WELT!).

Bryodixonia, a monospecific genus endemic to New Zealand, is clearly related to the widespread genus *Ulota*, with which it shares differentiated marginal cells in the lower half of the lamina (i.e. cells with thick anticlinal walls), caudine parenchyma cells with very-thick, orange-red walls, and crisped to flexuose leaves. *Bryodixonia perichaetialis* is however easily distinguished from species of *Ulota* by the highly differentiated perichaetial leaves with prorate basal cells, the diminutive calyptra, the immersed capsule and the flat operculum (Sainsbury, 1945; Goffinet, 1997a). Since these characters all pertain to the perichaetium or the sporophyte it is not clear

to what extent these changes (compared to *Ulota*) are correlated or independent. The *rbcL* sequence divergence between *Bryodixonia* and two species of *Ulota* is similar to that found within other genera of the Orthotrichaceae (*Groutiella* or *Schlotheimia*; Goffinet, 1997a), suggesting that *B. perichaetialis* is an ingroup of *Ulota*. Furthermore, comparisons of *rbcL* sequences indicate that *B. perichaetialis* is phenetically closer to the Australasian *U. lutea* than to the North American-East Asian *U. obtusiuscula*. Molecular data thus *a priori* do not support segregating *Bryodixonia* at the generic level, and *B. perichaetialis* is at present best considered a patristically derived species within the genus *Ulota*.

9. *Schlotheimiaeae* Goffinet trib. nov.

Macromitreae affinis. Plantae epiphytiae, dioicae vel phyllo dioicae, cladocarpae. Calyptrae mitriformes laeves lobatae uni- vel pluristratosae. Peristomium duplex bene evolutum.

Type: *Schlotheimia* Brid.

A sister group relationship of *Schlotheimia* with regard to the other Macromitrioideae was strongly supported based on analyses of *rbcL* sequence data. Though this relationship was not supported in the most parsimonious scenario using morphological characters, *Schlotheimia* was the only large genus the monophyly of which withstood both analyses (Goffinet, 1997a). Morphologically the genus is well differentiated from the *Macromitrium*-complex (Vitt, 1993; Vitt, Koponen, & Norris, 1993), and the invariably well developed peristome as well as the lack of papillae on the upper leaf cells may suggest that *Schlotheimia* is the least derived genus in the Macromitrioideae.

10. *Matteria* Goffinet gen. nov.

Macrocomae (Broth.) Grout affinis. Plantae epiphytiae autoicae acro- et cladocarpae. Folia patula vel squarrosa. Cellulae costales adaxiales elongatae. Peristomium duplex. Sporae multicellulares.

Type: *Matteria gracillima* (Besch.) Goffinet

Macromitrium subg. *Trachyphyllum* Broth. in Engl. & Pr., Nat. Pfl. 1(3): 478, 1902. *Macrocoma* (Hornschr. ex. C. Müll.) Grout subg. *Trachyphyllum* (Broth.) Vitt, Bryologist 83: 433, 1980.

This new genus is named in honour of C. Matteri (BA) for her contributions to our knowledge of the South American moss flora.

a. *Matteria gracillima* (Besch.) Goffinet comb. nov.

Schlotheimia gracillima Besch., Bull. Soc. Bot. France 32: LXI. 1885. Type: 'Patagonie occidentale, île Wellington: Port Eden, 24 janvier 1879 (Dr. Savatier, no. 1838 e. p.)' (holotype—BM-Besch.).

Macromitrium gracillimum (Besch.) Broth. in Engl. & Pr., Nat. Pfl. 1(3): 478, 1902. *Macrocoma gracillima* (Besch.) Vitt, Bryologist 83 (4): 433, 1980.

b. *Matteria papillosa* (Thér.) Goffinet comb. nov.

Macromitrium papillosum Thér. in Herz., Arch. Esc. Farm. Fac., Cienc. Med. Cordoba 7: 50, 1939. Type: 'Prov. Chiloé, Dep. Llanquihé: Pétrohué, leg. C.C. Hosseus (n. 521)' (lectotype — PC-Thér.; isotype — J!).

Macrocoma papillosa (Thér.) Vitt, Bryologist 83 (4): 433, 1980.

In addition to the characters presented by Vitt (1980), *Matteria* (as *M.* subg.

Trachyphyllum) is easily distinguished from *Macrocoma* (as *M.* subg. *Macrocoma*) by the multicellular spores. The calyptra of *Matteria* is subentire (i.e. lacerations along the plications, are restricted to the basal portion of the calyptra) but has one major slit expanding beyond the middle. This situation has also been observed in species of *Macrocoma* (e.g. *M. orthotrichoides* Pedersen 13393 — ALTA). Whether the calyptrae are actually cucullate is difficult to ascertain, and more material is needed to clarify this. The cucullate nature of the calyptrae however may not be surprising considering that the rostrum is somewhat oblique and that in other genera of the Orthotrichaceae (e.g. *Zygodon*), cucullate calyptrae are often found in species with oblique rostra.

10.5 KEY TO THE GENERA OF THE ORTHOTRICHACEAE

1. Stems orthotropic, sympodially branched	2
1. Stems plagiotropic, monopodially branched, rarely also sympodially branched	13
2. Leaf laminal cells smooth	3
2. Leaf laminal cells papillose	6
3. Calyptrae cucullate, seta dextrorse below capsule, capsule exserted	
	<i>Codonoblepharon</i>
3. Calyptrae mitrate, seta sinistrorse or capsule immersed	4
4. Leaf lamina bistratose, capsule smooth, spores multicellular	
	<i>Muelleriella</i>
4. Leaf lamina unistratose, if bistratose, capsule ribbed and spores unicellular	5
5. Dorsal cells of costa linear, with pointed ends, leaves flexuose	
	<i>Stoneobryum</i>
5. Dorsal cells of costa irregularly rectangular, not linear, leaves erect appressed, rarely flexuose	
	<i>Orthotrichum</i>
6. Basal marginal cells of leaf differentiated from inner cells, hyaline, quadrate, with strongly thickened, horizontal anticlinal walls	
	<i>Ulota</i>
6. Basal marginal cells of leaf not differentiated from inner cells	7
7. Basal laminal cells dimorphic, strongly incrassate, nodose or porose, yellowish cells alternating with evenly thickened, hyaline cells	
	8
7. Basal laminal cells monomorphic, if nodose, then cells not forming alternating bands of yellowish and hyaline cells	
	9
8. Capsule smooth, long exserted, gymnostomous, perichaetial leaves weakly differentiated, endemic to high elevations in East Africa and Borneo	
	<i>Leptodontiopsis</i>
8. Capsule ribbed, emergent, peristome double, perichaetial leaves strongly differentiated, endemic to Central Chile	
	<i>Pleurorthotrichum</i>
9. Laminal cells strongly bulging	10
9. Laminal cells flat	11
10. Leaves green, short, ovate oblong, broadly obtuse, capsule ribbed	
	<i>Bryomaltaea</i>
10. Leaves orange-brown to green, ovate lanceolate, narrowly obtuse, capsule smooth	
	<i>Leratia</i>

11. Dorsal cells of costa linear, with pointed ends, leaves flexuose, calyptrae cuculate, seta dextrorse *Zygodon* 14
11. Dorsal cells of costa irregularly rectangular, not linear, leaves erect appressed, rarely flexuose, calyptrae mitrate, seta sinistrorse 12
12. Capsule globose, constricted at mouth, stomata cryptoporic, restricted to upper half of capsule *Orthomitrium*
12. Capsule cylindric to ovate, if globose, than not constricted at mouth, stomata phaneroporic or cryptoporic, restricted to the lower half of the capsule *Orthotrichum*
13. Calyptrae lobate at base 14
13. Calyptrae entire to lacerate 16
14. Basal marginal cells of leaf hyaline, quadrate, with strongly thickened, horizontal anticinal walls, forming multiseriate margin, calyptrae unistratose *Ulota*
14. Basal marginal cells of leaf not differentiated, or if differentiated, than not quadrate and not in several rows, calyptrae bi- to tristratose 15
15. Leaves decurrent, decurrencies composed of two to three rows of large, inflated hyaline, papillose to tuberculate cells, dioicous *Cardotiella*
15. Leaves not decurrent, and hyaline basal marginal cells not papillose or tuberculate, phyllodioicous *Schlotheimia*
16. Stem and branch leaves monomorphic (only shape is considered not the size) 17
16. Stem and branch leaves conspicuously dimorphic 21
17. Basal marginal cells hyaline, quadrate, with strongly thickened, horizontal anticinal walls, forming multiseriate margin, calyptrae unistratose *Ulota*
17. Basal marginal cells not differentiated, or if differentiated, then not quadrate and not in several rows, calyptrae bi- to tristratose 18
18. Abaxial layer of the costa composed of strongly bulging, papillose, isodiametric cells almost to the base *Florschuetziella*
18. Abaxial layer of costa composed of substereids (*i.e.* flat, smooth, linear cells) 19
19. Basal laminal cells rectangular, dimorphic, strongly incrassate, nodose, porose, yellowish cells alternating with evenly thickened hyaline cells, calyptrae cucullate *Stenomitrium*
19. Basal laminal cells, except for the most proximal ones, isodiametric, monomorphic 20
20. Leaves squarrose-recurved, to widely spreading, abaxial surface of costa composed of elongate cells into apex, spores multicellular *Matteria*
20. Leaves erect-appressed, abaxial surface of costa covered with isodiametric cells, spores unicellular *Macrocoma*
21. Marginal cells of leaf linear and hyaline, forming a distinct border at least in the lower third of the leaf *Groutiella*
21. Marginal cells of leaf not differentiated into a border of linear, hyaline cells 22
22. Leaf laminal cells smooth 23
22. Leaf laminal cells papillose 26
23. Leaf laminal cells flat *Schlotheimia*
23. Leaf laminal cells bulging 24

24. Perichaetial leaves aristate, capsule immersed	<i>Ceuthotheca</i>
24. Perichaetial leaves not with long excurrent costa, if aristate, capsule exserted	25
25. Leaves decurrent, decurrent tissue composed of two to three rows of large, inflated hyaline, papillose to tuberculate cells, basal cells not differentiated, short, isodiametric, oblate, chlorophyllose	<i>CardotIELLA</i>
25. Leaves not decurrent, if decurrent then cells linear and chlorophyllose, or decurrent tissue composed of a single row of hyaline, bulging cells, basal cells differentiated from upper cells, rectangular, hyaline	<i>Macromitrium</i>
26. Branch leaves of sterile and perichaetium-bearing branches dimorphic	<i>Desmotheca</i>
26. Branch leaves monomorphic	27
27. Basal laminal cells long rectangular, hyaline	<i>Macromitrium</i>
27. Basal laminal cells, except the most proximal ones, short, isodiametric to oblate, chlorophyllose	28
28. Leaves decurrent, decurrent tissue composed of two to three rows of large, inflated hyaline, papillose to tuberculate cells, basal cells not differentiated, short, isodiametric, oblate, chlorophyllose	<i>CardotIELLA</i>
28. Leaves not decurrent, if decurrent then cells linear and chlorophyllose, or decurrent tissue composed of a single row of hyaline, bulging cells, basal cells differentiated from upper cells, rectangular, hyaline	29
29. Capsule ribbed, dioicous	<i>Leiomitrium</i>
29. Capsule smooth, phyllodioicous	<i>Macromitrium</i> subg. <i>Cometium</i> Mitt.

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11. Genetic analysis of a hybrid zone in *Mielichhoferia* (Musc.)

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11.1 SUMMARY

Previous work demonstrated that the 'copper mosses' *Mielichhoferia elongata* and *M. mielichhoferiana* are genetically and morphologically distinct, but hybridize at five sites in Scandinavia and the United States where they grow in mixed populations. The present report describes genetic and morphological variation among 108 plants collected in a hybrid zone involving these two species at the Eureka Mine, San Juan County, Colorado. Allelic profiles at five polymorphic allozyme loci show that the relative proportions of *M. elongata*, *M. mielichhoferiana*, and recombinant gametophytes in the population are 19%, 58%, and 23%, respectively. The distribution of hybrid indices calculated from allozyme data suggest that genetically, recombinant gametophytes are skewed in the direction of the pure *M. mielichhoferiana* parental type. Morphological data are consistent with that interpretation; plants identified as recombinants based on allozyme data are intermediate between the parental species, but are closer (on average) to *M. mielichhoferiana* than to *M. elongata*. These observations suggest that primary recombinants derived from F₁ interspecific hybrids have backcrossed preferentially to *M. mielichhoferiana*. Alternatively, there may have been nonrandom mortality in the population, with *M. mielichhoferiana* types having a selective advantage. All three classes of gametophytes (*M. elongata*, *M. mielichhoferiana*, recombinants) form apparently functional archegonia and antheridia. In the population as a whole there was significant linkage disequilibrium (*D*) among all pairs of allozyme loci, suggesting that despite extensive hybridization, the two species remain genetically distinct. In contrast, a complete absence of linkage disequilibrium in the recombinants is attributable to recombination among the unlinked allozyme loci following interspecific hybridization. Sporophytes sampled from the population appear to belong exclusively to gametophytes of *M. elongata*. A testable hypothesis to explain the genetic and morphological patterns observed in this study is proposed: hybridization in the population is asymmetric with regard to male and female parentage, with *M. elongata* acting as the predominant female parent and *M. mielichhoferiana* as the predominant male parent. This hypothesis leads to the prediction that recombinant gametophytes contain nuclear genes from both parents but have maternally inherited chloroplast genes predominantly or exclusively from *M. elongata*. This prediction can be tested using molecular markers from the chloroplast and nuclear genomes.

KEYWORDS: Allozymes, hybridization, hybrid zones, introgression, linkage disequilibrium, *Mielichhoferia*.

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11.2 INTRODUCTION

The frequent occurrence of interspecific hybridization in vascular plants has long been recognized. Well-documented natural moss hybrids are, in contrast, relatively few and are mostly limited to crosses between taxa with immersed and exserted capsules because intermediate hybrid sporophytes can be readily identified. Anderson (1980) stressed that all hybrid mosses are, by definition, sporophytic. Gametophytes derived from such hybrid sporophytes are expected to display segregation and although they would contain genetic material from both parental species, they are not strictly comparable to vascular plant hybrids with a complete chromosome complement from each parent. In discussions that follow, *F₁ primary hybrids* refer to first generation hybrid sporophytes derived from interspecific crosses whereas gametophytes derived from such hybrid sporophytes are referred to as *recombinants*.

The long-term evolutionary significance of hybridization in plants has been debated. Stebbins (1959) argued that hybridization is important in plant evolution because it increases recombinational variation among the offspring of crosses between genetically differentiated taxa (or races with different adaptive norms). Recent molecular investigations have confirmed the common and widespread occurrence of hybridization in vascular plants (reviews in Arnold, 1992, 1994; Reiseberg, 1995; Reiseberg & Brunsfeld, 1992; Reiseberg & Wendel, 1993), but the *importance* of hybridization in plant evolution is a more difficult issue to resolve. Interspecific hybridization may produce increased levels of genetic variation as a result of both inter- (Lotsy, 1916) and intra-genic recombination (Golding & Strobeck, 1983) and such infusions of variation could promote general adaptation and even the ability to occupy completely new habitats. Hybrid zones may serve as barriers to gene flow between genetically differentiated taxa (Levin & Kerster, 1967; Barton & Hewitt, 1983) or, in the case of introgressive hybridization, may contribute to the merging of previously distinct species either locally (DePamphilis & Wyatt, 1990; Klier, Leoschke, & Wendel, 1991) or over the whole range of restricted taxa (Reiseberg *et al.*, 1989). Hybridization can also lead to the origin of new species, either with or without subsequent chromosome doubling (Grant, 1981; Reiseberg, 1995).

The evolutionary significance of interspecific hybridization depends on the pattern and extent of hybridization, including the ecological and reproductive characteristics of hybrid individuals and their offspring. To date, all cases in which spore viability has been assessed indicate that moss hybrids are evolutionary dead-ends; spore inviability prohibits the establishment of recombinant gametophyte progeny (Nicholson, 1905; Andrews & Hermann, 1959; Anderson & Lemmon, 1972; Rushing & Snider, 1985; Hedderson, 1986). (Reese and Lemmon, 1972, reported a low percentage of germinating spores from *Weissia* × *Astomum* hybrids, but mature gametophytes were not obtained.) Anderson & Lemmon (1972) attributed hybrid sterility to genic imbalances in crosses between *Astomum ludovicianum* (Sull.) Sull. and *Weissia controversa* Hedw. (*i.e.* chromosome pairing during meiosis in hybrid sporophytes appeared to be normal). In crosses between *Ditrichum pallidum* (Hedw.) Hampe and *Pleuridium acuminatum* Lindb., however, Anderson & Snider (1982) found evidence of chromosomal incompatibility evidenced by irregular pairing at meiosis.

Wyatt and coworkers (Wyatt *et al.*, 1988; Wyatt, Odrzykoski, & Stoneburner, 1992, 1993a, b) have recently provided allozyme evidence of allopolyploid origins for species in the genera *Plagiomnium* and *Rhizomnium*. Indeed, it may turn out that

contrary to earlier assumptions (e.g. Anderson, 1980; Smith, 1978a), many polyploid mosses may be allo- rather than autoploid in origin. This would appear to create a contradiction; hybrid speciation involving allopolyploidy may be important in mosses, yet all known naturally occurring moss hybrids are sterile. However, morphological detection of hybrid mosses is only possible in groups where related species differ substantially in sporophyte structure, a requirement that is met in few genera of acrocarps and even fewer of pleurocarps. Moreover, even when the sporophytes of congeneric species differ, hybridization is only one of several possible explanations for intermediate morphologies. In order to unambiguously identify hybrids, genetic markers are needed that are diagnostic, or at least partially diagnostic, for related species.

Genetic evidence of hybridization and the subsequent establishment of viable recombinant gametophytes has been provided for species of *Sphagnum* L. (Cronberg, 1994) and *Mielichhoferia* Hornsch. ex Nees, Hornsch. & Sturm. (Shaw, 1994). Patterns of morphological variation in several species complexes within *Sphagnum* are consistent with hybridization followed by segregation among recombinant gametophytes. Indeed, the apparently noncorrelated patterns of variation in multiple 'diagnostic' traits distinguishing closely related species are exactly those expected from hybridization, segregation, and clonal proliferation of particular recombinant types. Allozyme evidence suggests that hybridization between *S. capillifolium* (Ehrh.) Hedw. and *S. rubellum* Wils. occurs at least occasionally when the two grow sympatrically (Cronberg, 1994). Similarly, interspecific recombinants between sympatric *Mielichhoferia elongata* and *M. mielichhoferiana* have been identified using allozyme markers that are diagnostic for the species in all allopatric populations (Shaw, 1994).

The present paper describes a genetic analysis of *Mielichhoferia* plants from one of the five populations where *M. elongata* and *M. mielichhoferiana* are known to hybridize and produce viable recombinants (Shaw, 1994). In addition to diagnostic allozyme loci used to identify recombinants in the previous more extensive (less intensive) study, three additional loci (*Pgm1-3*) were utilized in this study to identify plants in the population as *M. elongata*, *M. mielichhoferiana*, or recombinants. The following specific questions were addressed. 1. Are recombinant gametophytes, identified by genetic markers, morphologically intermediate between the parental taxa? 2. Are recombinant gametophytes, on average, midway between the parental taxa with regard to genetic markers? That is, are recombinants likely to be first generation gametophytic progeny formed by F_1 hybrid sporophytes (in which case they should, on average, be midway between the parental species), or are they more likely to be later generation offspring produced by reproduction involving primary recombinants? 3. Do recombinant gametophytes form functional gametangia; that is, do they at least show the potential for reproductive success? 4. Is there genetic or morphological evidence that *M. elongata* and *M. mielichhoferiana* are merging at this site because of interspecific hybridization?

11.3 MATERIALS AND METHODS

11.3.1 The collection site and plant sampling

The site from which plants were sampled for allozyme analyses was referred to as EUM in Shaw (1994). The site is a narrow, shaded ravine (Eureka Gulch) at the

south end of extensive tailings from the (abandoned) Eureka Silver Mine, San Juan Co., Colorado. The elevation is 3,200 m (10,500 ft). Two soil samples from the gulch had pHs of 3.12 and 4.53; both had elevated levels of 'available' copper (70.7 and 21.1 ppm), and one sample had a relatively high concentration of aluminum (Shaw & Owens, 1995). One small population of *Mielichhoferia macrocarpa* (Hook. ex Drumm.) Bruch & Schimp. ex Jaeg. & Sauerb. occurs on the underside of overhanging rocks in the gulch, but *M. elongata* and *M. mielichhoferiana* are more abundant on the vertical sides, especially along the south wall. (The gulch is so narrow that north- and south-facing sides are ecologically similar.)

The Eureka Gulch empties eastward into the wide glacial valley of the Anamas River approximately 9.7 km (six miles) north-east of Silverton. The mountains on either side of the valley reach 3,660–4,115 m (12,000–13,500 ft) elevation, and are dotted with abandoned silver and gold mines throughout the area. The 'ghost town' of Eureka is represented by a few buildings in the Anamas River valley near the site of the Eureka Mine. Other populations of *M. elongata* occur at at least three other abandoned mine sites in the valley, and additional sites for the species are known from adjacent valleys in San Juan Co. (Hartmann, 1969; Shaw & Schneider, 1994). *Mielichhoferia mielichhoferiana* is not known from any other site in Colorado, nor in fact from any other site in the United States south of Alaska. Plants from Burns Gulch, on the opposite (east) side of the Anamas River valley from the Eureka Gulch, appear somewhat intermediate in morphology between *M. elongata* and *M. mielichhoferiana*, but genetic analyses have not been completed.

One hundred and twenty-two samples of *M. elongata* and/or *M. mielichhoferiana* were collected from the Eureka Gulch during August 1995. Each sample was approximately 1 cm² and consisted of 5–20 stems. Sampling was haphazard, but collections were made along a rough transect extending from the east-facing rock wall just outside (and to the north) of the entrance to Eureka Gulch, into the gulch, and along the south wall. Plants representing the two species are especially abundant along the south wall, forming small (e.g. 5 cm²) to larger (e.g. 1 m² or larger) patches.

11.3.2 Morphological and allozyme analyses

The samples were returned to the laboratory and refrigerated. Before they were extracted for the allozyme analyses, ten stems were removed for morphological measurements and assessments of reproductive condition. For each of the ten stems, the presence and numbers of perichaetial and perigonial buds were recorded, and four of the largest leaves were removed for morphological measurements. The lengths and maximum widths of the four leaves were measured under a compound microscope, and the lengths and widths of two upper median cells were measured on three of the leaves. The lengths of the longest marginal teeth on either side of the leaf were measured on the same three leaves. Mean values for these multiple measurements per sample were used in subsequent statistical analyses. Comparable leaf and cell measurements were made on 20 additional plants that bore sporophytes. The latter were not included in the genetic analyses, and attempts at culturing spores from these field-collected sporophytes were unsuccessful.

The remaining plants from each sample were extracted for allozyme analyses. Extraction, electrophoresis, and staining of allozymes were conducted according to

the protocols described in Shaw (1994). Previous work (Shaw, 1994; Shaw & Schneider, 1994) showed that banding patterns for two enzymes, PGI and MDH, are diagnostic for *M. elongata* and *M. mielichhoferiana* in all allopatric populations from both Europe and North America. In previous work, the three bands for MDH were interpreted as three independent loci; in the present study, however, a more conservative interpretation of one MDH locus was employed. As previous work at the EUM site showed that two additional enzymes, G-3-PDH and PGM, are polymorphic, these systems were also stained in the present study. Only one individual was found to contain the rare *G-3-pdh* allele that was detected previously and this locus will not be considered further here.

Three bands were expressed for PGM (hereafter, *Pgm1*, *Pgm2*, and *Pgm3*, as in previous papers). It was apparent that two three-locus banding patterns predominated in the population, and that these patterns were correlated with alternative alleles at the *Pgi* and *Mdh* loci that are universally diagnostic for *M. elongata* and *M. mielichhoferiana*. Outside the EUM site, one of these two PGM banding patterns is fixed in *M. mielichhoferiana*; *M. elongata* is polymorphic for two of the three *Pgm* loci. Because of strong associations between alleles at the three *Pgm* loci at the EUM site (confirmed statistically; see below), and also because these three loci showed strong associations with alleles at the diagnostic *Pgi* and *Mdh* loci, *Pgm* was considered to be a reliable marker for distinguishing *M. elongata* and *M. mielichhoferiana*. There were therefore five informative allozyme loci for identifying plants at the EUM site as *M. elongata*, *M. mielichhoferiana*, or recombinant.

11.3.3 Statistical analyses

Samples were classified as *M. elongata*, *M. mielichhoferiana*, or recombinants on the basis of their allelic profiles at the diagnostic (*Pgi*, *Mdh*) and partially diagnostic (*Pgm1-3*) loci (Table 1). Some recombinants could, by the random processes of segregation and independent assortment in hybrid sporophytes, have the parental genotypes for the five allozyme loci. Thus, the estimated frequency of recombinants in the population is a minimum estimate. To evaluate the degree of interspecific mixing of plants, all samples were assigned a 'hybrid index' based on the presence of alleles characteristic of *M. elongata* or *M. mielichhoferiana*. At a particular locus, a zero was assigned for an allele characteristic of *M. elongata* and a one for an allele characteristic of *M. mielichhoferiana*. The hybrid index for each sample was the mean of the values for the five loci, and therefore ranged from zero (pure *M. elongata*) to one (pure *M. mielichhoferiana*). Recombinants had hybrid indices of 0.2, 0.4, 0.6, or 0.8.

Direct estimates of linkage disequilibrium (*D*), a measure of the nonrandom association of alleles at two loci, between all pairwise combinations of loci were calculated from allele frequencies at each locus. *D* is defined as $(p_1q_1 \times p_2q_2) - (p_1q_2 \times p_2q_1)$ where p_1q_1 is the frequency of the two-locus genotype with allele 1 at loci p and q, p_2q_2 is the frequency of the genotype having allele 2 at both loci, and p_1q_2 and p_2q_1 are the alternative combinations of alleles (Hartl & Clark, 1989). Linkage disequilibrium refers to the associations of alleles in gametes (i.e. haploids) and must be estimated indirectly from data on diploids. Maximum likelihood approaches are typically employed to estimate *D* in diploids since one cannot simply count up the

contributions of different gametic types (Barton & Gale, 1993). In contrast, mosses have the advantage that D can be computed directly from allozyme data, and uncertainty in the estimate is associated only with sampling sufficient plants to get sound estimates of allele frequencies in the population.

The maximum possible value for linkage disequilibrium in a population is constrained by allele frequencies at the two loci. The largest possible value, 0.25, occurs when allele frequencies at both loci are 0.5. Linkage disequilibrium is therefore sometimes expressed as D/D_{\max} where D_{\max} is the maximum value D can have given the allele frequencies at the two loci in the population. Both D and D_{\max} were computed for plants at the EUM site. Separate estimates were obtained for the population as a whole, including both the pure species and recombinants, and for the recombinants alone. Statistical significance of linkage disequilibrium was evaluated using chi square tests for association of alleles.

Morphological and reproductive variation among plants at the site was evaluated by computing mean values for each class of plants (*i.e.* *M. elongata*, *M. mielichhoferiana*, recombinants). Significant differences in morphological traits among the classes were assessed using analyses of variance (ANOVAs) for each trait separately.

11.4 RESULTS

11.4.1 Allozyme analyses

Ten samples yielded expression of two bands for one or more loci that typically expressed only one (*e.g.* two *Pgi* bands, or >3 *Pgm* bands). These samples were interpreted as mixtures of more than one genetic individual and were excluded from subsequent analyses. Four additional samples were excluded because allozyme data were unavailable for one of the loci. The data set on which the following results were obtained therefore contained 108 samples.

Based on allelic profiles, 20 plants (19%) were identified as pure *M. elongata*, 63 (58%) as pure *M. mielichhoferiana*, and 25 (23%) as interspecific recombinants. Eleven different multilocus genotypes were identified among the recombinants (Table 1). The mean hybrid index for the recombinants in the population was 0.64, closer to pure *M. mielichhoferiana* than to *M. elongata*. This bias in the direction of the *M. mielichhoferiana* parental type is reflected in the distribution of hybrid indices in the population (Fig. 1). If the recombinants were derived exclusively from F_1 hybrid sporophytes and there has been no differential mortality among plants with differing degrees of relatedness to the two parental taxa, we would expect approximately four out of the 25 to have a hybrid index of 0.2, four to have an index of 0.8, and eight each to have indices of 0.4 and 0.6 (with a mean index for the population of recombinants as a whole of 0.5). The distribution of hybrid indices is not consistent with that expected distribution ($G = 34.51$, $P \leq 0.01$). Evidently, the current relative frequency of different classes of recombinants is the result of more complex patterns of reproduction and/or differential survival.

There was significant linkage disequilibrium (at $P \leq 0.05$) among all pairwise combinations of the five allozyme loci in the population as a whole (Table 2). Values for D/D_{\max} show that nonrandom associations among alleles at different loci are

Table 1. Allelic profiles at 5 allozyme loci for 13 multilocus genotypes identified in the hybrid zone at the Eureka Mine.

	<i>Mdh</i>	<i>Pgi</i>	<i>Pgm1</i>	<i>Pgm2</i>	<i>Pgm3</i>
<i>M. elongata</i>	c	c	a	b	e
<i>M. mielichhoferiana</i>	b	d	b	e	b
Recombinants	c	c	a	e	b
	c	c	b	b	b
	c	d	b	b	b
	b	c	a	b	e
	b	d	a	b	e
	b	d	b	e	e
	b	c	a	b	e
	b	d	a	e	b
	b	d	b	b	b
	b	c	b	b	b

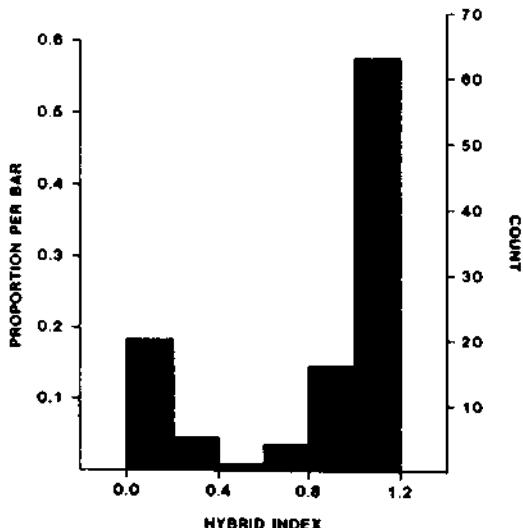


Figure 1. Histogram showing the distribution of 'hybrid indices' for 108 samples of *Mielichhoferia* from the Eureka Mine, computed from allelic profiles at five isozyme loci. Plants with a hybrid index of 0 (shown as a bar spanning the range from 0.0 to 0.2) are pure *M. elongata*; those with an index of 1 (shown as a bar spanning the range from 1.0 to 1.2) are pure *M. mielichhoferiana*; intermediate indices represent interspecific recombinants. See text for additional explanation of how hybrid indices were computed.

very strong. D/D_{\max} values range from 56% for *Pgi* and *Pgm2* to 87% for *Pgm1* and *Pgm3* (Table 2). In contrast to the population as a whole, when comparable calculations were made for just the recombinants, there was no evidence of linkage disequilibrium among any pair of loci (data not shown). These results suggest that the five allozyme loci are unlinked, and that recombination during meiosis in hybrid sporophytes broke down associations that did exist because of species differences.

Table 2. Linkage disequilibrium between five allozyme loci in the Eureka Mine hybrid zone. Linkage disequilibrium values (D) are given above the diagonal; below the diagonal values are presented for D/D_{max} (see text for additional explanation of calculations).

	<i>Mdh</i>	<i>Pgi</i>	<i>Pgm-1</i>	<i>Pgm-2</i>	<i>Pgm-3</i>
<i>Mdh</i>	XXX	0.1428	0.1450	0.1447	0.1401
<i>Pgi</i>	0.8100	XXX	0.1365	0.1171	0.1333
<i>Pgm-1</i>	0.8219	0.7321	XXX	0.1544	0.1542
<i>Pgm-2</i>	0.8184	0.5627	0.7470	XXX	0.1305
<i>Pgm-3</i>	0.7924	0.7561	0.8722	0.7381	XXX

11.4.2 Morphological analyses

The two 'pure' species, identified by their allelic profiles, differ significantly in leaf and cell length and width, as well as in the ratios of leaf and cell length/width (= 'leaf shape') computed from the primary data (Table 3). Although *M. mielichhoferiana* tends to have larger marginal teeth on the leaves, the difference was not significant. For leaf length, leaf width, and leaf shape (length/width), the recombinants are intermediate between *M. elongata* and *M. mielichhoferiana* but are closer to the latter (Table 3). For cell width, the recombinants are virtually identical to the *M. mielichhoferiana* parental type. For cell length, in contrast, the recombinants are virtually identical to the *M. elongata* parental type. Because of these opposite patterns, the recombinants are almost exactly midway between the parental types in cell shape (Table 3).

The data are not sufficient to determine if recombinant plants with different degrees of relatedness to the two parental species (i.e. hybrid indices of 0.2, 0.4, 0.6, or 0.8) show correlated morphological similarities to one or the other parental type. There was too much variation within each class of recombinants, as well as in the parental species. In fact, there was no evidence that the recombinants as a class were more

Table 3. Means (\pm standard errors) for morphological traits in Eureka Mine plants of *Mielichhoferia elongata*, *M. mielichhoferiana*, and recombinant gametophytes. Identifications are based on allelic profiles at five allozyme loci (*Mdh*, *Pgi*, *Pgm-1*, *Pgm-2*, *Pgm-3*). * = differences between *M. elongata* and *M. mielichhoferiana* significant at $P \leq 0.05$; ** = $P \leq 0.001$.

Trait	<i>M. elongata</i>	<i>M. mielichhoferiana</i>	Recombinants
Leaf length**	566.15 ± 20.85	$1,151.81 \pm 28.36$	$1,018.82 \pm 46.68$
Leaf width**	249.55 ± 7.85	309.33 ± 5.66	302.25 ± 9.33
Leaf shape**	2.30 ± 0.09	3.76 ± 0.08	3.42 ± 0.15
Cell length*	65.17 ± 2.49	72.00 ± 1.41	65.95 ± 1.96
Cell width**	12.59 ± 0.32	10.25 ± 0.17	10.78 ± 0.30
Cell shape**	5.32 ± 0.23	7.22 ± 0.17	6.48 ± 0.30
Tooth length	6.82 ± 0.18	7.31 ± 0.20	7.18 ± 0.24
Frequency of male stems	0.045 ± 0.017	0.071 ± 0.019	0.185 ± 0.035
Number of perigonia per male plant	0.675 ± 0.363	0.479 ± 0.122	0.598 ± 0.194
Frequency of female stems	0.085 ± 0.044	0.039 ± 0.014	0.014 ± 0.011
Number of perichaetia per female plant	0.382 ± 0.187	0.178 ± 0.053	0.083 ± 0.057

(or less) variable than either parental species. ANOVAs done on coefficients of variation for each morphological trait did not indicate any significant differences between *M. elongata*, *M. mielichhoferiana*, or recombinants, except in cell length, for which *M. mielichhoferiana* was more variable than *M. elongata* and recombinants (analyses not shown).

Twenty plants bearing mature sporophytes collected from the hybrid zone had the general appearance of *M. elongata* (relatively soft texture, lighter green colour). They also matched *M. elongata* in leaf shape, although in cell shape they fell more within the range of *M. mielichhoferiana* (Fig. 2). The cells of perichaetial leaves of most bryaceous species are longer and narrower than those of vegetative leaves, and it is likely that the plants bearing sporophytes belong to *M. elongata* (or possibly recombinants).

Mielichhoferia elongata, *M. mielichhoferiana*, and the recombinants formed perichaeta and perigonia. The recombinants had the highest frequency of male stems but the lowest frequency of female stems (Table 3). Although the frequency of male stems in *M. elongata* was only about 25% of the frequency in recombinants, each male plant of *M. elongata* had (on average) as many perigonia. With regard to females, the recombinants not only had a lower frequency of female stems, but each female stem also contained (on average) only about 20% as many perichaeta as *M. elongata*. (*M. mielichhoferiana* was intermediate in this regard.) Compared to *M. mielichhoferiana*, *M. elongata* formed more male stems, more perigonia per male stem, more female stems, and more perichaeta per female stem (Table 3).

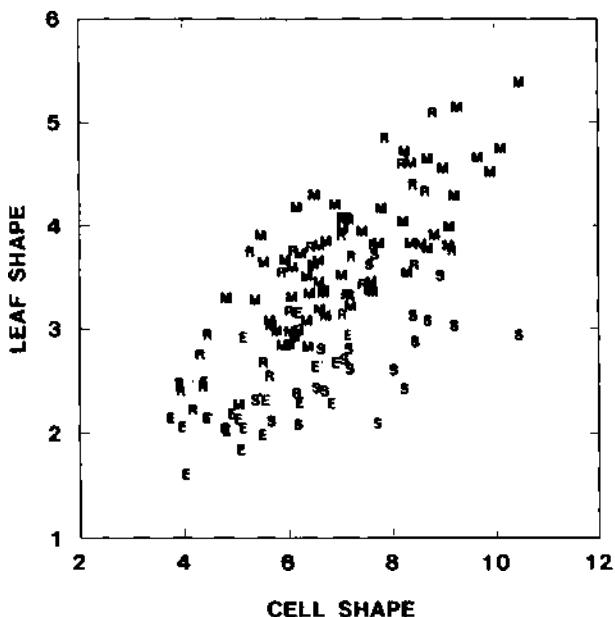


Figure 2. Scatter diagram showing variation among plants of *Mielichhoferia* from the Eureka Mine in relation to leaf shape (length/width) and leaf cell shape (length/width). E, M, and R = pure *M. elongata*, pure *M. mielichhoferiana*, and recombinants, respectively, based on isozyme profiles. S = gametophytes bearing sporophytes (genetic data not available).

11.5 DISCUSSION

The recombinant gametophytes in the Eureka Mine hybrid zone are morphologically intermediate between *M. elongata* and *M. mielichhoferiana*. For several of the morphological traits measured, however, they are not midway between the parental species. The expression of morphological traits in hybrid sporophytes and recombinant gametophytes depends on a number of factors. Perhaps most important is the genetic control of particular traits. Polygenic traits can be expected to display intermediate expression in both diploids and haploids whereas traits governed by one or two genes are more likely to show parental expression (Reiseberg & Ellstrand, 1993). In F_1 hybrid sporophytes, the expression of traits controlled by one or two genes for which there is strong dominance will be like that of one or the other parent whereas traits governed by genes without dominance are more likely to yield intermediate expression. Dominance does not, of course, affect the expression of genes in haploid gametophytes, but patterns of intergenic interactions can yield intermediate, parental, or extreme (transgressive) expression. In a survey of morphological patterns in natural and experimental hybrids of flowering plants, Reiseberg & Ellstrand (1993) found that hybrids are no more likely to display intermediate morphological expression than parental ones.

Experimentally produced hybrid moss sporophytes generally have intermediate morphology, but may be more similar to one of the two parental species (Wettstein 1923). In crosses between *Physcomitrium pyriforme* (Hedw.) Hampe and *Funaria hygrometrica* Hedw. sporophyte morphology, including peristomial structure, appears to be biased in the direction of the maternal parent. Naturally occurring hybrids among several species of *Weissia* also tend to display morphologies that are biased toward the maternal parent (Nicholson, 1905, 1906). These patterns suggest complex inheritance that includes maternal effects. Such biased patterns could result from nongenetic maternal effects on developing sporophytes, non-nuclear genetic factors, and/or nuclear-cytoplasmic interactions. Complex interactions of genetic and nongenetic effects are also possible (Montalvo & Shaw, 1994).

In the absence of epistatic interactions between nonallelic genes, we would expect the mean value for a particular trait in a population of gametophyte progeny produced by an F_1 hybrid sporophyte to be midway between the parental means. Indeed, departures from this expectation can be used as evidence for nonadditive gene action (*i.e.* epistasis; Burnett, 1975). At the Eureka Mine, it is clear that the mean of recombinants does not fall midway between the parental species for several morphological traits.

A morphological bias in the direction of *M. mielichhoferiana* could reflect a genetic bias among recombinants in that direction, or, even if the recombinants are genetically intermediate between the two parents, complex patterns of gene expression could result in non-intermediate phenotypes (*cf.* above). The distribution of hybrid indices, however, shows that the population of recombinants are in fact more genetically similar to *M. mielichhoferiana* than *M. elongata*. There are two possible explanations for the genetic bias toward *M. mielichhoferiana*.

One possibility is that all recombinants are progeny of F_1 hybrid sporophytes, but differential mortality among gametophytes of varying genetic make-up has

resulted in a skewed distribution toward *M. mielichhoferiana* among survivors. Support for this hypothesis comes from the observation that *M. mielichhoferiana* is more abundant than either *M. elongata* or recombinants in the population. If the greater abundance of *M. mielichhoferiana* reflects a higher level of fitness in the environment of the Eureka Gulch, recombinants that (by chance, during meiosis in hybrid sporophytes) inherit a greater genetic similarity to *M. mielichhoferiana* are at a selective advantage over those closer to the *M. elongata* parental type. On the other hand, although *M. mielichhoferiana* is more abundant than *M. elongata* in the Eureka Gulch, gametophytes of *M. mielichhoferiana* display a lower reproductive capacity in terms of gametangial production by both male and female stems than do those of *M. elongata*. While survival ability and the capacity to proliferate asexually may not be correlated with levels of gametangial formation, this observation does not provide support for differential mortality as an explanation of the skewed distribution of recombinants toward *M. mielichhoferiana*.

The second possible explanation is that recombinant gametophytes in the Eureka Gulch are not solely the offspring of F_1 hybrid sporophytes. If primary recombinants produced by F_1 sporophytes survive, form gametangia, and successfully parent sporophytes, they are most likely to mate with *M. mielichhoferiana* gametophytes because of the numerical abundance of this species relative to *M. elongata* at the site. Preferential backcrossing between recombinants and *M. mielichhoferiana* would produce the pattern of genetic relatedness observed among recombinants at the Eureka Mine. Moreover, the fact that recombinants form both perichaetia and perigonia shows that they are reproductively viable and could be involved in subsequent matings within the population.

Two observations must be reconciled with the latter hypothesis. All sporophytes collected to date at the Eureka Mine appear to be attached to gametophytes of *M. elongata*. Although the gametophytes could be recombinants (which can be difficult to distinguish by morphology alone), they are unlikely to be pure *M. mielichhoferiana*. Nevertheless, *M. mielichhoferiana* must be involved in crosses, and if recombinants backcross more commonly to *M. mielichhoferiana* than to *M. elongata*, the former must be commonly involved in matings.

We can propose a testable hypothesis to explain this apparent discrepancy. *Mielichhoferia mielichhoferiana* is often or always the paternal parent in crosses, either with *M. elongata* or recombinants. First generation recombinants produced by F_1 hybrid sporophytes tend to cross predominantly with *M. mielichhoferiana*, but because it acts as the paternal parent, sporophytes are always attached to recombinants. Similar asymmetries have been reported in other cases of interspecific hybridization in mosses. In eastern North America, *Weissia controversa* is the paternal parent in all reported hybrids with species of *Astomum* (*A. ludovicianum* — Reese & Lemmon, 1965; *A. muhlenbergianum* (Sw.) Grout — Williams, 1966; Anderson & Lemmon, 1972; Anderson & Snider, 1982). Interestingly, in Great Britain, however, *Weissia controversa* is reported to act as both maternal and paternal parent in crosses (Smith, 1978b). It is noteworthy that recombinant gametophytes at the Eureka Mine have few male stems, or perigonia per male stem, compared to *M. mielichhoferiana* (or *M. elongata*). Thus matings between

recombinants and *M. mielichhoferiana* are most likely to involve recombinants as the maternal gametophyte and *M. mielichhoferiana* as the paternal parent.

The hypothesis that *M. mielichhoferiana* is the recurrent paternal parent in crosses at the Eureka Mine can be tested using a combination of nuclear and cytoplasmic genetic markers. If this scenario is true, recombinants would primarily have maternally inherited chloroplast DNA markers from *M. elongata*, but would have a mixture of nuclear markers from both parental species. Comparable analyses have been used to assess patterns of interspecific gene flow in the angiosperm genus *Iris* (Arnold, Buckner, & Robinson, 1991).

Despite evidence of substantial hybridization between *M. elongata* and *M. mielichhoferiana* at the Eureka Mine (at least 1 out of 4 plants is a recombinant), the two species do not appear to be merging at the site. Strong linkage disequilibrium still exists among five allozyme markers characteristic of the two species, showing that *M. elongata* and *M. mielichhoferiana* have maintained significant genetic integrity. Moreover, statistically significant morphological differentiation between the species persists at the site. What factors maintain the integrity of these species in the face of hybridization? One possibility is that hybridization at the site is a relatively recent phenomenon, and that eventually the two will merge, at least locally. This would be significant, especially for *M. mielichhoferiana*, as the Eureka Mine is the only known North American site for this species south of Canada. Another possibility is that the recombinants are in fact all derived from F₁ hybrid sporophytes, and that although these primary recombinants are abundant at the site, they have not backcrossed to either parental species. If the genetic makeup of the recombinants is skewed toward *M. mielichhoferiana* because of differential mortality rather than recurrent matings with *M. mielichhoferiana*, there may be no reason to expect the two parental species to merge.

This work raises a number of questions that are presently being pursued. Chloroplast and nuclear DNA markers are being developed to test hypotheses about mating patterns in the population. The relative fitness of *M. elongata*, *M. mielichhoferiana*, and recombinants in the Eureka Gulch environment are needed to test differential survival as an explanation for the skewed genotypic mix found at the site. If localized introgression (interspecific gene flow because of backcrossing between recombinants and one or both parental species) is occurring within the hybrid zone at the Eureka Mine, has there also been movement of *M. mielichhoferiana* genetic material into seemingly pure populations of *M. elongata* elsewhere in and near the Anamas River valley? If spores or gametophyte fragments from recombinants have dispersed into other established populations of *M. elongata*, there could be step by step movement of *M. mielichhoferiana* genes into that species.

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12. Auxin structure-activity relationships in *Physcomitrella patens*

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12.1 SUMMARY

Auxin structure-activity relationships are significantly different in *Physcomitrella patens* (Hedw.) B.S.G. from those which characterize higher plants. Some differences observed in preliminary experiments derive from photodegradation of test substances in the culture medium. However, other results, obtained under conditions which protect auxins and their structural analogues from photodegradation, cannot be accounted for in this way. The order of activity of tested substances is the same when culture media with an initial pH of approximately 5 or approximately 7 are used. However, *P. patens* exhibits higher sensitivity to each substance at the lower pH. Similar results are obtained using wild type and *P. patens* mutants which are partially repairable by exogenous auxin. Mutants originally isolated selectively through their resistance to naphth-1-ylacetic acid (*NAR* mutants) are resistant to indol-3-ylacetic acid also. This finding supports the contention that naphth-1-ylacetic acid and indol-3-ylacetic acid act in *P. patens* through mechanisms which are, at least in part, shared.

KEYWORDS: Auxin, structure-activity relationships, mutants, *Physcomitrella*.

12.2 ABBREVIATIONS

2,4-D = 2,4-dichlorophenoxyacetic acid; (+)2,4-DP = D(+)-2-(2,4-dichlorophenoxy)-propionic acid; (-)2,4-DP = L(-)-2-(2,4-dichlorophenoxy)propionic acid; 2,4-DP = racemic mixture of (+)2,4-DP and (-)2,4-DP; IAA = indol-3-ylacetic acid; EtIA = ethyl indol-3-ylacetate; IBA = 4-(indol-3-yl)butyric acid; IPA = 3-(indol-3-yl)propionic acid; NAA = naphth-1-ylacetic acid; 2-NAA = naphth-2-ylacetic acid; NoA = naphth-2-yloxyacetic acid; PAA = phenylacetic acid; HOP = 4-hydroxyphenylacetic acid; PoA = phenoxyacetic acid; BA = 6-benzyladenine; ABP = auxin binding protein; abs = subcellular auxin binding site(s); D = darkness; ER = endoplasmic reticulum; PAR = photosynthetically active radiation; WL = white light; RL = red light.

12.3 INTRODUCTION

Since Kögl, Haagen-Smit & Erxleben (1934) identified a substance, isolated from human urine, which had identical diffusion and plant growth promoting properties to those of a native auxin obtained from grass coleoptile tips, as indol-3-ylacetic acid, numerous compounds with various degrees of structural relatedness to IAA have been tested for biological activity. In almost every case, auxin activity has been assessed by measuring, directly or indirectly, the effect of the test substance upon the elongation or expansion of various cells of flowering plants. Using the results of activity studies, several attempts have been made to develop a comprehensive theory of the relationship between biological activity and molecular structure for the multitude of substances investigated (see Stoddart & Venis, 1980, and references therein). This has led to predictions about the structure of hypothetical auxin receptors, postulated to mediate physiological responses to auxins, and about the nature of binding forces between auxins and auxin receptors. These ideas in turn have been useful during the development of assay systems designed to detect subcellular auxin binding sites (Hertel, Thomson & Russo, 1972) and auxin binding proteins (Napier & Venis, 1995). Additionally, a predicted correlation between the magnitude of the hormonal activity of a compound and its affinity for genuine auxin receptors, has constituted a major criterion for assessing the physiological relevance of abs and ABPs (Ray, Dohrmann & Hertel, 1977).

Despite the far-reaching significance of auxin activity studies and in contrast to the numerous and extensive investigations with higher plant material, there are few reports of similar studies using eukaryotic chlorophyllous cryptogams, and then only a very small number of compounds has been tested. Such an omission cannot be justified on the grounds that auxins have no role in these plants since there is ample evidence that auxins are involved in regulating the morphogenesis of cryptogams including bryophytes (Cove & Ashton, 1984) and endogenous IAA has been detected in several of them including *Marchantia polymorpha* (Schneider, Troxler & Voth, 1967), *Pellia epiphylla* (Thomas *et al.*, 1983) and *Physcomitrella patens* (Ashton *et al.*, 1985). Furthermore, mosses possess auxin influx and efflux apparatuses (Geier, Werner & Bopp, 1990; Ashton *et al.*, 1990a) as well as ER abs (Reichert, Ashton & Ray, 1982) which resemble closely the corresponding elements of higher plants.

In this paper, I report the relative effectiveness of some auxins and structurally related substances in promoting particular developmental changes, believed to be induced normally, *i.e.* in the absence of exogenous auxin, in wild type (normal) *P. patens* gametophytes by endogenous auxin(s) (Ashton & Cove, 1990; Ashton *et al.*, 1990b; Ashton, Zacharias & Fracchia, 1993). Developmentally normal strains and developmentally abnormal mutants, which are partially repairable with exogenous auxins, were used in the tests.

The existence of *P. patens* mutants, which in some cases are phenotypically repairable with the synthetic auxin, naphth-1-ylacetic acid and in other cases are insensitive to NAA (*NAR* strains) (Ashton, Grimsley & Cove, 1979; Ashton & Cove, 1990), makes this plant an attractive model system for investigations of the biosynthesis of endogenous auxin(s) and the molecular mode of auxin action. Initially, however, the identity of the endogenous auxin(s) of this moss needs to be ascertained and it should be established also that mutants repairable by, or resistant to, NAA are also

repairable by, or resistant to the auxin(s) present in the wild type. Although the identity of the major auxin in *P. patens* is currently unknown, the detection of IAA in gametophytes of this plant (Ashton *et al.*, 1985) has strengthened the candidacy of this substance, or a derivative of it, for this role. In this paper, data are presented which demonstrate that mutants which are repairable by, or resistant to, NAA are also repairable by, or resistant to, IAA.

12.4 MATERIALS AND METHODS

12.4.1 Plant material

Origins of *P. patens* strains used in this study are provided in Table 1. The combined information in the first two columns of this table constitutes the full haploid gametophytic genotypes of the strains listed. For simplicity, throughout this paper, strains are referred to by their partial genotypes, *i.e.* strain codes. The *BAR* strains, except *BAR 1*, were selected, following mutagenesis, by their resistance to the effects of the exogenously supplied cytokinin, 6-benzyladenine. *BAR 1* was isolated non-selectively and later shown to be cytokinin-resistant. Subsequently, the *BAR* mutants were found to be phenotypically repairable or partially repairable by exogenous NAA. The *NAR* strains were isolated by their resistance to exogenous NAA. All strains possessing a *thi-1* or *pabA3* allele require exogenous thiamine or *p*-aminobenzoic acid for growth.

Table 1. Origins of *P. patens* strains.

Code	Strain	Isolated from	Mutagen	Origin Isolation method	Selective reagent	Reference
<i>BAR 1</i>	<i>thi-1</i>		EMS	Non-selective	none	Ashton <i>et al.</i> , 1979
<i>BAR 281</i>	<i>pabA3</i>		NTG	Selective	BA	—
<i>BAR 471</i>	<i>pabA3</i>		NTG	Selective	BA	N. H. Grimsley pers. comm.
<i>NAR 34</i>	Wild type		NTG	Selective	NAA	—
<i>NAR 69</i>	<i>thi-1</i>		NTG	Selective	NAA	—
<i>NAR 87</i>	<i>thi-1</i>		NTG	Selective	NAA	Ashton <i>et al.</i> , 1979
<i>NAR 91</i>	<i>thi-1</i>		NTG	Selective	NAA	Ashton <i>et al.</i> , 1979
<i>NAR 112</i>	<i>pabA3</i>		NTG	Selective	NAA	Ashton <i>et al.</i> , 1979
<i>NAR 113</i>	<i>pabA3</i>		NTG	Selective	NAA	Ashton <i>et al.</i> , 1979
<i>NAR 185</i>	<i>pabA3</i>		NTG	Selective	NAA	—
<i>thi-1</i>	Wild type		EMS	Selective	none	Engel, 1969
<i>pabA3</i>	Wild type		NTG	Non-selective	none	Ashton and Cove, 1977
Wild type				From a single spore from a plant collected in Gransden Wood, Huntingdonshire		H. L. K. Whitehouse pers. comm.

12.4.2 Culture media and supplements

The standard ABC *P. patens* culture medium (Knight *et al.*, 1988) containing 1.8 µM *p*-aminobenzoic acid, 1.5 µM thiamine HCl and 0.5% (w/v) sucrose was used in this study with some minor modifications. The pH of the medium was adjusted to 7.3 (medium 1) or 6.2 (medium 2) before addition of 1.5% (w/v) Disco Bacto Agar. After autoclaving, the solidified media had a pH of approximately 6.9 or 5.3 respectively.

Ethanoic solutions of each auxin and auxin analogue were prepared at appropriate concentrations and diluted, 1 in 2000, into autoclaved and cooled culture medium just before pouring the medium into plastic Petri plates, approximately 50 ml per plate. The concentrations of the auxins and analogues given in the Results and Discussion sections refer to the concentrations of the compounds in the culture medium. The concentration of ethanol in all media, including the control medium, was 0.05% (v/v).

EtIA had no effect upon medium pH. Addition of the other auxins and analogues had no or little effect depending upon their concentration: at 50 µM, the largest effect upon the pH of medium 1 was a reduction of less than 0.1 of a pH unit. At 50 µM, the compounds assayed in medium 2 reduced the pH of this medium by 0.1 to 0.2 of a pH unit. When present in medium 2 at a concentration of 5 µM, 2,4-D, the strongest acid among the substances assayed in this medium, reduced the medium pH by less than 0.1 of a pH unit.

Care was taken, during the preparation of solutions and media containing auxins and auxin analogues and while inoculating the plates with moss, not to expose the solutions or media to strong light of any kind.

12.4.3 Culture conditions

Gametophytes were grown at 22–25°C under continuous light supplied by cool-white fluorescent tubes (Westinghouse, Regina, SK, Canada). For white light, either no filter was used or the Petri plates were covered with one layer of clear (Roscolux, No. 114, Hamburg frost; MacPhon Industries, Calgary, AB, Canada) resin filter. Red light was obtained by covering the plates with one layer of red resin filter (Roscolux, No. 27, medium red). Photon flux of PAR, measured with a quantum photometer (model LI-185A; Li-cor, Lincoln, Neb., USA) connected to a quantum sensor (model LI-190S), was, at the surface of the nutrient medium, 66–107 µmol m⁻² s⁻¹ for WL and 4.5–7.3 µmol m⁻² s⁻¹ for RL.

12.4.4 Spectrophotometry

Absorption spectra were obtained using Spectrosil cuvettes, 1 cm pathlength (Fisher, Edmonton, AB, Canada) in a Bausch and Lomb Spectronic 2000 double-beam spectrophotometer (Fisher). Sterile 100 µM solutions of chemicals to be examined spectrophotometrically were prepared by diluting ethanolic 100 mM stock solutions, 1 in 1000, in aqueous 1 mM potassium phosphate buffer, pH 6.9, which had been autoclaved and allowed to cool to room temperature. The UV absorption spectra of these solutions were monitored between 220 and 400 nm, as soon as possible after the solutions had been prepared. Petri plates containing 50 ml aliquots of each solu-

tion were maintained for up to 28 d at 22–25°C under WL and RL, conditions also used for incubating moss cultures, as well as in darkness, obtained by wrapping plates in aluminium foil. Samples were removed periodically for spectrophotometric examination. Solutions, which retained their original UV spectra after a prolonged period in all three incubation conditions, were subjected to further analysis. Samples (2.4 ml) of solution were mixed in acrylic cuvettes (Fisher) with 800 µl of 4 M HCl. Duplicate samples were mixed with 4 M KOH. The final concentration of HCl or KOH in each solution was 1 M. The samples of solution, thus treated, were transferred to Spectrosil cuvettes and their UV spectra obtained as described above.

12.5 RESULTS

12.5.1 Characteristic responses of *P. patens* grown in WL to exogenous auxins

NAA affects many aspects of wild type gametophytic growth and development. In brief, inhibition of growth is slight at 500 nM, pronounced at 5 µM and severe at 50 µM. Growth inhibition derives mainly from a reduction of chloronemata formation by NAA at 5 µM and its abolition at 50 µM. Conversely, caulinemata production is increased by 5–50 µM NAA. NAA causes caulinemata to become more darkly pigmented (red) and chloronemata to become pigmented (brown). Gametophore production is reduced by NAA but not severely at concentrations up to 5 µM. At 500 nM, most gameophores develop normally but a few remain as buds and generate many short, thick rhizoids ('spiky' buds). At 5 µM, most gametophores develop into 'spiky' buds or short, thick 'stems' with many long basal rhizoids. In contrast, IAA at concentrations up to 50 µM has little or no effect on the morphogenesis of *P. patens* grown in WL. 2,4-D induces similar effects to those of NAA but it is less potent. At 500 nM, 2,4-D has no apparent effect while gametophytes cultured on 5–50 µM 2,4-D resemble those grown on 500 nM NAA.

12.5.2 Sensitivities to NAA and IAA of a selection of NAR and BAR mutants

The strains listed in Table 2 were grown on medium 2 in RL. The effects of NAA and IAA on each strain were monitored after 4 weeks by comparing the strain's morphology on medium containing NAA or IAA at each concentration, 200 nM, 1 µM and 5 µM, with its morphology on a control medium lacking exogenous auxin. The sensitivity of each mutant to each concentration of NAA and IAA was then assessed relative to the sensitivity exhibited by the wild type.

The definitive phenotype associated with each mutant category has been described previously in detail (Ashton, Grimsley & Cove, 1979; Ashton & Cove, 1990). In brief, on medium lacking exogenous hormones, category 1, 2 and 4 mutants are morphologically abnormal, producing more chloronemal tissue, less (or no) caulinemal tissue and fewer (or no) gametophores than wild type. Category 1 mutants are resistant to both exogenous NAA and BA. Category 2 mutants are resistant to NAA, but can be repaired phenotypically by exogenous BA. *NAR 112* and *NAR 113*, previously reported to belong to mutant category 1 on the basis of their BAR phenotype in WL (Ashton, Grimsley & Cove, 1979), have been re-classified as category 2 strains since the morphologies of both mutants, as well as *NAR 34* and *NAR*

Table 2. *Sensitivity of P. patens strains to exogenous NAA and IAA.*

Strains	Mutant category	NAA (200 nM to 5 µM)	IAA (200 nM to 5 µM)
Wild type		Sensitive	Sensitive
<i>NAR 69</i>	1	Insensitive	Insensitive
<i>NAR 91</i>	1	Insensitive	Insensitive
<i>NAR 185</i>	1	Insensitive	Insensitive
<i>NAR 34</i>	2	Insensitive	Insensitive
<i>NAR 112</i>	2	Insensitive	Insensitive
<i>NAR 113</i>	2	Insensitive	Insensitive
<i>NAR 87</i>	2	Resistant relative to wild type	Resistant relative to wild type
<i>BAR 1</i>	4	Sensitive and partially repairable	Sensitive and partially repairable
<i>BAR 471</i>	4	Sensitive and partially repairable	Sensitive and partially repairable

87, are repaired almost completely when 100 nM BA is present in the medium and the cultures are grown in RL. Category 4 mutants are resistant to BA but sensitive to and partially repaired by NAA, i.e. when provided with NAA, they resemble more closely wild type grown in the presence of NAA. Wild type is sensitive to both chemicals.

12.5.3 Auxin structure-activity relationships in *P. patens* cultured in RL

The sensitivities of wild type *P. patens* and two NAA-responsive mutants, *BAR 1* and *BAR 281*, to a selection of compounds, which exhibit auxin activity in higher plant assays or which are structural analogues of such compounds, are compiled in Tables 3 and 4.

12.5.4 Stability of auxins and auxin analogues in various lighting conditions

There were no changes in the UV spectra of any of the tested compounds during incubation in RL or D up to including the latest time at which the solutions were examined (Table 5).

Spectra of the four indolyl substances changed during incubation in WL. The earliest changes in the spectrum of IAA occurred in the region 240–256 nm; in particular a peak was initiated at approximately 253 nm. Soon afterwards, a second peak arose at approximately 247 nm. The spectrum between 220 and 270 nm of IAA incubated for 11 d in WL resembled very closely the spectrum of 3-methylene-2-oxindole (3-Menox) (Evans & Ray, 1972). In particular, it exhibited a double peak at 247 and 253 nm which is very characteristic of 3-Menox. After 18 and 27 d in WL, the spectrum remained similar to that of 3-Menox, but by these times the peak at 247 nm had become larger than that at 253 nm.

The spectra of EtIA and IBA during the early stages of incubation in WL mimicked that of IAA by initiating a peak in the 250–256 nm region: in the case of EtIA at approximately 253 nm and in IBA at about 255 nm. In the case of IPA, however, no peak at or near 247 or 253 nm was observed during incubation under WL. The spectra of solutions of EtIA, IPA and IBA, after prolonged incubation in WL, did

Table 3. *Sensitivities to auxins and auxin analogues of P. patens grown in RL on medium of pH ~ 7. Phenoxy compounds are printed in italics.*

Developmental changes	Strains	Lowest concentrations at which developmental changes occur			
		50 nM	500 nM	5 μ M	50 μ M
\uparrow caulinemata and/or rhizoids	Wild type		IAA	IAAEt IBA NAA	IPA 2-NAA NoA 2,4-D 2,4-DP $(-)$ 2,4-DP
	<i>BAR 1</i>	NAA	IAA	IAAEt IBA 2-NAA	IPA 2,4-D 2,4-DP
	Wild type		NAA	IAA IAAEt 2-NAA	IPA IBA NoA 2,4-D 2,4-DP $(-)$ 2,4-DP
	<i>BAR 1</i>		IAA NAA	IAAEt IBA 2-NAA	IPA 2,4-D 2,4-DP
\downarrow chloronemata	Wild type		IAA	IAAEt IBA NAA	IPA 2-NAA
	<i>BAR 1</i>	NAA	IAA	IAAEt IBA 2-NAA	IPA 2,4-D 2,4-DP $(-)$ 2,4-DP
	Wild type		NAA	IAA IAAEt IBA	IPA 2-NAA
	<i>BAR 1</i>				
Abnormal gametophore development	Wild type		NAA	IAA IAAEt IBA	IPA 2-NAA NoA 2,4-D 2,4-DP

not resemble closely either that of IAA kept in WL or 3-Menox, although the spectrum of EtIA did exhibit shoulders at 246 and 252 nm and that of IBA had a peak at 255 nm.

The spectra of the three naphthalene derivatives also changed in WL (see Fig. 1 for NAA and NoA). This is especially interesting since it is commonly claimed that NAA is stable in light.

The spectra of the phenyl and phenoxy compounds were unchanged during incubation in WL (see Fig. 1 for 2,4-D). The spectra of solutions of these compounds exhibited a slight shift (0.5 to 2 nm at the peaks) towards lower wavelengths upon acidification with HCl. The spectra of PoA, 2,4-D, (+)2,4-DP, (-)2,4-DP and PAA in solutions made basic with KOH were very similar to the corresponding spectra of untreated solutions with a pH close to neutrality. In sharp contrast to PAA and the phenoxy compounds, solutions of HOP exhibited a very large (approximately 18 nm) spectral shift towards higher wavelengths when made basic with KOH.

Table 4. *Sensitivities to auxins and auxin analogues of *P. patens* grown in RL on medium of pH ~5.5. Phenoxy compounds are printed in italics.*

Developmental changes	Strains	Lowest concentrations at which developmental changes occur			
		50 nM	500 nM	5 µM	50 µM
↑ caulinemata and/or rhizoids	Wild type	IAA	NAA 2-NAA		2,4-D
	<i>BAR 28I</i>	IAA NAA		2-NAA	2,4-D
↑ red pigment	Wild type	IAA	NAA 2-NAA		2,4-D PAA
	<i>BAR 28I</i>	IAA	NAA	2-NAA	2,4-D
↓ chloronemata	Wild type	IAA		NAA 2-NAA	
	<i>BAR 28I</i>	IAA NAA		2-NAA	2,4-D
Abnormal gam. development	Wild type		IAA NAA	2-NAA	2,4-D
Max. ↑ in gam. production	<i>BAR 28I</i>		IAA	NAA	2,4-D

Table 5. *Stability of auxins and auxin analogues in white light (WL), red light (RL) and darkness (D).*

Compounds	Periods (d) from initiation of incubation when UV spectra monitored											°Earliest times (d) when spectral changes observed	
	1	2	3	7	11	18	20	21	23	27	28	D and RL	WL
IAA	✓	✓	✓	✓	✓	✓				✓		—	1
EtIAA		✓		✓	✓	✓	✓					—	7
IPA	✓	✓		✓	✓	✓	✓	✓				—	7
IBA	✓	✓	✓	✓	✓	✓	✓	✓				—	2
NAA	✓			✓	✓	✓	✓	✓				—	7
2-NAA	✓			✓	✓	✓	✓	✓				—	7
NoA	✓	✓		✓	✓	✓	✓	✓				—	2
PoA	✓			✓	✓	✓	✓	✓	✓	✓		—	—
2,4-D	✓			✓	✓	✓	✓	✓	✓	✓		—	—
2,4-DP	✓			✓	✓	✓	✓	✓	✓	✓		—	—
(-)2,4-DP	✓			✓	✓	✓	✓	✓	✓	✓		—	—
PAA	✓			✓	✓	✓	✓	✓	✓	✓		✓	—
HOP	✓			✓	✓	✓	✓	✓	✓	✓		—	—

* All compounds were in sterile, aqueous 1 mM potassium phosphate, pH 6.9, at a concentration of 100 µM.

† UV spectra of solution samples, made acidic with HCl or basic with KOH, were examined in addition to the spectra of untreated solutions.

‘—’ indicates that UV spectra did not change over the total period for which the solutions were monitored.

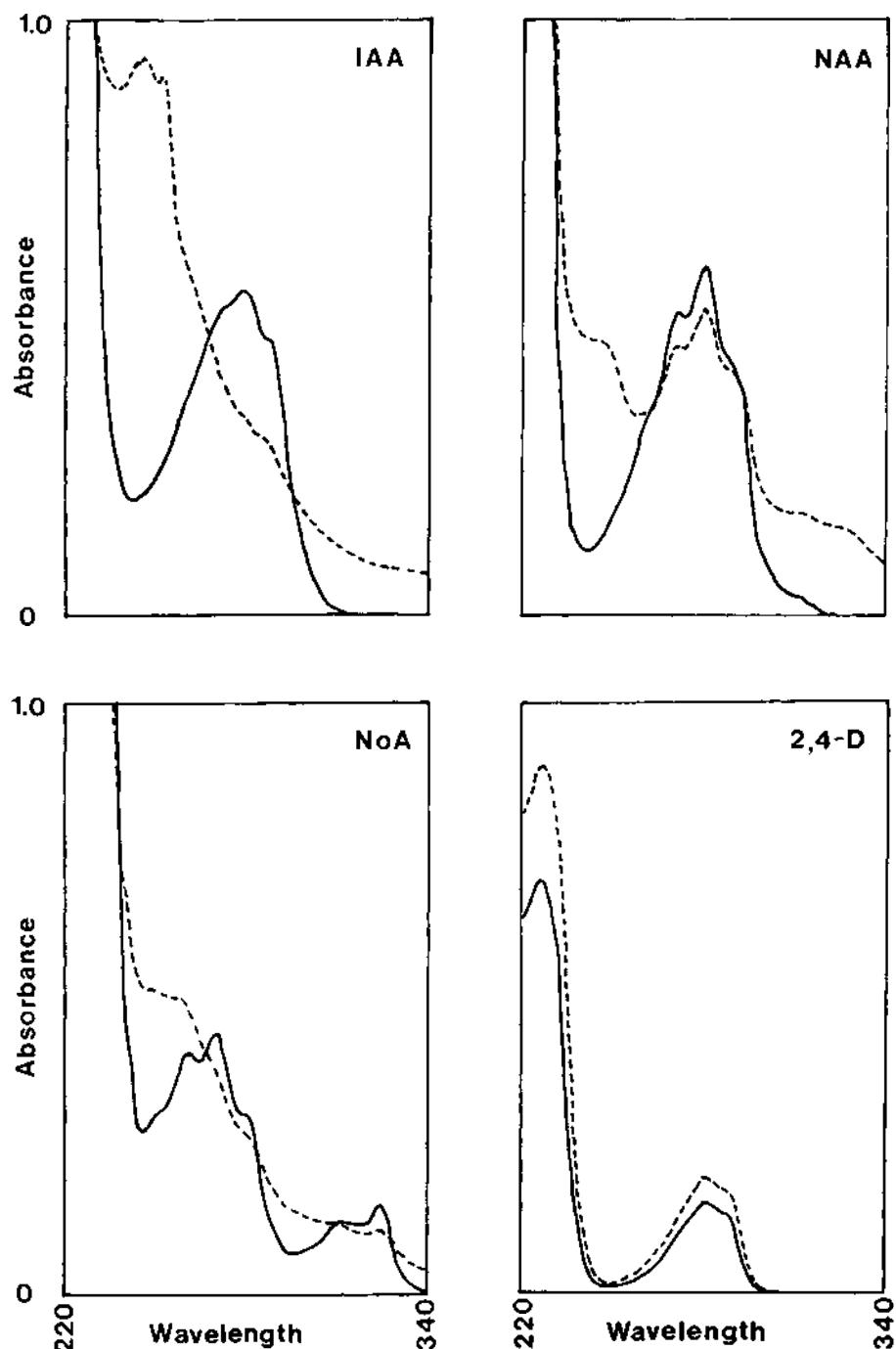


Figure 1. Absorption spectra of selected auxins in aqueous potassium phosphate buffer, pH ~6.9, incubated for 18 d (IAA, NAA and NoA) or 28 d (2,4-D) in WL (dashed lines) and in RL (solid lines). The latter spectra are identical to those of freshly prepared samples of the authentic substances as well as of samples stored in the dark. The ordinate represents absorbance from 0 to 1.0 A; the abscissa represents wavelength in nm.

Samples of solutions of phenyl and phenoxy compounds, subjected to prolonged incubation in WL, RL or D, yielded, after acidification or being made basic, the characteristic spectra of the authentic chemicals.

12.6 DISCUSSION

12.6.1 Auxin structure-activity relationships

The bioassays performed in continuous RL gave markedly different results from those carried out in WL, e.g. IAA is highly potent in RL but exhibits only weak auxin activity in WL; IPA has relatively weak but readily detectable activity in RL but elicits no response from *P. patens* in WL. These differences can be attributed to the differential sensitivities of some of the tested substances, especially the indolyl compounds, to photodegradation by WL.

Under conditions which protect auxins from photodegradation, auxin structure-activity relationships remain significantly different in *P. patens* from those characteristic of higher plants. For example *P. patens* exhibits a strong developmental response to 2-NAA, a substance which is weakly inhibitory in many higher plant growth assays, but it gives a relatively weak response to 2,4-D, which is a very potent growth promoter in higher plants (Ray, Dohrmann & Hertel, 1977). In general, the indolyl and naphthyl compounds tested were found to be potent auxins while the phenoxy derivatives exhibited little or no activity. I propose the following tentative explanations of these findings as a framework for further study:

(1) There may be structural differences between the auxin receptors of mosses and those of higher plants which are the result of evolutionary divergence.

(2) Different groups of substances which exhibit auxin activity in higher plants may exert their effects through different mechanisms. It follows that, for example, phenoxy auxins may act through a mechanism which is present in higher plants but absent in mosses, while clearly both plant groups possess the metabolic machinery required for sensitivity to indolyl auxins. This idea is especially attractive in view of Ray, Dohrmann & Hertel's data (1977) on the binding affinities of a wide selection of auxins and auxin analogues for the maize Site 1 (ER) abs, which is probably identical to ABP1 and presently held to be the best candidate for a physiologically relevant auxin receptor (Edgerton, Tropsha & Jones, 1995). *P. patens* structure-activity relationships accord better with this auxin binding data than do the structure-activity relationships for maize itself. For example, the three substances with the highest affinities for Site 1 abs are NAA, 2-NAA and IAA. 2,4-D has a low affinity for this site. Also, the site has a low affinity for both (+)-2,4-DP and (-)-2,4-DP. In *P. patens*, the (-)-form exhibits approximately the same low activity as a racemic mixture suggesting that the bryophyte responds similarly to the two stereoisomers. By contrast, the (+)-form is a potent growth promoter in maize, while the (-)-form is a weak auxin.

(3) There may be differences between higher plants and mosses with respect to the relative rates at which they catabolize the tested substances.

(4) Mosses and higher plants may differ with respect to their ability to take up the substances tested. This last explanation seems the least likely since *P. patens*

protoplasts accumulate ^{14}C -labelled 2,4-D and IAA equally well at pH 5, 6 and 7, while, at pH 4, the accumulation of 2,4-D is markedly greater than that of IAA (Ashton *et al.*, 1990a).

The relative potencies of the tested substances are the same when culture media with an initial pH of approximately 5 or 7 are used. However, *P. patens* exhibits higher sensitivity to each substance at the lower pH. This is consistent with expectations based on the discovery that auxin accumulation in mosses is pH-dependent, being higher at lower pHs (Ashton *et al.*, 1990a; Geier, Werner & Bopp, 1990).

PAA and HOP are presently the only realistic candidates for non-indolyl endogenous auxins in higher plants (see Bearder, 1980, and references within). Neither elicited any discernible response in *P. patens* making it unlikely that either functions as an endogenous auxin in this plant.

Essentially identical structure-activity relationships are observed with wild type and auxin-repairable *BAR* mutants. This strengthens the contention that the tested, exogenously provided compounds are functioning as authentic auxins, *i.e.* eliciting developmental transitions which are normally induced by endogenous auxin(s).

The dual insensitivity/resistance of *NAR* mutants to IAA and NAA provides strong support for the contention that the mechanisms of response to this pair of substances, and perhaps to indolyl and naphthyl auxins in general, possess at least one common component. Additionally, it enhances the potential utility of *NAR* strains for studies of the molecular mode of auxin action.

12.6.2 Protection of substances used in this study from photodegradation

Spectral analysis of solutions of the compounds incubated in RL verified that all of them are stable under these conditions, at least for the period of time required to perform bioassays. A literature search indicates that this study is probably the first of its kind in which steps have been taken to protect the substances being bioassayed from photodegradation, although photodegradation of auxins during lengthy bioassays using higher plants, *e.g.* the strawberry receptacle enlargement assay, has been previously recognized as a problem complicating the interpretation of data (Narayanan, Mudge & Poovaiah, 1981).

12.6.3 Photodegradation of test substances

Although this paper is not concerned primarily with the nature of the photodegradation of auxins and their analogues, some features of this process under the WL conditions employed in this study are noteworthy. As in riboflavin-sensitized degradation of IAA by visible light (Fukuyama & Moyed, 1964) and *in vitro* oxidation of IAA by some plant oxidase systems, *e.g.* that obtained from *Parthenocissus tricuspidata* crown gall tissue (Hamilton *et al.*, 1976), a major, though probably not exclusive or final, product of IAA degradation in WL appears to be 3-Menox. Monitoring aqueous solutions of the three other indolyl substances used revealed that, especially during the early phases of photodegradation, the spectra of EtIA and IBA, but not IPA, solutions kept in WL have some features identical or very similar to those observed in the spectrum of IAA solutions maintained under the same conditions. These common spectral characteristics are consistent with 3-Menox,

or a similar oxindole, being among the photodegradation products of EtIA and IBA although the evidence supporting this contention is less compelling than in the case of IAA. Whatever the nature of the photodegradation products, increasing the length of the side-chain attached to the indole ring retards the degradative process to some extent (Table 5). In the case of the naphthalene compounds, NoA appears to be the most susceptible to photodegradation although NAA and 2-NAA are also degraded in WL (Table 5; Fig. 1). As far as I can ascertain, the photodegradation of these three compounds has not been previously reported and the case of NAA is especially interesting since it is often assumed that NAA, unlike IAA, is stable in visible light. Crosby & Tutass (1966) have reported that 2,4-D in aqueous solution is degraded rapidly by UV light at 254 nm producing 2,4-dichlorophenol and several other intermediates with one or more hydroxyl groups, in place of the chlorine atoms and/or acetic acid side-chain originally present, all of which are converted eventually into coloured (brown) polymeric humic acids. They obtained similar results with sunlight. Boval & Smith (1973), using polychromatic UV light, confirmed that 2,4-dichlorophenol is a product of the photodegradation of 2,4-D and also provided evidence that, under the lighting conditions which they employed, the aromatic ring is ruptured. Spectral analysis of solutions of 2,4-D kept in WL provided strong assurance that this compound is not degraded under these conditions even after prolonged incubation. The spectra of samples in 1 M KOH or 1 M HCl were particularly informative in this respect. Degradation of 2,4-D of the kind described by Crosby & Tutass (1966) would have been readily apparent from a comparison of the UV spectrum of an untreated sample of solution with that of a sample in 1 M KOH, since the oxide ions of aromatic alcohols, such as 2,4-dichlorophenol, have UV spectra which differ markedly from those of the corresponding undissociated molecules (Parke & Williams, 1955). The small spectral shift towards lower wavelengths exhibited by 2,4-D solutions upon acidification with HCl provides further assurance that the acetic acid side-chain is not lost during incubation in WL since spectral shifts of this kind are probably a consequence of protonation of the acid's conjugate base which predominates greatly at and above a pH of 5. Observation that the spectra of fresh solutions of authentic 2,4-D are identical to those of 2,4-D solutions subjected to prolonged incubation in WL (Fig. 1) also precludes the possibility that 2,4-D decomposes under these conditions into 2-chlorophenoxyacetic acid or 4-chlorophenoxyacetic acid since these mono-chlorinated derivatives of PoA have spectra which differ from those of 2,4-D (Phillips, 1959). Spectral analysis of solutions of the other phenoxy and phenyl compounds indicates that they too are stable in WL. As expected, all solutions of HOP, the only one of these substances possessing a hydroxyl group attached to the ring, exhibited a large spectral shift towards visible wavelengths when made basic with KOH as a consequence of the formation of oxide ions.

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13. Origin of centrioles in Anthocerote spermatogenous cells

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13.1 SUMMARY

Although there have been many investigations of the locomotory apparatus in anthocerote spermatogenous cells, much less attention has been given to the *de novo* origin of centrioles in these cells. In this report, we describe the stages in spermatogenous cell development leading up to and including the development of centrioles in three anthocerote species. In somatic cells, and in spermatogenous cells up until the penultimate division, the microtubule organizing centre (MTOC) of the axial microtubule system and of the mitotic spindle is electron-opaque material from which the microtubules apparently emanate. Prior to the penultimate division, within the electron-opaque MTOC that has accumulated at each pole, structures begin to differentiate that, at first, are only faintly recognizable as a pair of centrioles. The imbrication of the microtubule triplets is in opposite orientation in the two halves of the centriole, indicating that their growth is at the ends. The pair of centrioles and associated pericentriolar material serve as the spindle MTOC for the penultimate divisions. For the final division, the pair of centrioles divide, so that each daughter cell receives a single centriole. After the final spermatogenous cell division, the centrioles sever at the mid point to become the basal bodies of the two flagella, whereas the pericentriolar material differentiates into the lamellar strip of the multilayered structure, an MTOC associated with spline development. These data indicate that the centrioles of anthocerotes are generated in material that served as an MTOC in previous cell divisions and that modification of these MTOCs allows for both centriole formation and the development of the locomotory apparatus. The time of origin of centrioles and the presence of microtubule arrays involved in marking the plane of the subsequent division in anthocerotes are unique from other bryophyte taxa, underlining their isolated position taxonomically.

KEYWORDS: Anthocerote, spermatogenous cells, centrioles, microtubule organizing centres.

13.2 INTRODUCTION

The presence of centrosomes or centrosome analogues in spermatogenous cells of land plants was the subject of numerous investigations by many botanists/cytologists in the late 1800s and early 1900s (reviewed by Lepper, 1956). Sharp (1914) used the term 'blepharoplast' to denote that special centrosome analogue associated with the

development of the locomotory apparatus and that apparently served as a spindle MTOC during the penultimate and spermatid mother cell divisions. Despite the clear proof of the presence of this structure in some systems, others did not find similar structures in their studies and dismissed the idea that there were indeed centrosomal analogues in plants. Some of these negative findings were almost surely due to the small size of the bryophyte spermatogenous cells, the resolution of structures using standard light microscopic techniques of the day, and the difficulty in finding good samples of these cells for microscopy (a problem that continues today!). Still, Lepper (1956) summarized the consensus of these early light microscopic studies as supporting a view that spermatogenous cells are unique amongst land plants in producing a centrosome, even though the majority of plant cells had a relatively broad, unfocused spindle devoid of a centrosome.

With the advent of transmission electron microscopy, it was shown clearly that centrioles are present in the spermatogenous cells of a number of lower land plants (Moser & Kreitner, 1970; Moser, Duckett & Kreitner, 1977). In the bryophytes, these take the form of bicentrioles, a compound structure in which the two centrioles are joined by a central hub, with opposite imbrication of the microtubule triplets on each side (Moser & Kreitner, 1970). Although it is well known that centrioles arise *de novo* in all archegoniates, the origin of bicentrioles is not well documented in any bryophyte taxa, the only exceptions being the studies of Robbins (1984) and Renzaglia & Duckett (1987), on two hepatic species. Research in this area has centred on comparisons of the mature aspects of the locomotory apparatus to establish phylogenetic and taxonomic-relatedness between the various bryophyte taxa (reviewed in Carothers & Duckett, 1980). To the best of our knowledge, the origin of bicentrioles has never been described in any anthocerote, even though the presence of a bicentriole was demonstrated as early as 1970 by Moser & Kreitner. Because of the importance of sperm locomotory apparatus in determining land plant phylogeny (Graham, 1993) and the relationships between the lower land plants (Renzaglia & Duckett, 1988), the origin of these structures is also potentially important in taxonomic and phylogenetic considerations within the lower land plants, and certainly merits investigation (see Renzaglia *et al.*, 1994, for an example of earlier stages of spermatogenous cell development yielding potentially important phylogenetic information).

In this study, we examine the stages of development preceding and during the *de novo* appearance of bicentrioles in three anthocerote species. Each of these species grows well under simple culture conditions and all produce large numbers of antheridia under these conditions. Thus, we were able to collect relatively complete developmental series for three species and more limited, but confirmatory, series for several other species. From these observations, we find that the origin of bicentrioles in anthocerotes is distinct from that found in the two hepatic species documented previously.

13.3 MATERIALS AND METHODS

13.3.1 Plant material

Notothylas breutelli was collected in greenhouses at Southwest Louisiana State University, Lafayette, LA, USA. *Phaeoceros laevis* was collected in fields in

Stoneville, MS, USA and on logging trails in Andover, NH, USA. *Folioceros appendiculatus* was supplied by Jiro Hasegawa (Japan). All were subsequently cultured in terraria receiving natural lighting, for at least several months. When gametophytes exhibited antheridia, portions of the gametophytic tissue including the antheridia were processed for microscopy.

13.3.2 Microscopy

Antheridia with surrounding gametophytic tissue were fixed for 2 h at room temperature in 6% (v/v) glutaraldehyde in 0.05 M PIPES buffer (pH 7.4). The samples were then washed twice, 15 min each in 0.10 M cacodylate (pH 7.2), and post-fixed in 2% (w/v) osmium tetroxide for 2 h. After a brief distilled water rinse, the samples were stained en bloc with 2% (w/v) uranyl acetate for 24 h at 4°C. After a distilled water rinse, the samples were dehydrated in an acetone series and transferred to propylene oxide. Samples were embedded in a 1:1 mixture of Spurr's and Epon resins (without catalyst), by increasing the amount of resin by 25% increments over 6 h and then allowing the propylene oxide to evaporate slowly over 18 h, thereby increasing the concentration of plastic very gradually to 100%. The samples were then transferred to fresh resin and the vials agitated on a shaker for 24 h. The samples were then transferred to BEEM capsules and the resin mix with catalyst was added to the capsules for 2–4 h at room temperature under vacuum. Other samples were embedded in peel-away embedding moulds for flat embedding. Polymerization of either flat-embedded or capsule-embedded samples was carried out at 68°C for 24–48 h.

Blocks were sectioned with a diamond knife using a Reichert Ultracut ultramicrotome. Light monitor sections (~0.35 µm) to determine the presence and developmental stage of the antheridium were mounted on glass slides and stained with 1% (w/v) toluidine blue in 1% (w/v) borax. Thin sections (grey to pale gold reflectance) were mounted on uncoated 300 mesh copper grids and examined in a Zeiss EM 10 CR electron microscope after post-staining with 2% (w/v) uranyl acetate and Reynold's lead citrate, each for 7 min. Some samples were also serially-sectioned and mounted on Formvar-coated slot grids to determine bicentriole numbers in a given cell.

13.4 RESULTS

Prior to the appearance of bicentrioles, the interphase spermatogenous cells are cuboidal to slightly rectangular and contain a number of mitochondria, elements of the endomembrane system, a centrally-located nucleus, and a relatively undifferentiated plastid (Fig. 1A). Mitosis in these cells involves the coordinated division of both the single plastid and the nucleus. The anthocerote antheridial cells utilize both the plastid-associated axial microtubule system (AMS), that lies between the nucleus and the plastid (Figs 1B, 2), and a modified pre-prophase band (PPB) of microtubules, that lies between the plasmalemma and the plastid (Fig. 2A), to establish the division plane. At the isthmus of the dividing plastid, electron-opaque globular material accumulates and microtubules emanate from this material in the direction of the division of the plastid (Fig. 2). Presumably, the microtubules of the AMS provide the force to separate the plastid into two masses at the poles of the cell. Our best

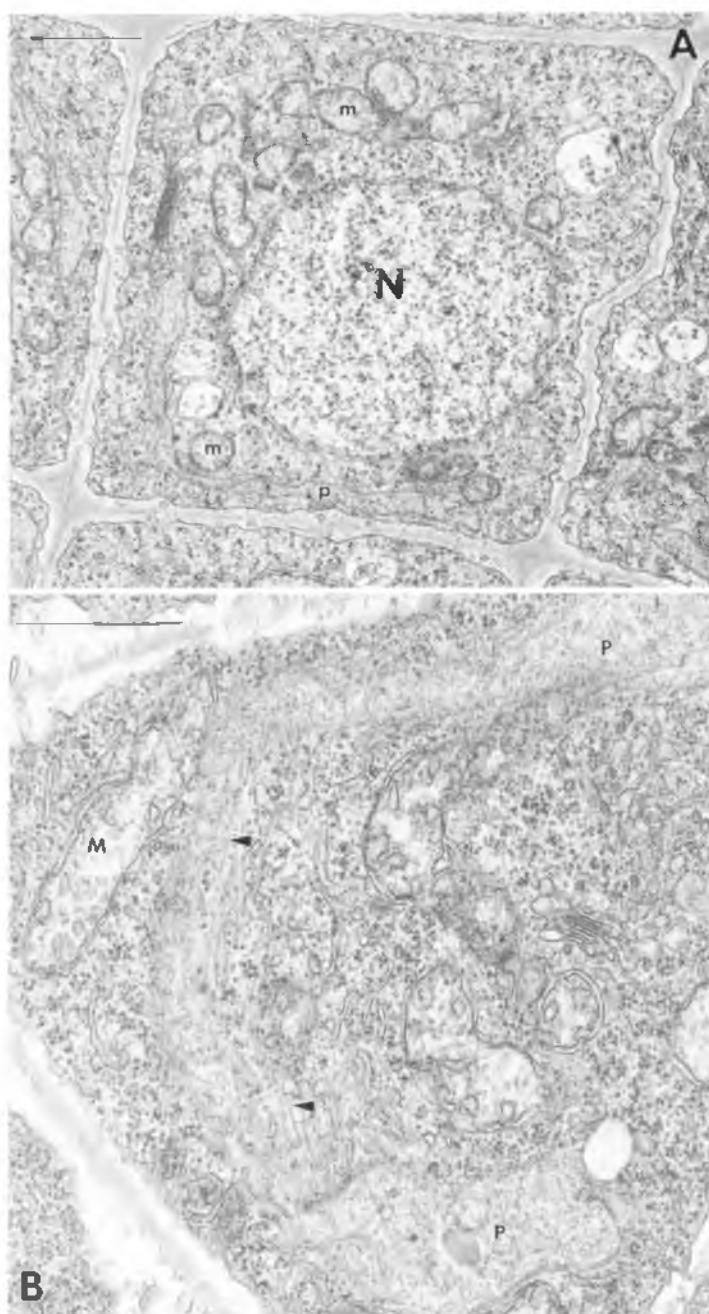


Figure 1. A. Electron micrograph of a spermatogenous cell of *Phaeoceros laevis* from a cell generation prior to the penultimate division. Numerous mitochondria (m), a centrally-located nucleus (N), and a relatively undifferentiated plastid (p) are visible. B. Electron micrograph of a spermatogenous cell of *Notothylas breutellii* revealing the presence of the axial microtubule system (some marked with arrowheads) that are involved in the migration of the two halves of the plastid (p) to the poles. M = mitochondrion. Bars = 1.0 μm in A; 0.5 μm in B.

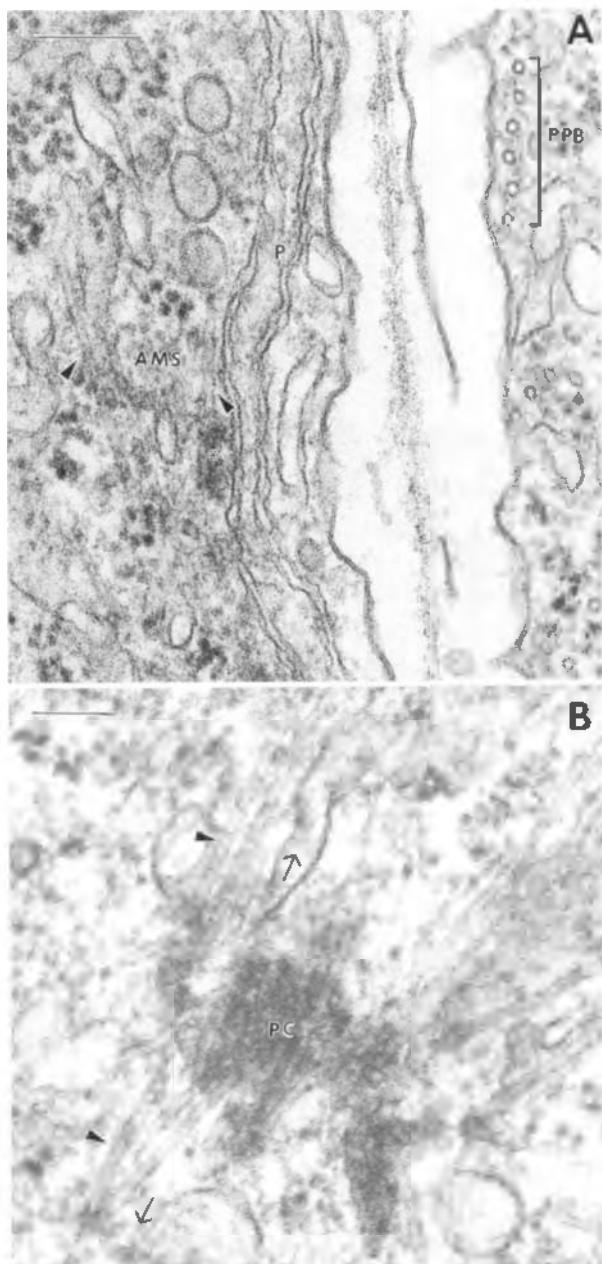


Figure 2. A. Electron micrograph of two adjacent sperm cells of *Notothylas breutellii* revealing the two microtubule systems involved in establishing cell polarity. The AMS directs microtubules (one marked with an arrowhead) in the direction of the long axis of the plastid (P) and is apparently organized by electron-opaque pericentriolar material (*). A pre-prophase band (PPB) of microtubules occurs along the plasmalemma in the adjacent cell. B. Electron micrograph through the centre of the AMS microtubule array in *Phaeoceros laevis*. The microtubules (arrowheads) are directed towards the two poles (arrows) and apparently emanate from the electron-opaque pericentriolar material (PC). Bars = 0.2 μm in A; 0.1 μm in B.

interpretation of the static electron micrographs is that the electron-opaque MTOC material, which organizes the AMS, separates in two, migrating along the outline of the plastid envelope until it reaches the poles, where it serves as the spindle MTOC for nuclear division (Fig. 3A). Cytokinesis involves final separation of both the nuclear contents and the two separated halves of the plastid.

Just prior to the penultimate division, instead of directly organizing the microtubules for a division, the electron-opaque MTOC begins a series of differentiations into a pair of bicentrioles and associated pericentriolar material (Fig. 3). The 'A' microtubules of the triplet organize into the nine-fold symmetry typical of centrioles, and eventually microtubules are constructed until the classic triplet cartwheel arrangement is formed (Fig. 3B-D). These bicentrioles grow out in both directions from a central hub to about $0.5 \mu\text{m}$ in total length (Fig. 4), with the triplets imbricated in opposite directions in the two halves of the bicentriole (Moser & Kreitner, 1970). The bicentrioles and the surrounding electron-opaque pericentriolar material serve as the spindle MTOC for the last two spermatogenous cell divisions (Figs 5 & 6). In the first of these divisions, there are two bicentrioles at each pole (Fig. 5), with two of them contributing to each daughter cell. In the final spermatogenous cell division, the pair of bicentrioles separate and migrate to the poles of each cell so that each daughter cell contains but one bicentriole (Fig. 6). The last division occurs at an oblique angle to the previous division, resulting in two wedge-shaped daughter cells.

In the young spermatid, the bicentrioles migrate from their position near the nucleus at the poles to a slightly more peripheral position, along the long axis of the spermatogenous cell wall. At this time, the bicentriole severs at the hub, and re-orientates to become the basal bodies of the flagella; the pericentriolar material begins to differentiate into the lamellar strip of the multilayered structure, an MTOC associated with spline formation. At mid-stage in development, the locomotory apparatus, consisting of flagella, spline, and lamellar strip, becomes associated with the anterior mitochondrion and the nucleus (Fig. 7A). Electron-opaque material, similar in position to the distal fibres in the algal flagellar apparatus, connects the two basal bodies (Fig. 7B).

All three species of anthocerote formed the same kind of structures and in the same sequence. Partial series were constructed for three other species that match the observations described for the others, indicating the generality of these observations for all anthocerotes. A model summarizing these steps is shown in Fig. 8.

13.5 DISCUSSION

13.5.1 Unique aspects of anthocerote spermatogenous cells

Previous work on the ultrastructure of mid-stage to mature spermatids of anthocerotes has established a number of characters that are unique to this group, indicating an isolated taxonomic position (Moser, Duckett & Carothers, 1977; Renzaglia & Carothers, 1986; Renzaglia & Duckett, 1989; Renzaglia, 1982). These characteristics include:

- (1) basal bodies identical or nearly so;

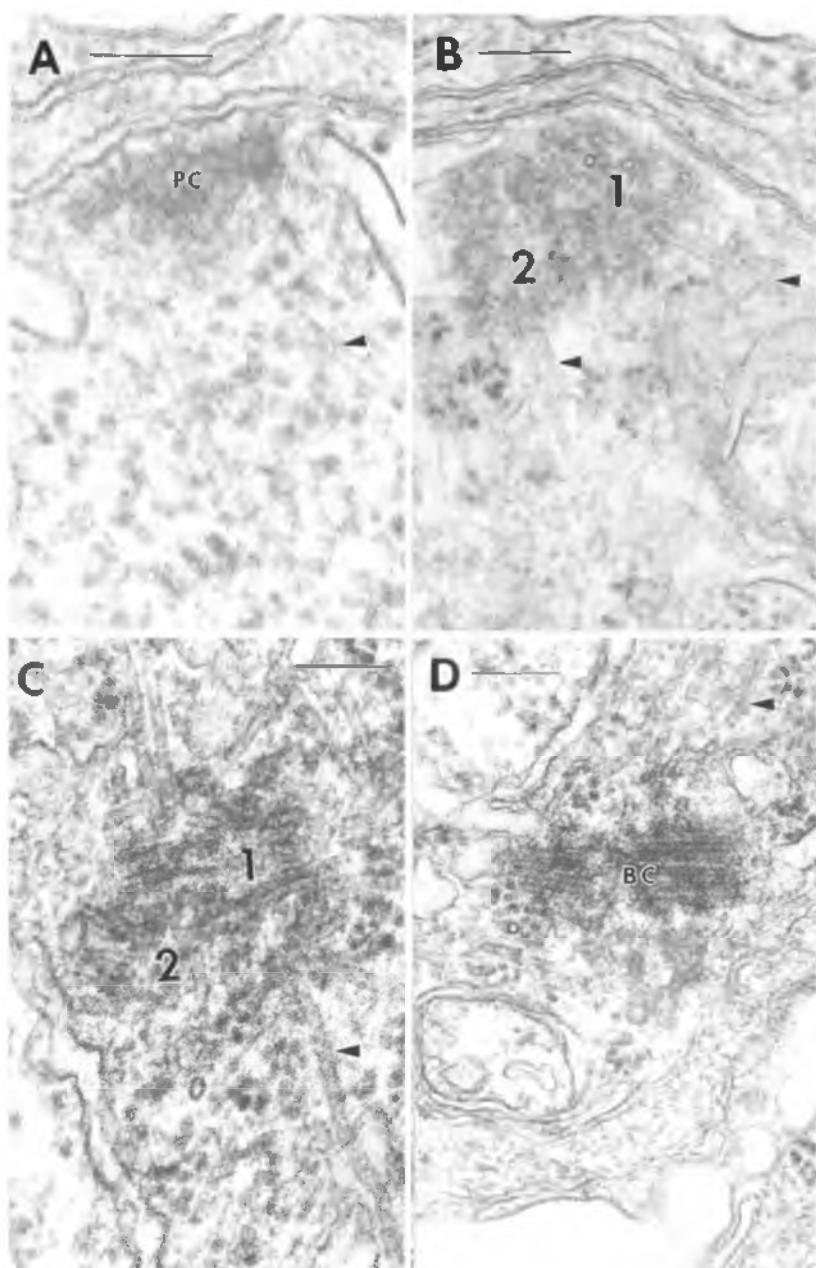


Figure 3. Micrographs illustrating the differentiation of bicentrioles in the penultimate division of anthocerote spermatogenous cells. A. Accumulation of pericentriolar material (PC) at the poles prior to the penultimate mitosis. Some microtubules may be seen emanating from this structure. B. Early stage of bicentriole formation. Within the pericentriolar material, two regions (denoted '1' and '2') appear to differentiate a few microtubules, vaguely arranged in a centriolar form. C. A slightly later stage, in which the two masses are now more clearly recognizable as centrioles. D. Nearly fully-formed bicentrioles (bc), in longitudinal section. Arrowheads denote microtubules emanating from the pericentriolar material. Bars = 0.1 μm in A-C; 0.2 μm in D.

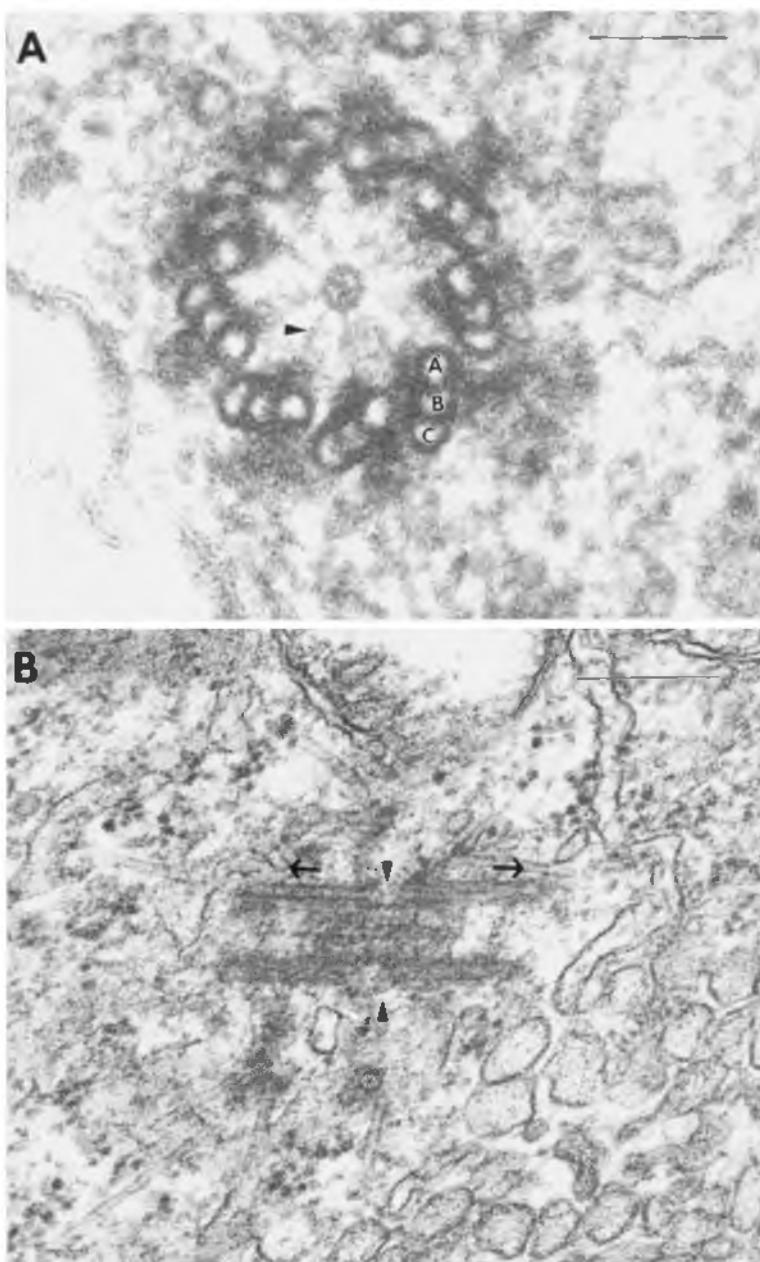


Figure 4. The ultrastructure of mature bicentrioles in cross (A) and longitudinal (B) sections. A. The mature bicentriole of *Notothylas breutellii* is strikingly similar in ultrastructure to other plant and animal centrioles. The microtubules display the characteristic nine-fold cartwheel symmetry observed in all centrioles, with the presence of fully-formed 'A' parts of the triplets and partially complete and fused 'B' and 'C' microtubules. Electron-opaque fibrillar structures (arrowhead) connect the triplets to the centre. B. The longitudinal section reveals that the bicentriole halves are connected together at the hub (arrowheads) and have grown out from this hub in the directions marked by the arrows. Satellite bits of pericentriolar material (*) can be seen apparently nucleating microtubules. Bars = 0.1 μ m.

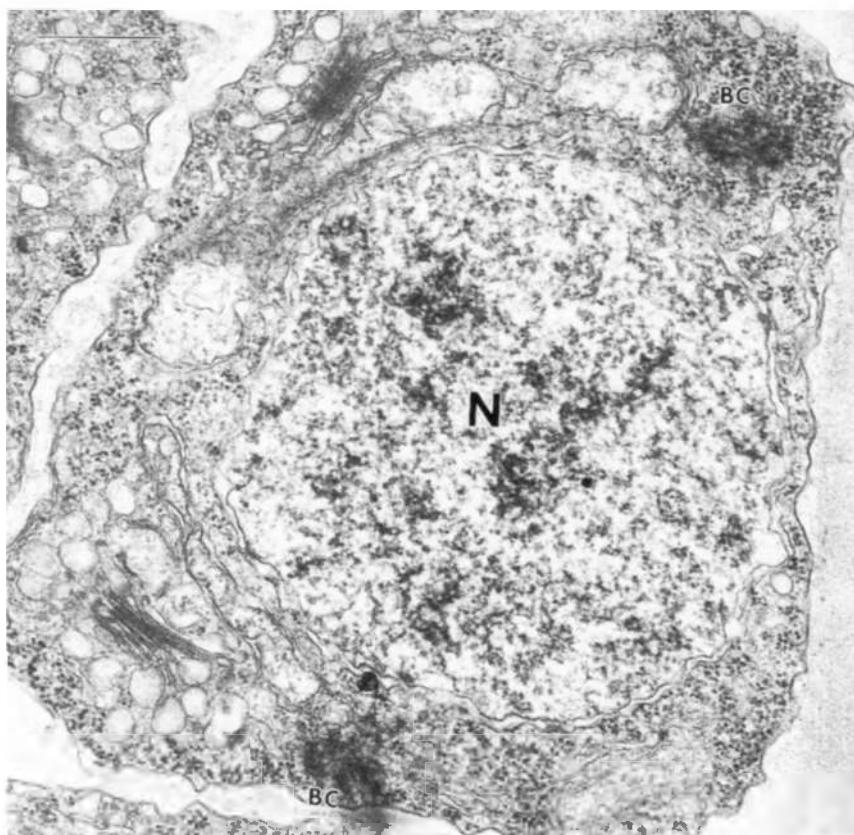


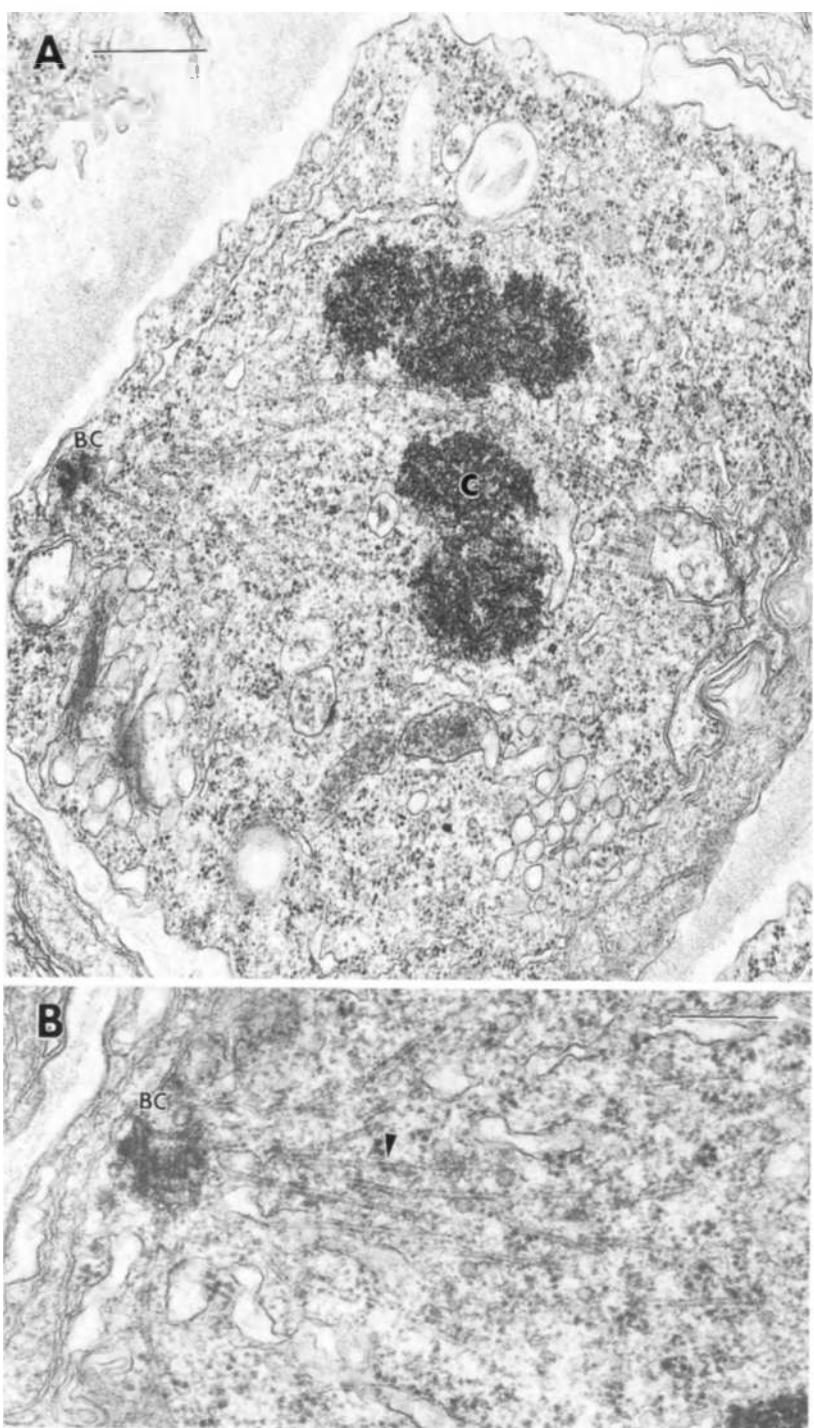
Figure 5. Spermatogenous cell just prior to the penultimate division with two bicentrioles (BC) at each pole. The pair of bicentrioles and associated pericentriolar material will serve as the spindle MTOCs for this division. N = nucleus. Bar = 0.5 μm .

- (2) lamellar strip of the multilayered structure elongated laterally;
- (3) spline containing twelve microtubules with no gaps (inapertuate);
- (4) transition zone of flagella short and lacking a stellate pattern (the latter shared with *Sphagnum*);
- (5) nucleus of mature sperm coiled sinistrally.

To this list we can add two other characteristics from our earlier stages of spermatogenous cell development described herein. These are:

- (1) presence of AMS and PPB microtubule arrays that mark the plane of subsequent cytokinesis in spermatogenous cell lines;
- (2) production of bicentrioles in the penultimate cell division.

The characteristics of the mid-stage spermatogenous cell have been utilized in cladistic analyses to establish phylogenetic relationships between the various land-plant lineages (Garbary, Duckett & Renzaglia, 1993; Mishler *et al.*, 1994; Garbary & Renzaglia, this volume) and recent additions to the analyses, which include characters described herein, further emphasize the position of anthocerotes as an isolated taxon, basal to all land plants. Similar results were obtained by Hedderson, Chapman and



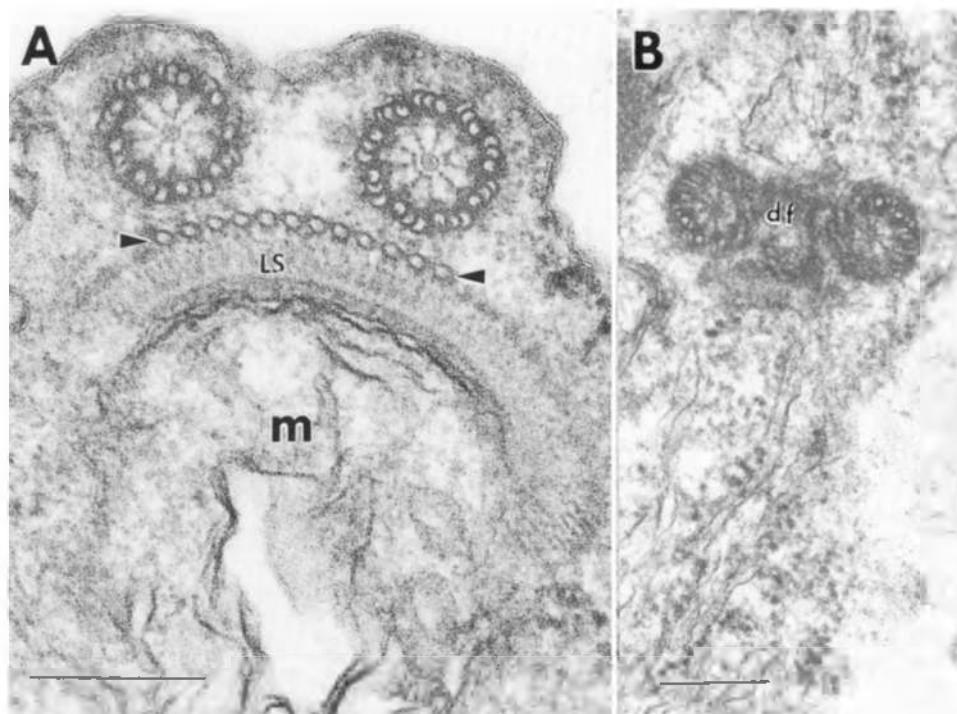


Figure 7. Electron micrographs of the locomotory apparatus in mid-stage spermatids of *Phaeoceros laevis*. A. The bicentrioles are now split to form the two equivalent basal bodies. The pericentriolar material has differentiated into the lamellar strip (LS) from which a layer of spline microtubules (bordered by arrowheads) has been organized. The LS is tightly associated with the anterior mitochondrion (m). B. A view of the electron-opaque material, similar to distal fibres (df) in algae, that connect the two basal bodies. Bars = 0.2 μ m.

Rootes (1996), utilizing 18sRNA sequences. In the cladogram created from the sequence analyses, anthocerotes are basal to all land plants, with the mosses and hepaticas clustered as sister taxa to the tracheophytes. Thus, there is at least agreement on the basal position of the anthocerotes based upon two very different data sets.

The absence of microtubule arrays that determine the plane of subsequent cytokinesis in male gametophytic tissue is a character found throughout the land plants surveyed hitherto (Brown & Lemmon, 1991). Thus, the occurrence of both AMS and modified PPB arrays in the spermatogenous cells of anthocerotes is of special significance. The retention of these MT arrays may be related to the special nature of anthocerote nuclear divisions such that, without the microtubule systems to ensure proper plastid division, nuclear division could not occur. In a comparison of polyplastidic and monoplastidic mitosis in two species of *Lycopodium*, Renzaglia *et al.* (1994) also found plastid-associated microtubules (although seemingly not as extensive as

Figure 6. A. The final spermatogenous cell division occurs in a plane oblique to that in previous divisions and is organized by a single bicentriole (BC) and the surrounding pericentriolar material at each pole. c=chromosome. B. A higher magnification micrograph revealing the bicentriole (BC) as the focus of the spindle microtubules (one marked with an arrowhead). Bars=0.5 μ m in A; 0.2 μ m in B.

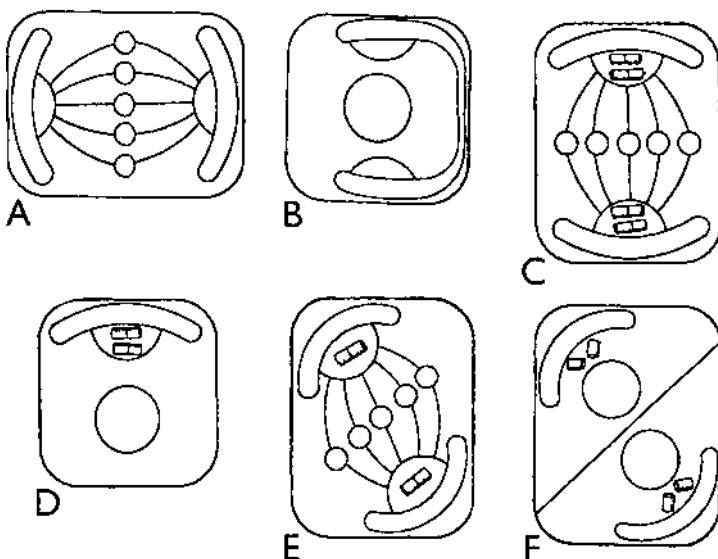


Figure 8. Schematic interpretation of the origin of bicentrioles in anthocerote spermatogenous cells. In A, the pericentriolar material associated with the plastid envelope serves as the spindle MTOC for mitosis. Just prior to the penultimate division (B), the plastid has separated into masses at each pole and pericentriolar material accumulates at the site for eventual spindle formation. A pair of bicentrioles forms within the pericentriolar material at each pole (C) and the penultimate division commences with a bicentriole and associated pericentriolar material serving as the spindle MTOC. In D, the cell just prior to the final spermatogenesis cell division contains a pair of bicentrioles. For the final division, the bicentrioles separate and rotate to poles oblique to those in the penultimate division (E). The final (F) cytokinesis, produces two wedge-shaped daughter cells and the bicentrioles are now split, each forming the basal bodies of the two flagella.

those described herein for the anthocerote spermatogenous cells) only in the monoplastidic species. Therefore, the presence of plastid-associated microtubule systems represents a plesiomorphic characteristic related to mechanisms required for coordinated plastid/nuclear divisions in monoplastidic cell lineages.

13.5.2 Bicentrioles in anthocerotes arise in pericentriolar material

In anthocerotes, the division of the plastid and the division of the nucleus are tightly linked (Brown & Lemmon, 1998; Vaughn *et al.*, 1992), ensuring that the cell division will result in two cells with one plastid each, rather than the production of biplastidic and aplastidic cells. This link begins with the movement of the plastid to a plane anticipating the direction of the subsequent cell division. In the centre of the division plane, two bands of microtubules, the AMS and a relatively unfocused PPB develop. The microtubules of the AMS are organized by electron-opaque material that accumulates at first at the isthmus of the plastid division. Subsequently, this band of MTOC material splits and begins to move towards the two poles, causing a movement of the two halves of the plastid to opposite ends of the cell. This assemblage of MTOC material, now concentrated at opposite poles, also organizes the mitotic spindle for the subsequent division. Prior to the penultimate spermatogenous

cell division, a pair of bicentrioles develops at each pole from this undefined pericentriolar material. The spermatogenous cells then proceed to divide twice, using the pericentriolar material and the associated bicentrioles as the spindle MTOC. Without duplication, the pair of bicentrioles splits before the spermatid mother cell divides, and the individual bicentrioles rotate to an angle such that cell division is oblique to the plane of the previous cell division. A diagrammatic model of the origin and subsequent development of bicentrioles is shown in Fig. 8.

13.5.3 The development of bicentrioles in anthocerotes follows a route different from hepatics or mosses

In the mosses and hepatics that have been studied, the bicentrioles apparently arise in an MTOC area (Vaughn & Renzaglia, unpublished; Bernhard & Renzaglia, unpublished) and one pair of bicentrioles arises in this structure just prior to the final division. Once formed, the bicentrioles and associated pericentriolar material separate for the final spermatogenous division, this ensuring that each daughter cell will have only one bicentriole. In the anthocerotes, two pairs of bicentrioles form at each of the electron-opaque spindle areas prior to the penultimate division. In the penultimate division, the pairs of bicentrioles and associated pericentriolar material act as the spindle MTOC, and, with no further duplication of bicentrioles, each daughter cell receives a pair of bicentrioles. Prior to the final spermatogenous cell division, the bicentrioles and associated pericentriolar material migrate to form a division plane oblique to those in previous divisions so that wedge-shaped cells result, similar to those in other bryophytes. Thus, the time of production of bicentrioles appears to differ between the anthocerotes and other bryophytes, although the relatively few species monitored and the lack of serial section reconstructions on these other species limits our ability to generalize with complete certainty.

Certain other aspects of bicentriole formation are similar in the three groups, however. All of these groups produce the bicentrioles at sites which were previously utilized as the site of the spindle MTOC of the previous division. In anthocerotes, this is the electron-opaque substance associated with the plastid-associated AMS band of microtubules. In the hepatics, an accumulation of electron-opaque substance at the polar organizer and, in mosses, at the site of the last spindle poles (Bernhard & Renzaglia, unpublished) are the sites of bicentriole formation. Once formed, the bicentrioles never duplicate, as do centrioles in mammalian centrosomes.

13.5.4 Development of centrioles/basal bodies/flagella follows several different routes in lower land plants

This study is part of a more extensive investigation of spermatogenous cell development in bryophytes (Vaughn, Sherman & Renzaglia, 1993; Vaughn & Renzaglia, in preparation) and pteridophytes (Vaughn, Sherman & Renzaglia, 1993; Hoffman, Vaughn & Joshi, 1994; Hoffman & Vaughn, 1995). Two basic patterns of centriole/basal body formation have come out of this work (Robbins, 1984).

In organisms with two flagella, such as the bryophytes, the bicentrioles arise at the site occupied by the previous spindle MTOC, such as the polar organizer of hepatics (Vaughn & Renzaglia, in preparation) and the electron-opaque MTOC of

anthocerotes (this study). Centrin, a calcium-binding protein associated with centrosomes, accumulates at this site, prior to the formation of the centriole (Vaughn & Renzaglia, in preparation). Bicentrioles arise either in the penultimate (anthocerotes) and or final (hepatic and mosses) divisions and no precursor or template is found, with the possible exception of the hub area between the two halves of the bicentriole.

In pteridophytes (except *Phylloglossum*; Renzaglia & Whittier, 1993) and seed plants with many-flagellated sperm, bicentrioles do not form directly, but rather form an intermediate structure such as the blepharoplast, which serves as a template for basal body formation. The blepharoplast contains neither tubulin or centrin, but does react with MPM-2 and C-9 monoclonal antibodies that recognize the pericentriolar material in mammalian centrosomes (Hoffman, Vaughn & Joshi, 1994). The blepharoplast clearly acts as an MTOC during the final two divisions in spermatogenesis, organizing both interphase and mitotic arrays (Hepler, 1976; Hoffman, Vaughn & Joshi, 1994). Only after reorganization of the blepharoplast following the final mitosis do basal bodies form and does centrin begin to accumulate (Vaughn, Sherman & Renzaglia, 1993; Hoffman, Vaughn & Joshi, 1994). Thus, both biochemically and structurally, the origins of centrioles in biflagellated and multiflagellated sperms appear to follow different developmental pathways, although both involve conversion of previously utilized MTOCs. Critical investigations are now required of organisms that produce spermatazoids with more than two but less than the thousand flagella found in some of the advanced ferns and gymnosperms (e.g. Renzaglia & Whittier, 1993) to clarify the relationships between the bryophytes and the more advanced taxa.

13.6 ACKNOWLEDGEMENTS

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14. Microtubules and gametophyte morphogenesis in the liverwort *Marchantia paleacea* Bert.

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14.1 SUMMARY

The microtubules (MTs) in the developing gametophytes of *Marchantia paleacea* are directly or indirectly involved in the mechanisms controlling: (a) the establishment of cell polarity, (b) cell shaping, (c) cell differentiation, and (d) determination of the cell division plane. These phenomena, which underlie gametophyte morphogenesis, have been studied in developing oil-body cells, mucilage papillae, air pores and photosynthetic filaments.

In meristematic cells MT reorganization precedes the establishment of a new polar axis, which in turn is directly related to the determination of the division plane. During prophase structurally defined microtubule organizing centres (MTOCs) are activated on the polar axis, in contact with the nuclear envelope, which persist up to prometaphase. This axis determines the initial orientation of the cell plate. The final orientation of the cell plate, i.e. the sites of its fusion with the parent walls, are predicted before the onset of mitosis by typical or atypical preprophase microtubule bands (PPBs), or atypical PPBs and independent cortical MT bundles.

In differentiating cells cortical MTs define the orientation of newly deposited cellulose microfibrils and directly control cell shaping. In addition, endoplasmic MT systems are involved in the spatial distribution of the organelles. These MT activities are obvious in the club-shaped mucilage papillae, in photosynthetic filament cells and in air pore cells. The cortical MTs controlling protodermal cell shaping, are also involved in the mechanism of intercellular space formation in the protoderm. This is a unique phenomenon in the gametophyte of Marchiales.

The participation of MTs in cell differentiation is obvious in the oil-body cells, in which endoplasmic MTs participating in the directed movement of the dictyosome vesicles, are intimately involved in the *de novo* formation of the oil-bodies. In addition, in the advanced stages of the oil-body cell differentiation typical MTs are replaced by other tubules with a larger diameter, which may provide mechanical support for the oil-body.

KEYWORDS: *Marchantia paleacea*, morphogenesis, microtubules, oil bodies, mucilage papillae, intercellular spaces, air chambers, cellulose microfibrils.

14.2 INTRODUCTION

The MT* cytoskeleton is the main tool by which higher plant cells control their morphogenesis (Seagull, 1989; Lloyd, 1991; Cyr, 1994). Information on the MT cytoskeleton in the different vegetative cell types of developing gametophytes of liverworts is limited to the organization and function of MTs in: (a) the mitotic and cytokinetic apparatus (reviews by Steer, 1984; Duckett, 1986; Duckett & Renzaglia, 1988), (b) developing gemmae of *Odontoschisma denudatum* (Duckett & Ligrone, 1995), and (c) some cell types of *Marchantia paleacea* gametophytes. Axial arrays of endoplasmic MTs have recently been described in putative food conducting elements of *Asterella* (Ligrone & Duckett, 1994a).

This chapter attempts to review the existing information and presents new data, particularly on the use of drugs that disrupt MTs, on MT function in the gametophyte development of *Marchantia paleacea*. Particular attention is given to the specific role of MTs in: (a) oil body initiation and development, (b) morphogenesis of mucilage papillae, photosynthetic filaments and air pores, (c) development of intercellular spaces in protoderm and (d) determination of the plane of cell division. The data presented have been derived from transmission electron microscopy and tubulin immunolabeling. The supporting micrographic data may be found in earlier papers by the authors.

14.3 OIL BODY DEVELOPMENT

The oil bodies (OBs) of liverworts are cytoplasmic sacs limited by a single membrane containing lipophilic globules suspended in a matrix rich in carbohydrate (reviews by Duckett, 1986; Duckett & Renzaglia, 1988; Schuster, 1992). Their distribution and development varies between groups. In Marchiales, OBs are restricted to idioblasts scattered through the thalli whereas in other groups they are present in virtually every vegetative cell. In the latter they originate from the endoplasmic reticulum (Duckett & Ligrone, 1995). However, in *Marchantia*, and probably in Marchiales generally, the OBs are formed by a unique process in which dictyosomes, endoplasmic reticulum and MTs all appear to participate (Galatis & Apostolakos, 1976; Galatis, Apostolakos & Katsaros, 1978; Galatis, Katsaros & Apostolakos, 1978). In *Marchantia* the OB cells develop in the dorsal epidermis, in the inner tissues of the thallus and in the scales. At maturity they encompass a large OB (Fig. 1), displaying one or more lipophilic globules embedded in a matrix mainly of polysaccharidic composition, and filling most of the cell lumen (Galatis *et al.*, 1978b, c). Histochemical tests also reveal the existence of polyphenols and the absence of proteins. The presence of polysaccharides is a strong indicator of dictyosomal participation in OB development. Polysaccharides have not been localized in OBs of other liverworts (Duckett & Ligrone, 1995).

OB cell differentiation starts in the apical thallus region with the establishment of polarity in young OB cells. This is expressed by the displacement of the nucleus and

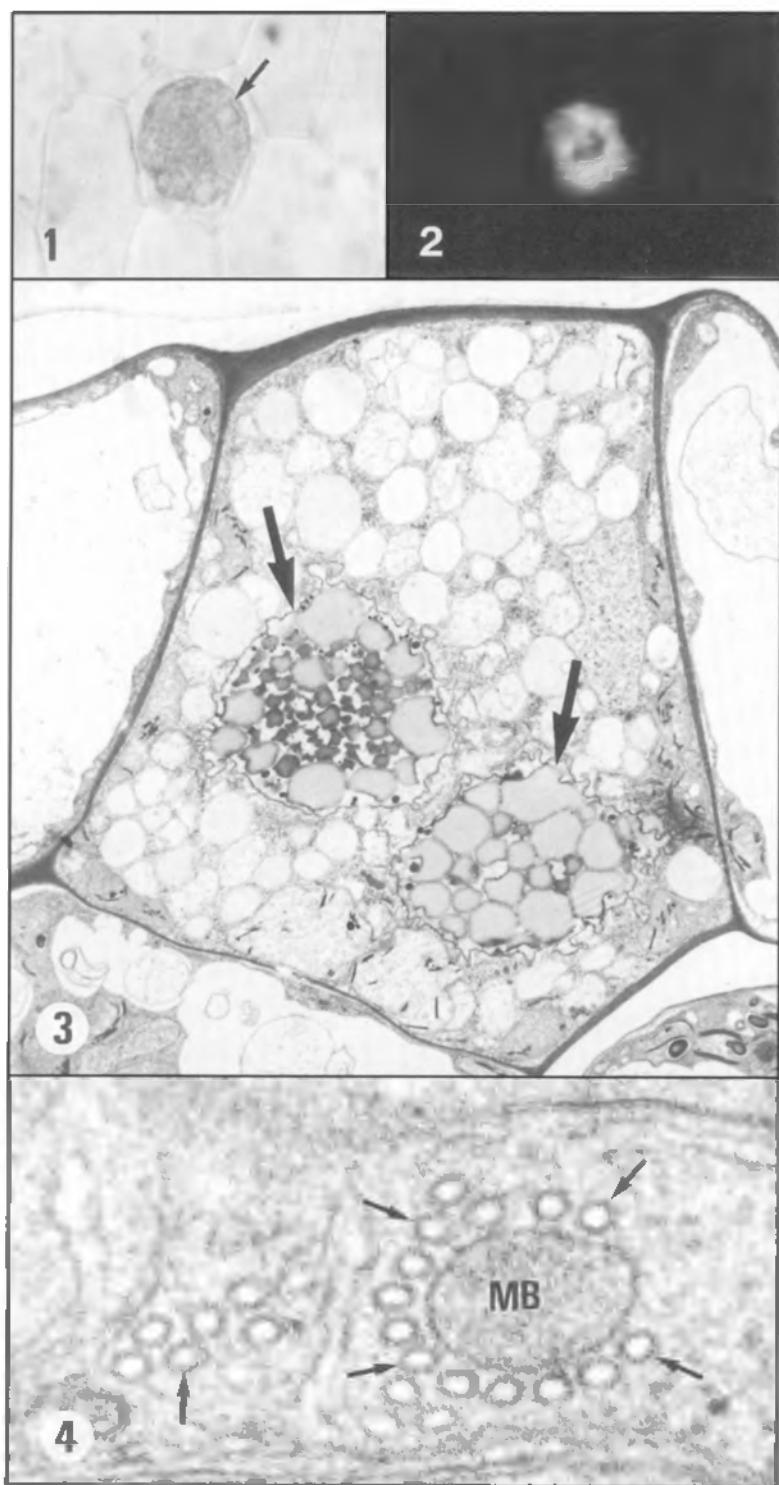
* Abbreviations: AC air chamber, AP air pore, IS intercellular space, MP mucilage papilla, MT microtubule, MTOC microtubule organizing centre, OB oil body, PF photosynthetic filament, PPB preprophase microtubule band.

larger organelles towards the cell periphery and the formation of a clear cytoplasmic region at the cell centre. This region is traversed by endoplasmic MTs, that are focused on a site where numerous dictyosome vesicles are gathered. Dictyosome vesicle fusion produces a nascent OB from which MTs emanate (Galatis *et al.*, 1978a; see also Fig. 2). The newly formed OB is surrounded by profiles of endoplasmic reticulum. As it grows, by the continuous contribution of materials from the endoplasmic reticulum and dictyosomes, it becomes filled with a granular matrix in which lipophilic globules gradually appear.

The MTs in OB cells appear to be involved in the formation of the clear cytoplasmic area and the movement of dictyosome vesicles towards the region of MT convergence, where the OB is initiated. MTOCs may function in this region. Their activation is probably the first event in OB development. The essential role of MTs in this process is supported by the fact that in thalli treated with anti MT-drugs, OB development is severely disrupted (Apostolakos & Galatis, unpublished data). After treatment of developing thalli with 20 µM oryzalin for 20–48 h or with 2 mM colchicine for 48–72 h the MTs are destroyed, while more than one OB appears in each OB cell (Fig. 3). They develop independently of each other or fuse to form a larger irregular OB. Thus, MTs are clearly involved in a *de novo* formation of this particular cytoplasmic compartment.

At the stage of synthesis of the lipophilic globules in the OB compartment, the MTs are replaced by another system of tubules (Fig. 4), which forms a dense network around the OB (Galatis & Apostolakos, 1976; Galatis *et al.*, 1978b). They traverse the cytoplasm surrounding the OB, parallel to its membrane, often in bundles, and form complexes with elongated microbodies. Each tubule consists of several subunits and has a diameter of 33–37 nm. The fact that these tubules: (a) are visible after tubulin immunolabeling, (b) are disorganized after treatment with oryzalin and colchicine, and (c) reappear in thalli recovering after removal of the anti-MT drugs, strongly suggests that they consist of tubulin (Apostolakos & Galatis, unpublished data). These unusual tubules are probably assembled by more than 13 protofilaments, and to our knowledge, this is the first case of 'MTs' displaying a larger number of protofilaments in plant cells. MTs with a diameter of approximately 30 nm, and consisting of 15 protofilaments have been observed during the final stages of differentiation of epidermal cells in *Drosophila* (Tucker *et al.*, 1986), where they are formed by MTOCs residing on the plasma membrane (Mogensen & Tucker, 1987; Mogensen, Tucker & Stebbings, 1989). Our observations favour the hypothesis that the nucleation sites of the large tubules in *Marchantia* are localized on the OB surface.

This system of large tubules seems to provide mechanical support for the OB, while at the same time it may align the elongated microbodies parallel and close to the OB surface. Another possibility is that they may prevent the OB from merging with the plasmalemma. In colchicine- and oryzalin-affected OB cells, in which the absence of the large tubules allows the OBs to form projections reaching the plasmalemma, images suggesting the fusion of the OB membrane with the plasmalemma have been observed. In colchicine- or oryzalin-treated thalli many OB cells are disrupted as a consequence of breakage of the cell walls. This is probably the result of mechanical forces exerted by the growing OB on the cell wall, the growth of which is delayed in the treated OB cells and/or by fusion of the OB membrane with the plasmalemma.



14.4 MUCILAGE PAPILLA DEVELOPMENT

The mucilage papillae (MP) in *Marchantia* are elongated club-shaped cells (Fig. 5 inset), which secrete large quantities of mucilage. Mucilage in *Marchantia* (Galatis & Apostolakos, 1977) as in other liverworts and mosses (Ligrone, 1986; Duckett, Renzaglia & Pell, 1990) is produced by the endomembrane system and secreted by exocytosis of dictyosome vesicles. The active MP are polarized cells, in terms of cell shape and organelle distribution. The base of the MP is occupied by vacuole(s), the nucleus, plastids and mitochondria are localized at the centre, while the apical cytoplasm is rich in dictyosomes and endoplasmic reticulum (Galatis & Apostolakos, 1977). The latter form extensive stacks with numerous active dictyosomes around their periphery.

In *Marchantia*, the MP are produced by marginal cells of developing scales. Before division these cells (MP mother cells) become polarized and form a tubular protrusion rich in cytoplasm. By an asymmetrical division this protrusion is separated from the mother cell and differentiates into a MP (Galatis & Apostolakos, 1977). During polarization the MP mother cell undergoes a controlled expansion. The axis of cell growth is perpendicular or oblique to the scale periphery. While in unpolarized cells MTs are found along all the walls, in outgrowing MP mother cells they are absent in the apical region. They are found at the base of the cell protrusion, where they form a ring transverse to the polar axis (Apostolakos & Galatis, 1993). A similarly aligned system of cellulose microfibrils is deposited in the wall externally to this MT ring. This ring thus represents a 'morphogenetic tool' establishing the new growth axis, and the wall region that will expand in a polar fashion.

The MTs in MP form distinct cortical and endoplasmic systems, the organization of which changes continuously during MP development (Apostolakos & Galatis, 1993). In the young MP most of the cortical MTs form a ring occupying a median cell region and are aligned transversely to the cell axis. The apical cell region lacks cortical MTs, while the basal part contains axially orientated cortical MTs. Relatively few endoplasmic MTs are found in the central and basal portion of the young MP.

In differentiating MP the cortical MTs are realigned, while the endoplasmic ones proliferate. At this stage the cortical MT cytoskeleton consists of oblique and/or axial MTs, running along the whole cell surface up to the MP tip. Relatively few MTs retain the initial transverse orientation. The endoplasmic MTs form distinct bundles traversing the whole length of the cell. MP growth is accompanied by deposition of wall material along the whole surface of the cell except for the basal portion. The newly deposited cellulose microfibrils are co-aligned with the underlying cortical MTs.

In developing MP the cortical MTs controlling the orientation of the newly-deposited cellulose microfibrils determine the course of cell shaping. In particular,

Figures 1–4. Fig. 1. Epidermal OB cell containing a large OB (arrow). Living tissue. $\times 1,100$. Fig. 2. A young OB cell as it appears after tubulin immunolabeling. MTs diverge from the nascent OB surface. $\times 900$. Fig. 3. OB cell taken from a thallus treated with colchicine. The absence of MTs results in the formation of two OBs (arrows). $\times 4,500$. Fig. 4. Portion of an OB cell being at the stage of synthesis of lipophilic material. The arrows show cytoplasmic tubules. MB microbody. $\times 150,000$.

the central, circumferentially located MT ring defining the deposition of similarly aligned microfibrils, favours longitudinal expansion, preventing cell bulging (Apostolakos & Galatis, 1993). In this way the MP becomes club-shaped (Fig. 5 inset). Circumferential cortical MT arrangements related to a directed cell expansion have been described in tmema cells of the mosses *Funaria* (Bopp *et al.*, 1991; Sawidis *et al.*, 1991), *Calymperes* (Ligrone, Duckett & Eggunyomi, 1992), and *Bryum* (Goode *et al.*, 1993). Endoplasmic MT bundles in MP are possibly involved in the spatial arrangement of organelles, in a similar way to that in the apical cells of moss protonemata (reviews by Schnepf, 1982, 1986) and in food-conducting cells of bryoid mosses (Ligrone & Duckett, 1994b). Well organized cortical and endoplasmic MT and microfilament systems, seem to be associated with the organelle distribution, particularly plastid partitioning, during formation of the tmema cells in the moss *Bryum* (Goode *et al.*, 1993).

Key participation of MTs in the shaping and polarization of MP is supported experimentally (Apostolakos & Galatis, unpublished data). In colchicine- and oryzalin-affected MP both cell shape and organelle distribution are disturbed. The apical region bulges, the nucleus is displaced to the swollen region of the cell, while the plastids and the vacuoles no longer occupy their normal positions (Fig. 5). In addition, large quantities of wall material are uniformly deposited over the surface of the swollen cell region (Fig. 5). This thickened wall region appears to prevent mucilage secretion, since large quantities of mucilage accumulate between the plasmalemma and the thickened wall, or are trapped within the latter.

14.5 PROTODERMAL INTERCELLULAR SPACE DEVELOPMENT

A unique phenomenon occurring in Marchantiiales is the formation, in the apical region of the thallus, of protodermal intercellular spaces (ISs). This is the first event in a long process, by which the air pores (APs) and the air chambers (ACs) are generated. ACs are giant subepidermal ISs communicating with the outside by means of the APs. In *Marchantia* the protodermal ISs open in the apical thallus region at positions where three or more cells meet. Sometimes, ISs open between two protodermal cells in the middle of their common anticlinal wall (Apostolakos, Galatis & Mitrakos, 1982; Apostolakos & Galatis, 1985a). The ISs together with the surrounding cells are defined as initial apertures.

The ISs of the initial apertures begin to form schizogeneously from the protoderm surface and proceed inwards (Fig. 6), a process also described in other liverworts (Kronestedt, 1982a, b). At the beginning of this process, the external periclinal walls of the protodermal cells bulge locally and form small surface cavities at the sites where the IS will open (Fig. 6a). The anticlinal wall below the cavity thickens. At this stage the cytoplasm below the walls delimiting the cavity is traversed by three MT 'systems' (Fig. 6a): (a) a 'system' of anticlinal MTs lining part of the wall below the cavity and reaching the external periclinal wall; (b) a periclinal MT system traversing the cortical cytoplasm which abuts on the anticlinal walls that terminate in the cavity; (c) another MT 'system' diverging into the endoplasm. These MT systems converge on the base of the surface cavity, where putative MTOCs may function (Apostolakos & Galatis, 1985a).

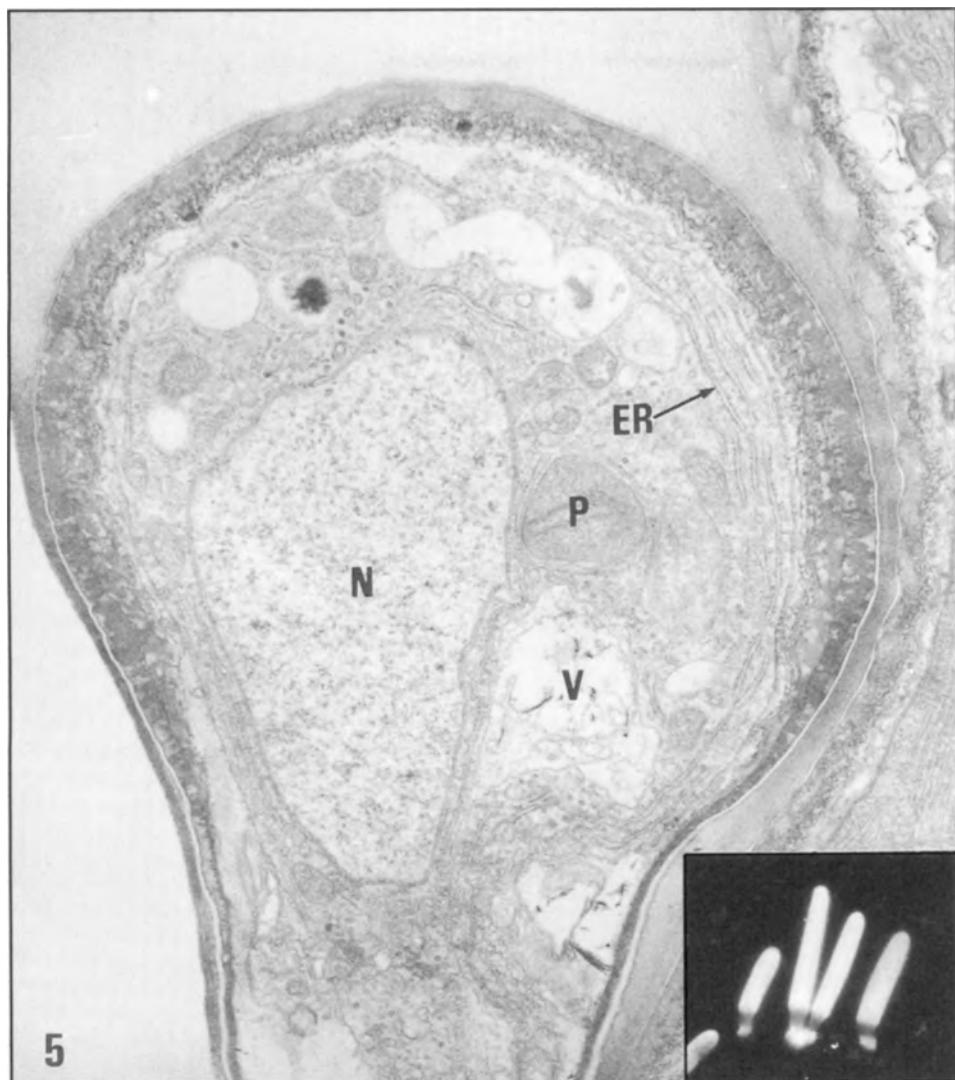


Figure 5. The swollen apical region of a MP affected by colchicine. *ER* endoplasmic reticulum; *N* nucleus; *P* plastid; *V* vacuole. $\times 15,000$. Inset: Normal MP, as they appear after calcofluor staining $\times 300$.

The wall thickening below the cavity expands gradually down to the subprotodermal cell layer, a phenomenon which is accompanied by a similar displacement of the putative MTOCs in the same direction. At the same time the initial aperture cells bulge outwards, a phenomenon associated with expansion of the anticlinal walls perpendicularly towards the protoderm surface, except for the thickened region of the wall below the cavity. Since the thickened region cannot follow the expansion of the other walls, it is forced to split apart along the middle lamella and thus initiates the IS (Apostolakos & Galatis, 1985a). Gradually, the IS extends towards the sub-

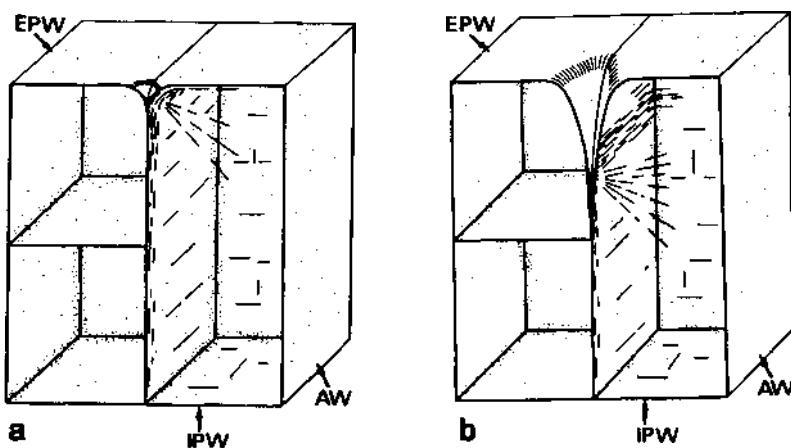


Figure 6. Three-dimensional diagram showing MT arrangement in an initial aperture cell during surface cavity (a) and IS (b) formation. The MTs in the other cells have not been drawn. *AW* anticinal wall; *EPW* external periclinal wall; *IPW* internal periclinal wall (from Apostolakos & Galatis, 1985a).

protodermal layer (Fig. 6b). During this process the cortical cytoplasm lining the base of the nascent IS is always traversed by the above MT systems (Fig. 6b).

The above findings suggest that the mechanism of protodermal IS formation is based on a localized wall reinforcement, which accurately controls the direction of expansion of the initial aperture cells. This directed expansion is defined by the cellulose microfibrils deposited in the thickened region of the anticinal wall, which will split, as well as and in other walls. The orientation of the microfibrils is controlled by cortical MT systems. Thus, the formation of the ISs in the protoderm of *Marchantia* keeps pace with cell shaping, a process controlled by the MT cytoskeleton.

14.6 AIR PORE DEVELOPMENT

The APs in *Marchantia* consist of four to seven superimposed cellular rings (Figs 7d, and 8b). Together these form a barrel-like structure, which delimits an intercellular canal (Figs 7d, 8a, b). Through this canal the AC communicates with the external environment. Half of the AP rings are elevated over the epidermis, while the remainder are immersed into the AC (Fig. 8a). In surface view the differentiated AP cells display more or less the form of a 'curved trapezium' (Fig. 8c), except for those of the inner ring, which exhibit a particular shape, due to the formation of projections which restrict the opening of the AP canal (Fig. 8c₅).

Both the APs and the ACs are produced by a common ontogenetic process (Apostolakos *et al.*, 1982), the first event of which is the formation of the ISs of the initial apertures (see previous section). Subsequently, a precise sequence of anticinal and oblique divisions in the protodermal and subprotodermal cells generates the ACs and the mother cells of the APs. The latter, which are usually four, border the entrance of the AC (Fig. 7a). At a later stage, periclinal divisions of the AP mother

cells initiate AP formation (Fig. 7b). Before their periclinal divisions the AP mother cells undergo considerable expansion above and below the thallus surface (Fig. 7a). By this process a new axis of polar growth is established in the protoderm, and this is followed by periclinal divisions (Apostolakos *et al.*, 1982).

The establishment of the new growth axis in the AP mother cells is a gradual process beginning with the reorganization of the cortical MTs (Apostolakos & Galatis, unpublished data). As a rule, the MTs in protodermal cells line the anticlinal walls transversely to the protoderm surface. However, in the AP mother cells distinct bundles of periclinal (*i.e.* parallel to the protoderm surface) MTs are formed. These line the middle of the anticlinal walls, bordering the entrance of the AC (Fig. 7a). The MTs lining the remainder of the anticlinal walls, which terminate at the entrance of the AC, converge in the same regions (Fig. 7a). Here the endoplasmic MTs also converge. At these sites the MTs are at their most numerous and form complexes with vesicles. These regions may function as MTOCs, or at least they have the ability to arrange the MTs.

The above cortical MT arrays appear to control the deposition of similarly aligned systems of cellulose microfibrils in the cell walls (Apostolakos & Galatis, unpublished data). As a result periclinal microfibrils are deposited in the middle of the walls which delimit the entrance to the AC, while in the anticlinal walls, which terminate there, the microfibrils are obliquely aligned relative to the protoderm surface. These cellulose microfibril systems allow the expansion of the anticlinal walls transversely to the protoderm, thus determining the growth of the AP mother cells along the same axis. Thus, the mechanism by which a new growth axis is established in the protoderm of *Marchantia* is a shift in cortical MT organization. The MT organization predicts a new cell polarity, which is followed by a change in the orientation of the cell division plane, which becomes periclinal.

Although the divisions of the AP mother cells are asynchronous, the periclinal

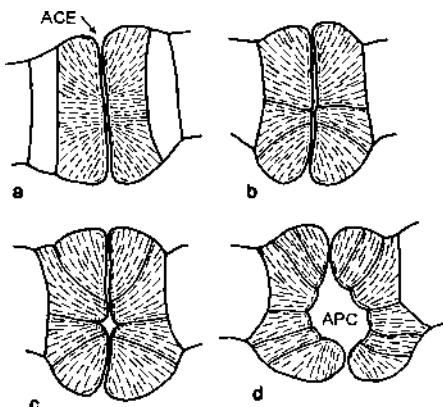


Figure 7. Diagrammatic representation of the successive developmental stages of the AP in transverse sections. (a) AP mother cells, (b) stage of the three-rings, (c) stage of the four rings, (d) differentiation completed AP. These diagrams show the arrangement of the MTs which line the walls delimiting the ACE (a, b) or the APC (c, d) as well as the walls terminated at the ACE (a, b) or the APC (c, d). They have not been made in the same magnification. The dots denote transversely sectioned MTs, while the lines denote longitudinally sectioned ones. *ACE* air chamber entrance; *APC* air pore canal.

daughter walls are aligned along the same plane. They meet the parent walls which delimit the entrance of the AC at their mid point (Fig. 7b), *i.e.* at the sites of intense wall expansion and of MT convergence. This fact suggests that in this region, where putative MTOCs are probably activated, a mechanism functions which affects the direction of growth of the cell plate. In this way the first two rings of the AP are formed.

The growth of the forming AP transversely to the thallus surface continues throughout the next developmental stages. At the two ring-stage the greatest expansion of the anticlinal walls perpendicularly to the protoderm takes place at the position where the walls delimiting the entrance of the AC meet the periclinal walls, which separate the two AP rings. At this position, bundles of periclinal MTs line the anticlinal walls delimiting the entrance of the AC (Fig. 7b). In the same position the MTs lining the walls terminating in this area converge (Fig. 7b). Therefore, the cellular conditions, prevailing before the periclinal divisions that allow the expansion of the anticlinal walls transversely to the protoderm, are re-established. As a consequence the cells of the external AP ring grow outwards, while those of the internal AP ring grow towards the AC (Fig. 7b, c). At the same time, the walls detach locally at the positions where the common periclinal walls meet those delimiting the AC entrance (Fig. 7c). Afterwards, the detached wall regions become locally thickened. By this process a rudimentary canal is formed at the middle of the developing AP (Fig. 7c). At the same time, the cells of the AP rings undergo oblique divisions, which lead to the formation of additional rings of the AP. In the daughter cells from these divisions the cortical MTs display the organization described above, which favours the further growth of the AP perpendicular to the thallus surface.

After their formation the AP rings grow in diameter, resulting in significant widening of the rudimentary AP canal (Fig. 7d). This process is concomitant with an increase in the size of the individual AP cells and a change in their shape. The cells of the newly formed AP appear triangular in surface view, but during their differentiation become curved trapezia. These changes in the cell shape are determined by the alignment of the cellulose microfibrils in the walls. From the detailed studies of Ziegenspeck (1941, 1942) it is clear that the microfibrils in the walls that delimit the AP canal are anticlinal (Fig. 8b), while those of the anticlinal and periclinal walls

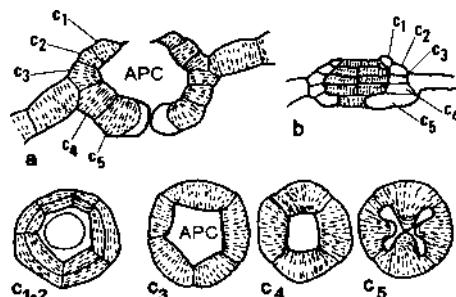


Figure 8. Diagram displaying the alignment of the cellulose micellae in the walls of a differentiated multilayered AP. (a) Micellae in the anticlinal walls terminating at the APC. (b) Micellae in the anticlinal walls bordering the APC. (c_1-c_5) Micellae in the periclinal walls of the cells of the successive AP rings. APC air pore canal (from Ziegenspeck, 1941).

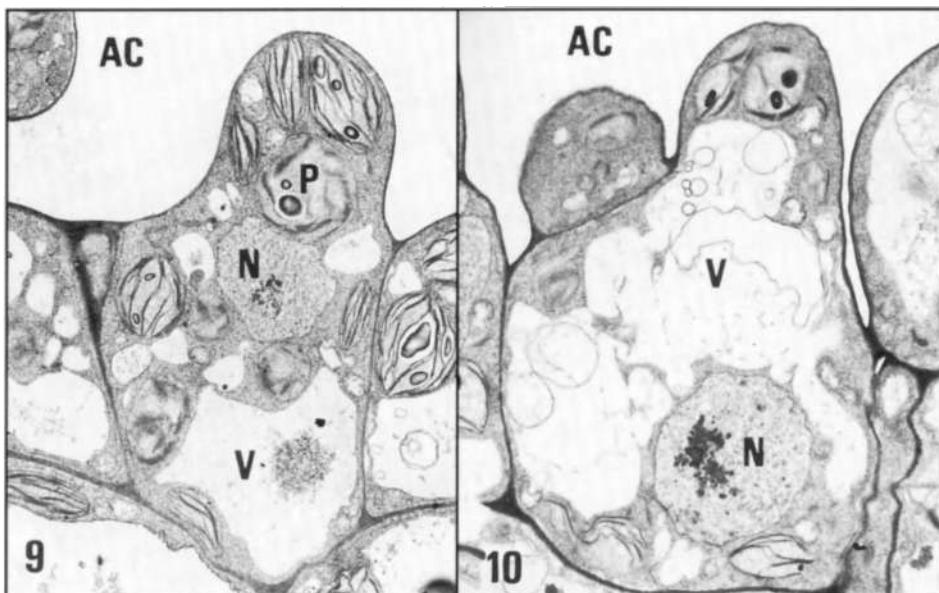
which terminate at the AP canal display a more or less radial arrangement (Fig. 8a, c). These microfibril systems allow the AP cells to assume the form of curved trapezia. The local thickening of the walls, outlining the AP canal, also contribute to this process. These walls, because of their thickenings, cannot follow the expansion of the opposite walls and therefore curve. The deposition of the above systems of cellulose microfibrils is controlled by the cortical MT systems (Apostolakos & Galatis, unpublished data). After the formation of the AP rings, the MTs lining the walls that delimit the AP canal are realigned and become anticinal (Fig. 7d). The MTs lining the other walls retain the orientation they had during the formation of the AP rings (Fig. 7d). Thus, the cortical MTs are a fundamental element in the morphogenesis of the multilayered APs.

The formation of the protrusions of the innermost AP ring cells (Fig. 8c₅) is also controlled by cortical MTs. A MT ring is organized at the base of the forming cellular projection (Fig. 7d). This, defines the deposition of a similarly oriented annulus of cellulose microfibrils, which in turn defines the position where the cellular protrusion is formed. Depolymerization of the MTs with anti MT-drugs inhibits the formation of the cellular projection (Apostolakos & Galatis, unpublished data).

14.7 PHOTOSYNTHETIC FILAMENT DEVELOPMENT

Photosynthesis in *Marchantia* is mainly carried out by a particular tissue, the photosynthetic filaments (PFs), which are formed within the ACs. Each PF is a multicellular, usually branched structure, which emerges from an epithelial cell (PF mother cell) of the AC and consists of a small number of cells. The terminal cell of the PFs is usually cylindrical in shape, while the remainder are more or less rounded. PFs are formed at an early stage in AC development. The PF mother cell grows into the AC and, by an asymmetrical division, separates a small spherical cell (initial PF cell). The latter elongates to become cylindrical and, by a series of divisions, gives rise to a PF (Apostolakos *et al.*, 1982).

Before its division, the PF mother cell becomes polarized. Part of the cell facing the AC expands to form a more or less tubular projection (Fig. 9). This is achieved by a local expansion of the external periclinal wall, presumably induced by forces generated by the growing protoplast. Most of the cytoplasm and organelles occupy the tip of the cell, while the nucleus lies at the base of the outgrowth (Fig. 9). The shaping of the tubular projection is controlled by a ring of cortical MTs, localized at its base, which seems to control the deposition of a similar annulus of cellulose microfibrils in the cell wall. The microfibril annulus determines (a) the establishment of a new growth axis in the cell, and (b) the site and the shape of the cellular protrusion (Apostolakos & Galatis, 1993). The cortical MTs in the mother cells of the gemmiferous filaments of *Odontoschisma* appear to have a similar organization and function (Duckett & Ligrone, 1995). Colchicine or oryzalin treatment inhibits the formation or disrupts the shaping of the tubular projection in the PF mother cells (Apostolakos & Galatis, unpublished data). In addition, the polarization of the affected PF mother cells is disturbed, as shown by the basal position of the nucleus (Fig. 10). Thus, the shaping and polarization of the PF mother cells are directly or indirectly controlled by MTs. It should be noted that this kind of MT organization



Figures 9–10. Fig. 9. Polarized PF mother cell displaying an outgrowth into the AC. Note the position of the vacuole (V), nucleus (N) and plastids (P). $\times 4,500$. Fig. 10. PF mother cell affected by oryzalin for 20 h. The cell outgrowth was formed before the treatment. The polar organization of the protoplast has been disturbed, a fact that is visible by the position of the nucleus (N) and the vacuole (V). $\times 4,700$.

and activity has not been described in branching moss protonemata, where new axes of growth are established (Schnepf, 1982; Doonan & Duckett, 1988; Doonan, 1991). In protonemata of *Funaria* a ring of cortical actin filaments defines the position of branch formation (Quader & Schnepf, 1989).

MTs also control cell shaping as well as the position of the nucleus in developing PFs. The initial PF cells and the terminal cells of the PFs display axial growth and assume a tubular form. Their cortical MTs are usually perpendicular to the cell axis. Most of them are localized at the middle of the cell surface and are arranged in a ring occupying an equatorial plane. This ring defines the deposition of circumferentially aligned cellulose microfibrils in the cell wall, which establish axial growth and favour the formation of tubular cells. Circumferential microfibrils also prevent an increase in cell diameter, resulting in the appearance of a shallow median constriction in the cells (Apostolakos & Galatis, 1993).

In the above cells the elliptical preprophase-prophase nucleus lies on the plane of the constriction, where mitosis and cytokinesis take place. As a result the daughter wall joins the preexisting walls at the cellular constriction. Similar phenomena have been observed in dividing terminal cells of growing gemmiferous filaments in the liverwort *Odontoschisma* (Duckett & Ligrone, 1995). In PF cells, the nuclear position in the area of the cell constriction seems to be controlled by endoplasmic MTs. During interphase these cells display a distinct perinuclear MT system, which is connected to the cell cortex by MT bundles, some of which terminate at the cell apex (Apostolakos & Galatis, 1993). The nucleus remains at this position during

preprophase-prophase probably because astral MT bundles connect the polar MTOCs with the apical and basal regions of the cell.

14.8 DETERMINATION OF THE CELL DIVISION PLANE

A ubiquitous characteristic of dividing cells of liverworts is the presence of structurally defined MTOCs in the polar regions of prophase cells (reviews by Steer, 1984; Duckett, 1986; Duckett & Renzaglia, 1988). These structures correspond to accumulations of endoplasmic reticulum membranes and vesicles, among which there lies electron-opaque material. MTs emanating from the polar MTOCs form a well organized bipolar prophase spindle, consisting of two interdigitating half spindles. The prophase spindle forces the nucleus, still with an intact envelope, to assume an elliptical shape (Galatis & Apostolakos, 1977; Fowke & Pickett-Heaps, 1978; Kronestedt, 1982a; Steer, 1984; Apostolakos & Galatis, 1985b, 1992, 1993; Brown & Lemmon, 1990, 1992; Duckett & Ligrone, 1995). During prometaphase dissolution of the nuclear envelope, the polar MTOCs disperse and the spindle MTs converge on several minipoles. In *Marchantia* the polar MTOCs are initiated in contact with the nuclear envelope. This fact, as well as the direct connections between the outer membrane of the nuclear envelope and the MTOC membranes, favour the suggestion that the former participate in the formation of the latter (Apostolakos & Galatis, 1985b). The simultaneous dispersion of the nuclear envelope and the polar MTOCs at prometaphase also suggests the existence of a particular relationship between these structures.

The available information on the mechanism of determination of the division plane in liverworts is limited to studies on *Reboulia*, *Odontoschisma* and *Marchantia*. In *Reboulia* the plane of cell division seems to be determined by interaction of the opposite arrays of MTs emanating from the polar MTOCs (Brown & Lemmon, 1990). Similarly, in gemmiferous filaments of *Odontoschisma* the equator of the elliptical prophase nucleus itself appears to determine the plane of division (Duckett & Ligrone, 1995). In *Marchantia* the mechanism by which the division plane is determined is more complex and is accomplished in two steps. During the first step the initial alignment of the cell plate is determined, while during the second its final orientation, *i.e.* the sites where the cell plate joins the parent walls, is fixed (Apostolakos & Galatis, 1985b, c, 1992, 1993).

The initial orientation of the cell plate is predicted by the equator of the elliptical prophase nucleus, *i.e.* by the plane of interdigitation of the half-prophase spindles. This plane is determined by the axis defined by the polar MTOCs. Both these are controlled by the polarity established in the premitotic cells. For example in the PF mother cells and the AP mother cells, the axis of the prophase nucleus predicts the forthcoming periclinal division (see previous sections).

The final orientation of the cell plate in *Marchantia* is controlled by polarized regions of the cortical cytoplasm. A mechanism that attracts or directs the expanding cell plate to join the parent wall at predetermined sites functions in these regions. These sites are predicted by the PPB and are determined before the onset of mitosis. PPBs have been observed in all cell types of *Marchantia*, but their organization varies. More or less typical PPBs have been observed in the MP mother cells, in the

mother and the dividing cells of PFs, as well as in scale cells. They are cortical MT rings in the final plane of cytokinesis (Galatis & Apostolakos, 1977; Apostolakos & Galatis, 1992, 1993). In contrast, in protodermal and inner thallus cells the PPBs are usually incomplete, i.e. they comprise independent cortical MT bundles lying on the plane of the future division rather than a complete annulus (Apostolakos & Galatis, 1985b, 1992). These incomplete PPBs are more highly organized at the cell edges; here the MTOCs of the PPBs may be located. Our observations show that the incomplete PPBs prevail in the dividing cells of *Marchantia*. This phenomenon may be explained by the fact that the PPBs are formed when the polar MTOCs are activated. Both PPB MTOCs and the polar MTOCs may therefore compete for the same pool of tubulin, which is insufficient for both the prophase spindle and the PPB. The formation of typical PPBs in PF mother cells, MP mother cells, and scale cells is probably due to particular conditions prevailing in these cell types. In the first two cell types a strongly marked polarity is established during interphase, which leads to the formation of a MT ring at the base of the cellular protrusion (see previous sections). The plane of this MT ring coincides with the future division plane. The interphase MT ring is retained after the activation of the polar MTOCs and behaves as a PPB. The scale cells, on the other hand, display a well organized interphase system of cortical MTs, which during preprophase-prophase may liberate sufficient tubulin to form both the prophase spindle and the typical PPB. The above mechanism of determination of the division plane is similar to that functioning in the dividing cells of vascular plants (Gunning, 1982; Galatis, Apostolakos & Katsaros, 1983, 1984a, b; Gunning & Wick, 1985; Wick, 1991a, b).

However, in protodermal cells of *Marchantia*, except for the PPB cortical site, there are other regions of the cortical cytoplasm, which affect the final orientation of the cell plate. Incomplete PPBs co-exist with independent MT bundles in most of the preprophase-prophase initial aperture cells. The independent MT bundles appear in the cortical cytoplasm lining the walls of the base of the developing ISs (Apostolakos & Galatis, 1985b). This pattern of the preprophase-prophase MT organization is unique and suggests that the cortical cytoplasm of the above cells is multi-polarized. It seems likely that the putative MTOCs at the base of the developing ISs (Apostolakos & Galatis, 1985a) remain active during preprophase-prophase and therefore the MT bundles persist in these sites. Multi-polarized preprophase cells have been described in a few cases and they are cells characterized by a particular organization (Galatis, Apostolakos & Palafoutas, 1986) or cells which are influenced by more than one intercellular morphogenetic stimulus from different directions (Galatis *et al.*, 1983). In dividing cells of the initial apertures of *Marchantia* all the polarized sites of the cortical cytoplasm have the ability to attract the growing cell plate margins. As a result the daughter wall is not straight but highly curved and in some planes meets the parent walls in the PPB cortical site, while in other planes at the base of the developing ISs (Apostolakos & Galatis, 1985c). The above suggests that the mechanism that controls the final cell plate orientation in higher plants does not only function in the PPB cortical region, but also in other sites of the cortical cytoplasm where MTOCs function during preprophase-prophase.

Aster completion of cytokinesis the interphase MTs reappear. The cortical MTs in post-cytokinetic cells of *Marchantia* reappear at first in the close proximity of the daughter wall (Apostolakos & Galatis, 1992), as occurs in *Sphagnum* (Schnepl, 1973,

1984). A similar pattern of cortical MT reappearance characterizes the post-cytokinetic root cells of ferns (Gunning, Hardham & Hughes, 1978; Jenni, Cattelan & Roos, 1990; Panteris, Galatis & Apostolakos, 1991). The dividing cells of lower land plants examined so far do not seem to follow the perinuclear pattern of cortical MT reformation of the flowering plants (for literature see Panteris, Apostolakos & Galatis, 1995). The reappearance of the MTs in the cortex of the post-cytokinetic cells of *Marchantia* may be related to the fact that at the end of cytokinesis the daughter nuclei have entered interphase and therefore their envelopes may have lost the ability to nucleate MTs. A similar temporal relationship between cell plate formation and daughter nuclei reconstruction exists in *Sphagnum* (Schneppf, 1973) and in the fern *Adiantum* (Panteris *et al.*, 1991), an event which could explain the cortical reappearance of the cortical MTs in those plants. Recent observations on *Triticum* post-cytokinetic root tip cells affected by taxol support the above hypothesis (Panteris *et al.*, 1995).

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15. Protonemal morphogenesis

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15.1 SUMMARY

This article reviews the structure, development and functions of protonemata from data on wild and cultured materials of over 200 different mosses. Protonemata are defined as multicellular filaments with new cells being added either by division of the apical cell or by the formation of side branch initials. This diagnosis includes chloronemata, caulonemata and rhizoids, all three interrelated developmentally but each with a wide range of distinctive attributes, embracing cytological organization, wall architecture, mode of growth and tropisms. Rhizoids and caudonemata elongate by tip growth whereas recent evidence from ultrastructure, time-lapse photography, effects of drugs and staining with DiOC₆(3) indicates that chloronemata more likely extend by general expansion growth.

The main functions of chloronemata are assimilation and propagation, by the production of gemmae, which are estimated to occur in approximately 25% of all mosses. These are liberated by at least five different abscission mechanisms, four of which involve specialized Tmema (abscission) cells. The main role of caudonemata is colonization whereas rhizoids variously function in colonization, propagation (by producing tubers), attachment and nutrient uptake. Their very fine (4–5 µm diameter) strongly thigmotropic ultimate ramifications appear to be similar functionally to mycorrhizal hyphae in vascular plants.

Following side branch formation profound cytological differentiation occurs along caudonemata and rhizoids. As the walls become thickened and pigmented the single large vacuole is replaced by numerous smaller, mainly apically located vacuoles. Plastids and mitochondria become suspended along axial arrays of endoplasmic microtubules. The nucleus increases in size, the nucleolus fragments and the genome replicates to about 8 C. The septa contain several thousand highly structured plasmodesmata. Hypomethylation of the genome of *Funaria* retards this nuclear and cytoplasmic differentiation. Cytologically rhizoids and caudonemata are closely similar to leptoids. Time-lapse photography reveals the vacuoles to be highly dynamic and that the chloroplasts move along the endoplasmic strands at similar rates to tip growth (20 µm/h).

Sphagnum, with its plate-like juvenile stage, and *Andreaea*, with the same comprising ill-defined thalli and filaments, lack protonemata as defined above. Other highly distinctive protonemal features are massive upright appendages, linking the Buxbaumiales and Polytrichales, and broad plates that distance Tetraphidales from

the Eubryidae. Gemma morphology may prove useful at the species level. Field observations reveal gemmiferous protonemata to be of two kinds: perennial and transient. The former ensure a continuous supply of propagules throughout the year the latter are associated with maximal exploitation of transient habitats.

KEYWORDS: Caulonemata, chloronemata, rhizoids, cytoskeleton, endoreplication, gemmae, intercalary expansion, solute transport, systematics, tip growth, vegetative propagation.

15.2 INTRODUCTION

Because of its simple, sequential and highly ordered development the moss protonema has long been a popular model system for investigating the cellular and molecular bases of plant morphogenesis (see for example reviews by Bopp & Atzorn, 1992; Ashton & Cove, 1990; Cove & Knight, 1993). Over the last two decades immunocytochemical and electron microscope studies in particular have made incisive contributions to understanding the cytoskeletal basis for key phenomena such as tip growth (Doonan, 1991), tropic responses (Walker & Sack, 1995a, b; Schwuchow, Kim & Sack, 1995; Meske & Hartmann, 1995) and gemma formation (Goode *et al.*, 1993a), whilst transformation technology has been used to dissect the molecular basis of development.

Looking now to exciting future revelations in developmental genetics our principal aim here is to describe the key features of moss protonemal development from essentially a cytological perspective; surprising though it may seem, lucid descriptions of the fundamental attributes of moss protonemata and how these differ from other filament systems (viz. root hairs, pollen tubes, filamentous algae and fungal hyphae) are impossible to find. For example Bhatla's (1994) recent book on protonemal differentiation nowhere defines the object under scrutiny. We also attempt what is probably the first comparative account of the morphological diversity of moss protonemata. This new information is then used to assess the possible relevance of protonemata in moss systematics and phylogeny. Finally we consider the roles of protonemata in the reproductive biology of mosses. Whereas virtually all published information on moss protonemata is based on axenically cultured protonemata of just three taxa, namely *Ceratodon purpureus*, *Funaria hygrometrica* and *Physcomitrella patens*, the present synthesis embraces observations and experiments on over 200 taxa in both culture and in nature.

15.3 MATERIALS AND METHODS

Photomicrographs in this review, unless detailed otherwise, are either from wild collected materials or protonemata established in axenic cultures from surface-sterilized (10–20 s in 1.5% sodium hypochlorite) spores, gemmae or gametophyte fragments and grown under standard conditions on Parker agar (Klekowski, 1969) viz. $20 \pm 2^\circ\text{C}$ and a 12/12 h light regime with illumination from fluorescent tubes giving an irradiance of approximately 50 W m^{-2} . Living specimens were photographed with a Leitz Ortholux microscope using differential interference contrast

optics. For transmission electron microscopy specimens were fixed in a mixture of formaldehyde and glutaraldehyde as described in Ligrone & Duckett (1994, 1996). Details of the preparation of critical point dried materials for scanning electron microscopy (SEM) are given in Duckett & Ligrone (1995a).

15.4 THE DIAGNOSIS OF PROTONEMATA

The key attributes of moss protonemata, based on *Ceratodon*, *Funaria* and *Physcomitrella* are illustrated in Fig. 1 and Table 1. Protonemata comprise multicellular filaments with new cells being added either by division of the apical cell (AC) or by the formation of side branch initials (SBI) usually situated immediately behind the cross walls along the primary axis. Occasionally, cells removed from the AC or SBIs may also undergo intercalary divisions this being one of the key features of protonemal brood cell formation (see Goode, Stead & Duckett, 1993b) often observed in ageing and/or desiccated cultures, or in response to the addition of abscisic acid. As far as we are aware this very precise division pattern, restricted in normal morphogenesis to apical cell and to side branch initials of fixed position, is unique to moss protonemata. As a consequence protonemata may be separated immediately from algae and from fungi in field collected specimens.

The filaments in mature protonemal colonies may be of two distinct kinds, chloro- and caulinemata, the one giving rise to the other and vice versa (illustrated schematically in Fig. 2) depending on the source of the material (spores or gametophore stems and leaves) or on the culture conditions (Bopp, 1990a, b; Johri & D'Souza, 1990; Goode, Duckett & Stead, 1992; Meske & Hartmann, 1995). Filaments intermediate in nature can thus occur but the extremes are very clearly distinct. The main features of chloro- and caulinemata are summarized in Table 1. Whereas previously the two kinds of filaments have been defined purely in terms of septal orientation, presence or absence of wall pigmentation and chloroplast shape, sometimes together with tropisms and growth rates (Bhatla, 1994; Russell, 1993), now we can incorporate a host of other cytological attributes. Table 1 also includes the rhizoid, the third kind of filament found in mosses, published diagnoses of which are even more

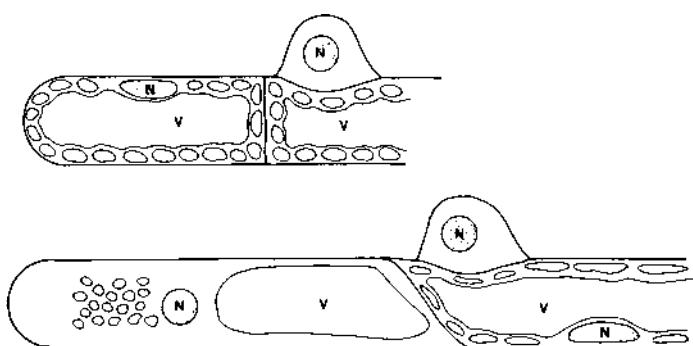


Figure 1. Diagrammatic representation of the key attributes of moss protonemata, chloronema above, caulinema below. N, nucleus; v, vacuole. The other cytoplasmic organelles illustrated are plastids.

Table 1. Summary of the principal features of chloronemata, caulonemata and rhizoids.

Feature	Chloronemata	Caulonemata	Rhizoids
Mature cells			
General features			
cytoplasmic polarity	absent	most organelles at apical end	most organelles at apical end
plasmodesmata	uniform diameter	dilated lumen	dilated lumen
sepia	transverse	oblique	oblique or transverse in ultimate ramifications
gravitropism	negative	negative?	positive?
thigmotropism	absent	weakly positive	strongly positive
vegetative propagules	gemmae and brood cells	-	tubers?
phototropism	strongly positive	positive-low irradiance	?
growth rate	slow (< 10 µm/h)	dia-high irradiance fast (> 20 µm/h)	fast?
bud formation	-	+	+
Walls			
pigmentation	-	++ main axes + side branches	++ thick, sometimes irregular internally
thickness	thin	+	+
Pellicle	-	+	
Plastids	spherical/ovoid	spindle-shaped/elongate	spindle-shaped/elongate along endoplasmic strands
shape	peripheral	along endoplasmic strands	spindle-shaped/elongate
position			central
Nuclei			endoreplicated?
shape	spherical	endoreplicated	elongate
position	peripheral	numerous	numerous
genome	haploid	endoplasmic	endoplasmic
Mitochondria	pleomorphic	frequent	frequent
Vacuoles	large, central	axial, endoplasmic	axial, endoplasmic
ER	peripheral		
Partially coated reticulum	rare		
Microtubules	cortical		

Apical cells	absent	cytoplasm at apical end
Polarity		
Plastids	large chloroplasts > 1 μm peripheral, uniformly distributed containing plastids and mitochondria	amyloplasts < 1 μm subapical, central and peripheral basally plastids and mitochondria absent
Apical dome	large central large central peripheral cortical	large basal and small distal to nucleus internal endoplasmic between nucleus and apex, cortical at basal end
Vacuole		
Nucleus		
Microtubules		
Functions	assimilation, propagation	colonization, solute transport
Mode of growth	general expansion growth	tip growth

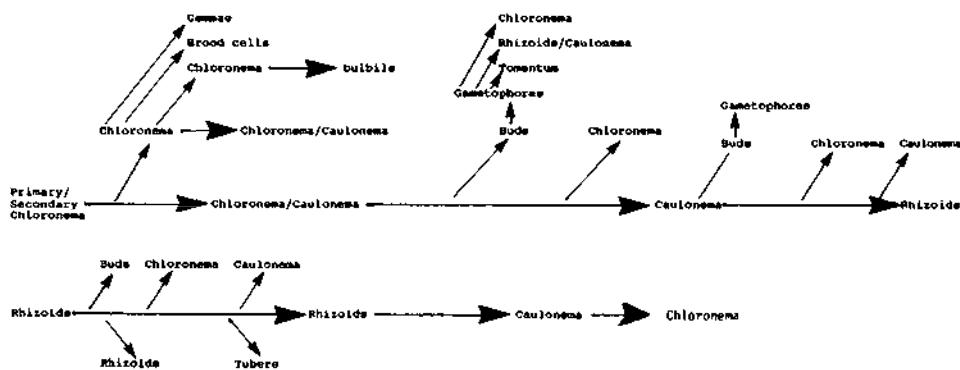


Figure 2. Diagrammatic representation of protonemal morphogenesis in mosses. The horizontal lines indicate the possible fates of a single axis derived either from a germinating spore (primary) or subcultured chloronema (secondary). Inclined lines indicate the various fates of side branches.

nebulous than those of chloro- and caulinema. Rhizoids are most commonly defined in positional terms, viz. filaments growing from mature gametophores, usually stems (Crundwell, 1979). They include the filaments attaching mosses to their substratum or clothing aerial stems as tomentum. Protonemal filaments of narrower diameter than chloro- and caulinemata and containing fewer chloroplasts are also referred to as rhizoids. Since they penetrate the substratum (either natural or agar) rather than growing along or away from it, it is tacitly assumed that they are either positively gravitropic or in the case of germ tubes negatively phototropic (Knoop, 1984). However, the critical experiments like those performed on chloro- and caulinemata have yet to be undertaken on mature rhizoids.

Morphologically rhizoids have inclined septa and walls which tend to be even more deeply pigmented than in caulinemata of the same species. Sometimes the walls may also be highly papillose (Fig. 4E) — a feature, as far as we are aware, unknown in caulinemata but which is sometimes expressed by protonemal gemmae (Fig. 6C) (Duckett & Ligrone, 1992). By far the most striking feature of rhizoids is the nature of their side branch systems. Whereas the different orders of chloro- and caulinemal branches have much the same diameter, rhizoidal branches become progressively narrower with ultimate ramifications down to 4–5 µm (Fig. 4I). Moreover these, the finest filaments found anywhere in bryophytes, are strongly thigmotrophic and coil tightly around any solid object (Goode *et al.*, 1992a; Duckett, 1994a; Duckett & Matcham, 1995a). The main axes (as do those of caulinemata) also tend to trace an undulating course and frequently wind around each other. Fine ramifications tightly wound around larger rhizoids form so-called 'rhizoid wicks' (Fig. 4J, K). These are particularly well developed in the Polytrichales (Wigglesworth, 1947). Like the transformations in chloronemal/caulinemal development the rhizoids may also give rise to other types of protonemal filament (Fig. 2).

15.5 THE GROWTH OF MOSS FILAMENTS

15.5.1 Caulonemata

Immunocytochemical and electron microscope studies together with experiments with drugs, in particular those that disrupt the cytoskeleton, have established beyond

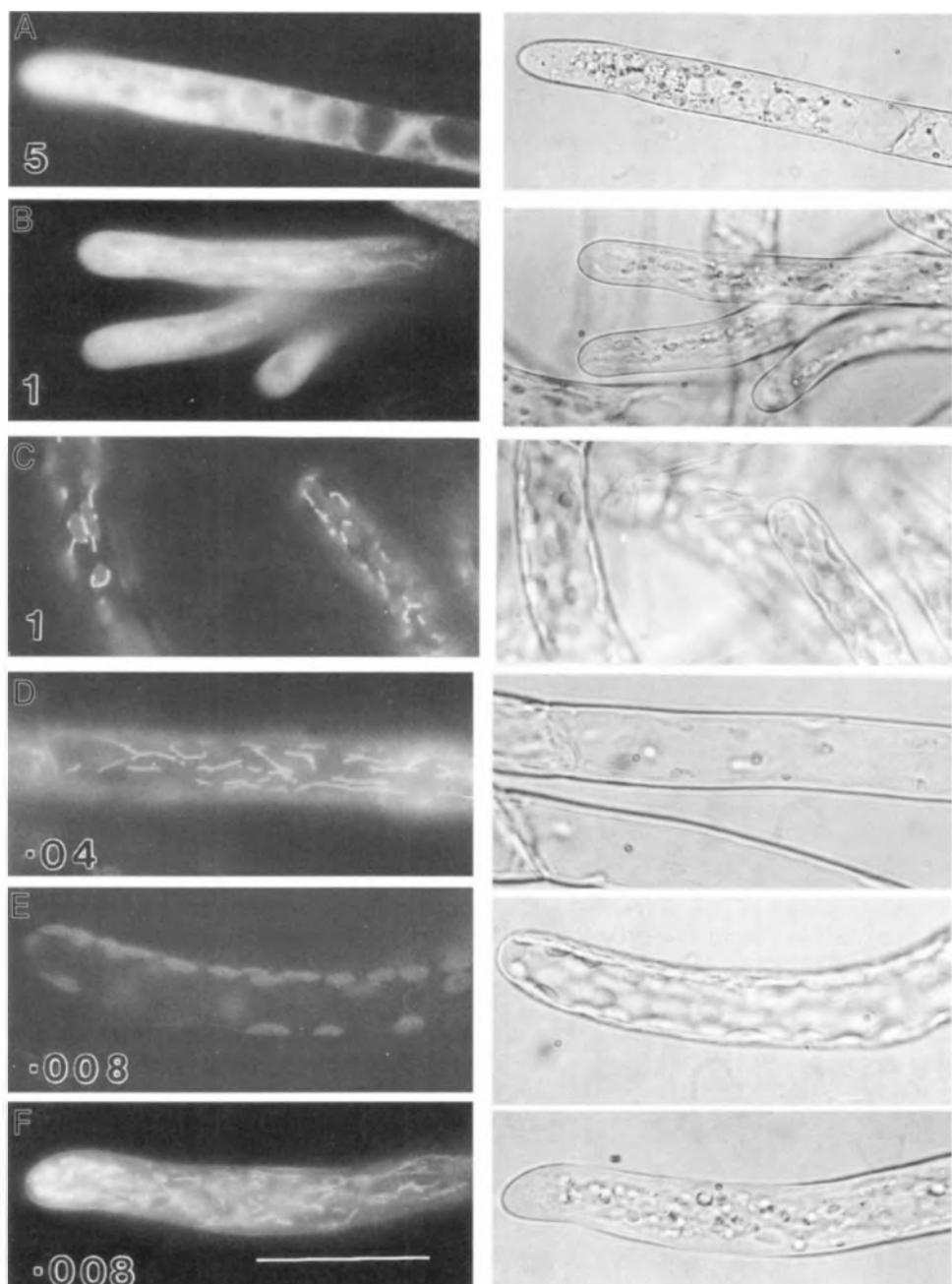
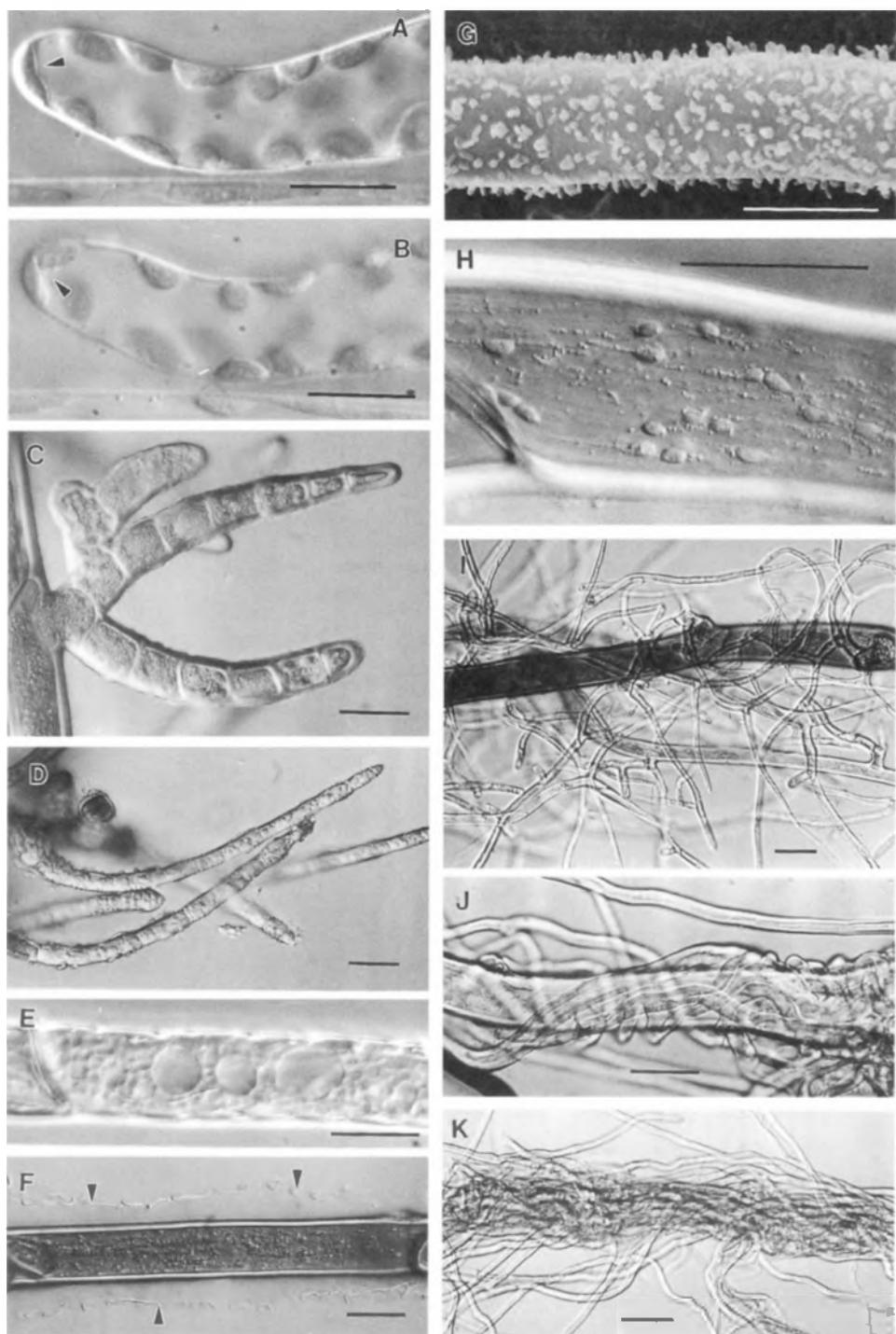


Figure 3. Staining of protonemata with $\text{DiOC}_6(3)$, fluorescence images on the left, corresponding bright field images to the right. The numbers refer to the concentration of $\text{DiOC}_6(3)$ in $\mu\text{g}/\text{ml}$. A, caulonemal tip cell with disrupted contents. B, intensive staining of caulonemal tip cells. C, staining of pleiomorphic mitochondria associated with the plastids (sainter autofluorescence) in chloronemal cells. D, staining of elongate mitochondria in cell 3 of a caulonema. E, chlorophyll autofluorescence alone in a chloronemal tip cell. F, clear staining of mitochondria in a caulonemal tip cell. Scale bar = 50 μm .



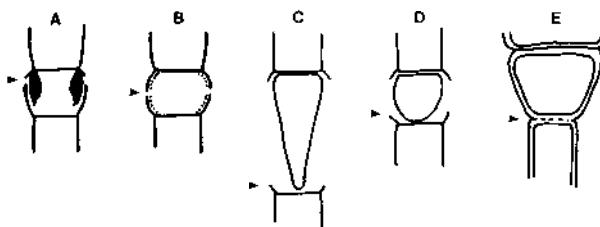


Figure 5. Liberation mechanisms of protonemal gemmae. The points of breakage are arrowed. A-D. Mechanisms involving abscission cells. A. Formation of a new, sometimes thickened, internal wall, breakage of the original wall (arrowed) and sliding apart of the old and new walls, e.g. *Calymperes*, *Schistostega*, *Syrrhopodon*, *Zygodon*. B. Dissolution of the original wall with little or no new wall formation e.g. *Orthotrichum*, *Bryum*. C and D. Formation of a new internal wall whose expansion severs the original wall. C. Extensive elongation of the new internal wall e.g. *Funaria hygrometrica*. D. Swelling of the new internal wall e.g. *Dicranella heteromalla*, *Ceratodon purpureus*. E. Schizolytic liberation by splitting along the boundary between existing walls e.g. *Pottiales*, *Rhizomnium*. Data from Bopp *et al.*, 1991; Duckett & Ligrone 1991, 1994; Duckett & Matcham, 1995a; Goode *et al.* 1993a and unpublished observations.

any reasonable doubt that caulinemata elongate by tip growth (Schmeidel & Schnepf, 1980; Schnepf, 1986; Wacker, Quader & Schnepf, 1988; Wacker & Schnepf, 1990; Meske & Hartmann, 1995). The cytological organization of caulinematal tips viz. numerous Golgi bodies and abundant ER plus arrays of endoplasmic microtubules and actin filaments converging at the tip, is closely similar to that in other tip growing filaments like pollen tubes, fungal hyphae and root hairs (Heath, 1990; Harold, 1997; Lancelli, Cresti & Hepler, 1997). Immediately behind this zone lie numerous mitochondria and amyloplasts associated with graviperception (Walker & Sack, 1990, 1991; Sack, 1991). A constant distance between the internally-located nucleus and the advancing tip (Jensen, 1981; Jensen & Jensen, 1984) is maintained by interactions between the endoplasmic microtubules and microfilaments (Doonan, 1991). Consequently the basal vacuole increases in length until the cell divides. The subapical cell is thus highly vacuolate with exclusively peripheral organelles.

Table 1 shows the cytological organization of growing rhizoid apices to be virtually identical to that of caulinemata, again indicating elongation by tip growth.

15.5.2 Chloronemata

Although literature accounts generally assume that chloronemata grow in the same manner as caulinemata our recent observations and experiments suggest elongation

Figure 4. Features of chloronemata (A-D), caulinema (E) and rhizoids (F-K). A and B. The same apical cell in *Physcomitrella patens*. B photographed 8 h after A. The chloroplast in the apical dome (arrowed) has divided. Note the large central vacuole and peripheral chloroplasts. C. Wild protonema of *Dicranum montanum* with thick-walled pointed chloronema. D. Wild protonema of *Weissia controversa*. The rough surface of these pointed filaments is due to the presence of surface waxes which render them non-wettable. E. Apical end of a dark-grown caulinemal cell of *Physcomitrella patens* packed with vesicles. F. Wild rhizoid of *Dicranum scoparium* showing the deeply pigmented wall and pellicle (arrowed). G. Highly papillose rhizoid of *Bartramia pomiformis*. H. Wild rhizoid of *Encalypta streptocarpa* showing small plastids along endoplasmic strands. The small spheres are lipid droplets. I. Very fine ultimate ramifications around a major rhizoid axis, wild *Dicranum scoparium*. J and K. Rhizoid wicks in *Polytrichum commune*. J. Forming wick; narrow side branches coiling tightly around a major axis. K. Mature wick; the major axis is completely obscured by fine rhizoidal branches. Scale bars = 20 μm .

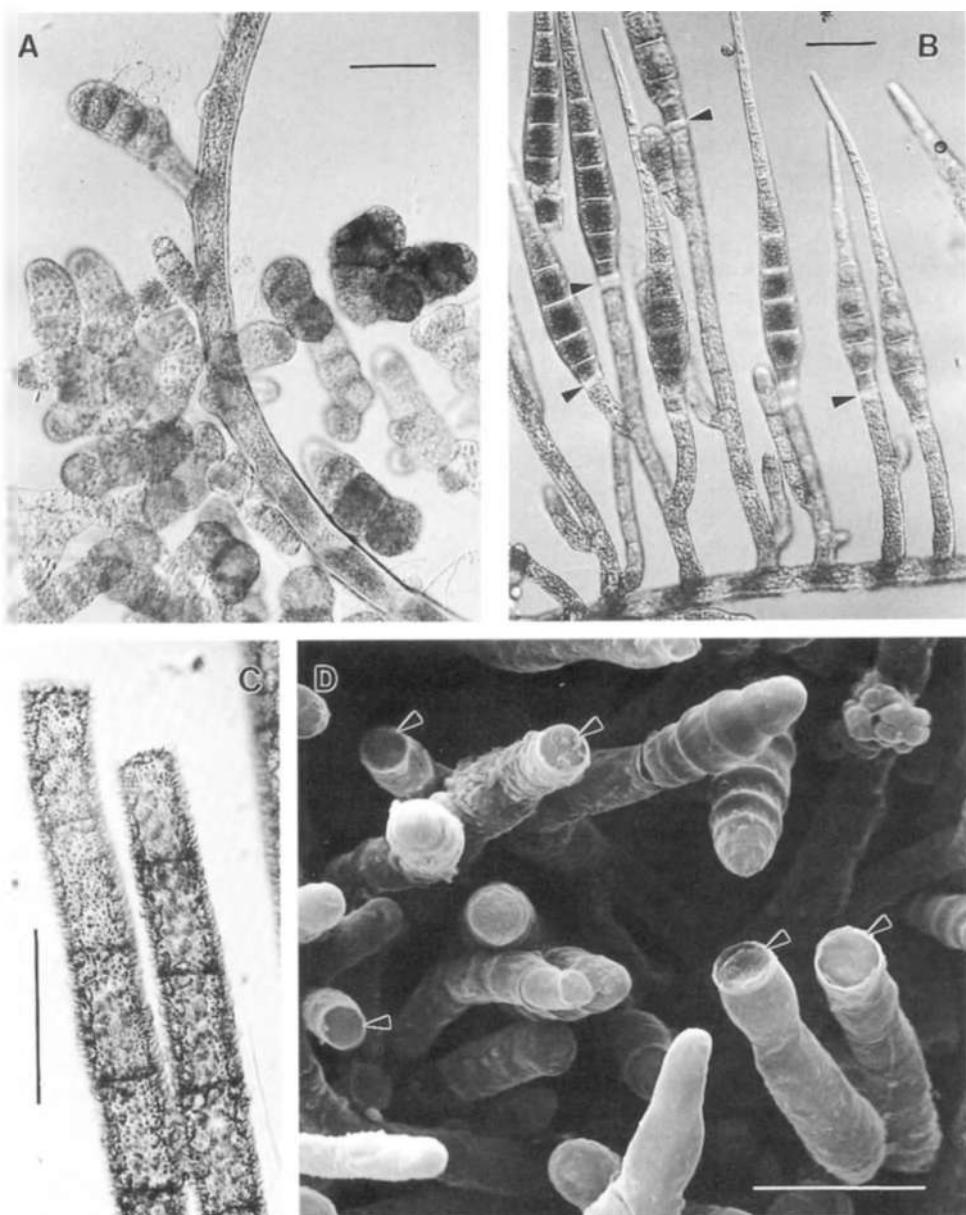


Figure 6. Wild gemmiferous protonemata. A. *Barbula rigidula*, rounded gemmae separating by schizolysis like those in the leaf axils of the same species. B. *Syrrhopodon texanus*, gemmae with sticky hyaline acumina. Tmemma cells arrowed. C. *Bryum ruderale*, papillose filamentous gemmae. D. *Zygodon conoideus* (SEM), note the scars of the Tmemma cells (arrowed) remaining after gemma detachment. Bar lines = 50 μ m.

by general intercalary wall expansion. The evidence may be summarized as follows:

(1) Spatially there is insufficient cytoplasm in the apical dome of chloronemata to accommodate the tip-growing apparatus of endoplasmic, axially-orientated microtubules and microfilaments together with highly differentiated endomembrane domains (Fig. 4A, B).

(2) Transmission electron microscopy confirms the absence of the tip-growing apparatus in the apices of chloronemata. Instead the dome is lined by cortical cytoplasm and contains both chloroplasts and mitochondria (Table 1).

(3) Whereas time-lapse photography reveals the organelles in caulinemal filaments to be highly dynamic, in contrast, during chloronemal elongation, the major organelles (e.g. chloroplasts) are remarkably static. Those in the apical dome tend to remain there — and even divide: not what might be predicted in a tip-growing cell (Fig. 4A, B). Particles of ink sticking to the tips of growing chloronemal apices remain there whereas those on caulinemata finish up far behind the apex after a few hours growth.

(4) Drugs that affect the cytoskeleton such as cytochalasins, oryzalin, cremart and carbamates have profound effects on caulinemal tips ranging from swelling to abnormal branching and even bursting (Doonan, 1991). In contrast those of chloronema remain unchanged with the only obvious effect being a cessation in the growth of the filaments.

(5) Staining of protonemata with different concentrations of the lipophilic cationic fluorochrome 3, 3'-dihexylocarbocyanine iodide ($\text{DiOC}_6(3)$) reveals major differences between the tip cells of chloronemata on the one hand and caulinemata and rhizoids on the other (Fig. 3, Table 2). This fluorochrome has previously been used to visualize mitochondria, Golgi bodies and ER in a variety of cells (Kawazu, Kawano & Kuroiwa, 1995; Terasaki & Reese, 1992). Organelle stainability in relation to concentration is also a good indicator of cell wall permeability (Duckett & Matcham, 1995a; Duckett & Read, 1991). Cells with high levels of exocytotic activity, such as those in root caps, mucilage papillae and tip-growing systems are readily stained by concentrations of $\text{DiOC}_6(3)$ as low as $0.0003 \mu\text{g/ml}$ whereas fully expanded cells or those growing by general intercalary expansion require much higher concentrations. Caulinemal and rhizoid tip cells clearly fall into the former category, chloronema tip cells into the latter. Table 2 also shows that $\text{DiOC}_6(3)$, even at high concentrations, does not penetrate the walls of mature caulinemal and rhizoid cells. The only chloronemal filaments staining with $\text{DiOC}_6(3)$ at concentrations as low as $0.008 \mu\text{g/ml}$, are those at the onset of brood cell formation. Here stainability may be attributed to a general loosening of the wall matrix together with exocytosis of additional materials as the walls begin secondary expansion (Goode *et al.*, 1993b).

Thus in terms of cell organization and dynamics the growth of chloronemata appears to be closely similar to the intercalary expansion of the photosynthetic filament cells in Marchantiaceae (Apostolakos & Galatis, 1992, 1993, 1998), the catenate gemmae in Jungermanniales (Duckett & Ligrone, 1995a) and the cauliniferous filaments in the moss *Aulacomnium androgynum* (Ligrone, Duckett & Gambardella, 1996).

The different modes of chloronemal and caulinemal elongation provide a ready explanation for their very different growth rates. In *Physcomitrella*, dark-grown eau-

Table 2. Staining of young protonemata of *Physcomitrella patens* with 3,3'-dihexylcarbocyanine iodide ($\text{DiOC}_6(3)$).

Cell type	DiOC ₆ (3) Concentration ($\mu\text{g/ml}$)						
	5.0	1.0	0.2	0.04	0.008	0.0016	0.00032
Caulonemata	- tip cells	+++D	+++	++	++	++	++
	- cells 2-5	++	++	++	++	+	+
	- cells 8 and older	-	-	-	-	-	-
Indeterminate	- tip cells	+++D	+++	++	++	++	+
Chloronemata	- tip cells	++	++	++	+	-	-
	- subapical cells	++	+	-	-	-	-
	- damaged filaments	+++D	++	++	+	+	+
Side branch initials		+++	++	++	+	-	-
Rhizoids	Main axes - tip cells	+++D	+++	++	++	++	+
	- cells 2-5	++	++	++	++	+	-
	- cells 8 and older	-	-	-	-	-	-
Attenuated side branches	- tip cells	+++D	+++D	+++	++	++	+
	- cells 2-5	++	++	++	++	+	-
	- cells 8 and older	-	-	-	-	-	-
Leaves	-	-	-	-	-	-	-

D cell organization disrupted

+++ very intense staining of cytoplasm and mitochondria

++ clear staining of mitochondria

+ faint staining of mitochondria

- staining absent

lonemata extend at up to 40 $\mu\text{m/h}$ compared with rates of less than 10 $\mu\text{m/h}$ for chloronemata (Russell, 1993). No data are presently available for rhizoids but it would seem likely that these have similar growth rates to caulinemata.

In terms of culture conditions, lack of nutrients, low light or darkness favour the production of rhizoids and caulinemata whereas chloronemata are most prolific under high irradiances and on nutrient-rich media (Goode *et al.*, 1992a). To date the developmental fate of side branch initials has focused on bud formation, and its hormonal control, for the future the switch between chloronemata and narrower rhizoidal ramifications is ripe for analysis.

15.6 DIFFERENTIATION AND FUNCTION

15.6.1 Chloronemata

The most conspicuous feature inside chloronemal filaments (Fig. 7A) is the numerous peripherally-disposed chloroplasts with dimensions and ultrastructure closely similar to those in leaf cells (Tewinkel & Volkmann, 1987). Such organization clearly suggests that the principal function of chloronemata is assimilation.

Typical illustrations of chloronemata show these as thin-walled with rounded apices. In some taxa this is not invariably the case. Tapering pointed filaments with

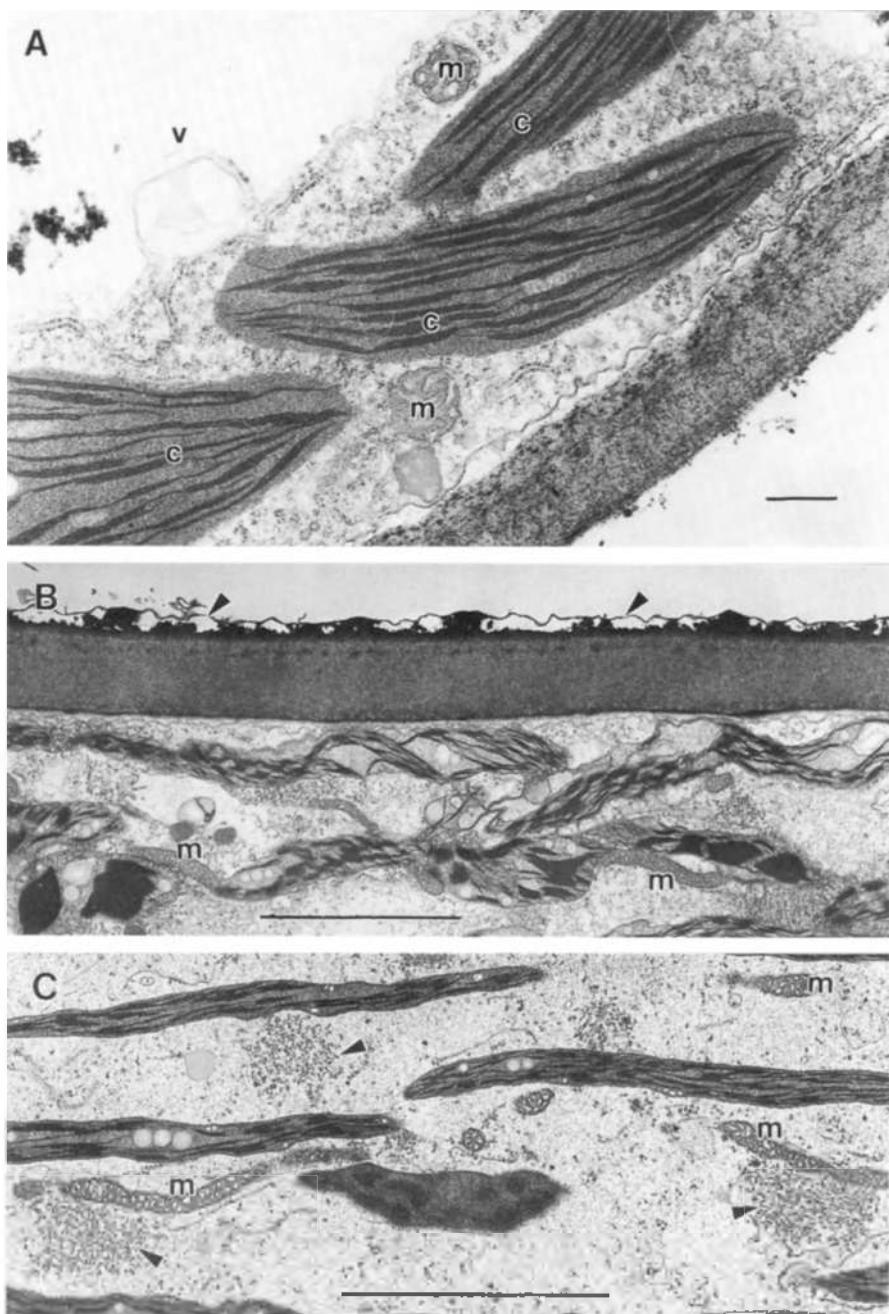


Figure 7. Protonemal ultrastructure, longitudinal sections. A. Mature chloronemal cell of *Funaria hygrometrica*. Note the large central vacuole and peripheral cytoplasm containing chloroplasts and mitochondria. B, C. Caulonemal cells of *Bryum tenuisetum*. B. Peripheral cytoplasm containing elongate mitochondria and chloroplasts with massive grana. Note the pellicle (arrowed). C. Central cytoplasm, containing elongate chloroplasts and mitochondria and aggregates of free ribosomes (arrowed). c, chloroplast; m, mitochondrion; v, vacuole. Bar lines = 0.5 μm , A; 5.0 μm , B & C.

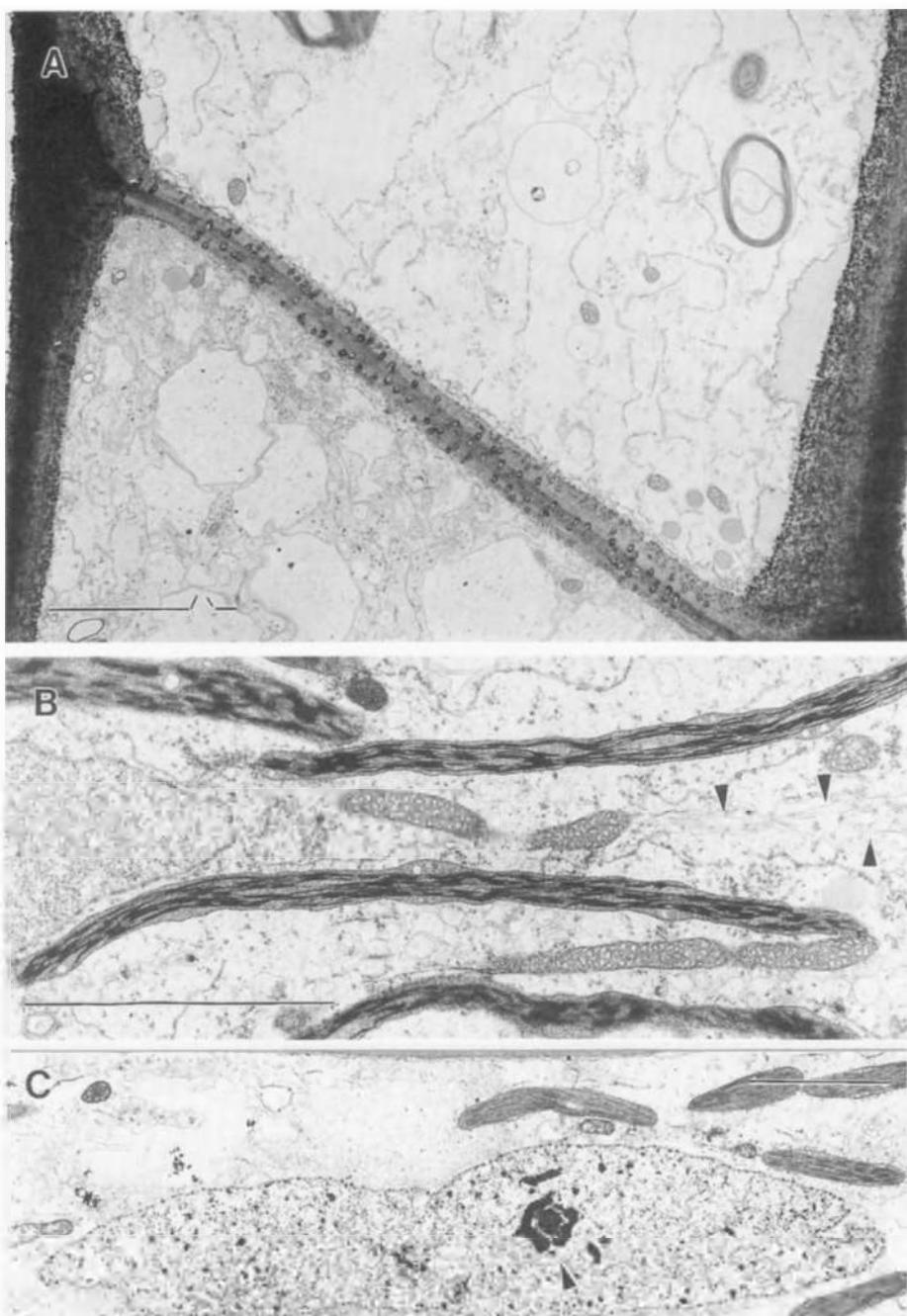


Figure 8. Caulonemal ultrastructure longitudinal sections, A *Funaria*, B & C *Bryum*. A. Cross wall containing numerous plasmodesmata, filament apex towards the top. Note the much denser cytoplasm at the apical end of the lower cell. B. Central cytoplasm. The endoplasmic microtubules (arrowed) extend from the nucleus to the end of the cell. C. Elongate nucleus over 10 μm in length. Note the small fragmented nucleolus (arrowed). Bar lines = 5.0 μm .

well marked thickening at the tips in the genus *Ephemerum* is a feature well known to taxonomists (Duckett, Goode & Stead, 1993). This feature is also found in a few dicranalean taxa and is widespread in the Pottiales (Fig. 4C, D). These pointed protonemata have two most interesting attributes: 1) they are non-wettable, a phenomenon sometimes enhanced by surface waxes. This is almost certainly related to prevention of waterlogging in situations where this would significantly depress photosynthesis, cf. the presence of cuticular waxes in leaves of mosses growing in rock crevices and on banks (Duckett & Ligrone, 1995b; Proctor, 1982). 2) Whereas ordinary chloronemata readily regenerate, this is not the case for the pointed filaments. These are determinate both structurally and developmentally. The pointed apical cells do not produce new filaments when subcultured and often all the sites for side branch formation have already produced similarly determinate cells.

The two other kinds of structural modifications exhibited by chloronemata are 1) redifferentiation to form chains of spherical to ovoid brood cells (Goode *et al.*, 1993b) and 2) the production of gemmae (Fig. 6), the latter defined as propagules with clearly differentiated abscission mechanisms (Duckett & Ligrone, 1992). Structurally these propagules may have cells virtually indistinguishable from those of non-gemmiferous chloronemata (e.g. *Funaria hygrometrica*), others have thickened, pigmented and sometimes papillose walls and conspicuous accumulations of lipids (e.g. *Bryum* spp.), whilst in a few cases hyaline sticky acumina are produced (e.g. *Syrrhopodon*, *Schistostega*, *Calymperes*) (Duckett & Ligrone, 1991, Ligrone, Duckett & Eggunyomi, 1992). In terms of development there is an interesting distinction between brood cells and gemmae. In the former the parent cells remain unpolarized with divisions symmetrical and median. In the latter reorganization of the cytoskeleton precedes a migration of the nuclei and chloroplasts to the basal ends of the cells marking a reversal in polarity at the onset of differentiation (Goode *et al.*, 1993a, b, d). This is then fixed and the lateral filaments, emerging when the propagules germinate, do so from the basal ends of the parent cells rather than in their normal terminal location behind the cross walls.

From the cytological standpoint one of the most interesting features of protonemal gemmae in mosses is their range of liberation mechanisms. As illustrated in Fig. 5 at least four different kinds of abscission cell can be recognized. To this may be added schizolytic separation involving detachment along preexisting cell walls, and paralleling the dissolution of chains of brood cells.

The production of brood cells and gemmae highlights clear-cut differences in developmental potential between chloronemata, caulonemata and rhizoids (Fig. 2). Chloronemata alone redifferentiate into brood cells and produce gemmae whereas bud formation, almost invariably at the base of first order side branches is restricted to caulonemata and rhizoids. Tubers (defined as propagules lacking special abscission mechanisms and separating only by breakdown of the parent filaments) usually develop in this position along rhizoids.

15.6.2 Caulonemata and rhizoids

Whereas chloronemal cells remain highly vacuolate, remarkable cellular differentiation takes place along caulonemata and rhizoids (Kingham *et al.*, 1995; Duckett & Matcham, 1995a). Immediate derivatives of the apical cell are highly vacuolate

with peripheral organelles. However, following side branch formation (Conrad, Steucek & Hepler, 1986; McCauley & Hepler, 1992; Quader & Schnepf, 1989) the walls become pigmented and thickened with the external development of a pellicle (Fig. 4F). The plastids become elongate or spindle-shaped and aligned along endoplasmic strands (Figs 3H, 7–9). The large single vacuole is replaced by numerous smaller vesicles mostly aggregated at the apical ends of the cells, *i.e.* these are now clearly polarized. The cytoplasmic changes are associated with endoreduplication of the genome to about 8C, with the nuclei increasing in volume and becoming spindle-shaped (Fig. 8C). When subcultured these cells rarely regenerate (Bopp, Zimmermann & Knoop, 1980). Transmission electron microscopy reveals the structural basis for this highly distinctive organization. Axial arrays of endoplasmic microtubules extend from the nuclear envelope to the cross walls (Fig. 8B). Aligned along them are the chloroplasts, elongate mitochondria (one or two microtubules only) (Fig. 9A) and variously-shaped tubules and vesicles (Fig. 9B). Profiles of partially-coated reticulum (Fig. 9C) are conspicuous together with local clusters of free ribosomes (Fig. 7C), otherwise the cytoplasm is electron-lucent and free of organelles apart from scattered lipid droplets (Fig. 4H).

These new ultrastructural data now provide ready explanation for the spindle to elongate shape of caulinemal plastids and their longitudinal alignment; these features are almost certainly a consequence of their association with the axial arrays of endoplasmic microtubules.

A final notable feature of the cytology of caulinemata is the plasmodesmata, numbering between 5 and 7000 in their oblique cross walls (Fig. 8A). Like those connecting leptoids (Ligrone & Duckett, 1994), and those associated with intensive trafficking of macromolecules in vascular plants (Lucas, Ding & der Schoot, 1993), these have expanded central lumina.

Overall the cytological organization of mature caulinemal and rhizoid cells bears an uncanny resemblance to that of the food-conducting leptoids in moss gametophores and sporophytes. The simplest explanation for such structural congruence is that caulinemata and rhizoids are food-conducting elements with their growing apices acting as sinks for assimilates. In the above-ground chloro/caulinemal system it may be envisaged that photosynthates, derived from the assimilatory chloronemata (whose bases are optimally situated adjacent to the cross walls), are transported symplastically along the caulinemal axes. The oblique orientation of the cross walls, accommodating the maximum number of plasmodesmata, is clearly in line with this notion.

Contrary to general statements in the literature (almost certainly based on studies of herbarium specimens), that rhizoids are short-lived with their principal function being attachment, our observations on wild rhizoid systems including tomenta reveal that these are long-lived (Duckett & Matcham, 1995a). This, together with their conducting-cell cytology and physiological data on nutrient uptake by terricolous mosses (Bates & Bakken, 1998), suggests that their main role in soft substrata at least, is the absorption and transport of mineral ions to the gametophores. Their extremely attenuated ultimate ramifications, which maximize the soil contact surface area, are clearly in line with such a function (*cf.* mycorrhizal hyphae in vascular plants which have similar diameters and the same function). However, in contrast

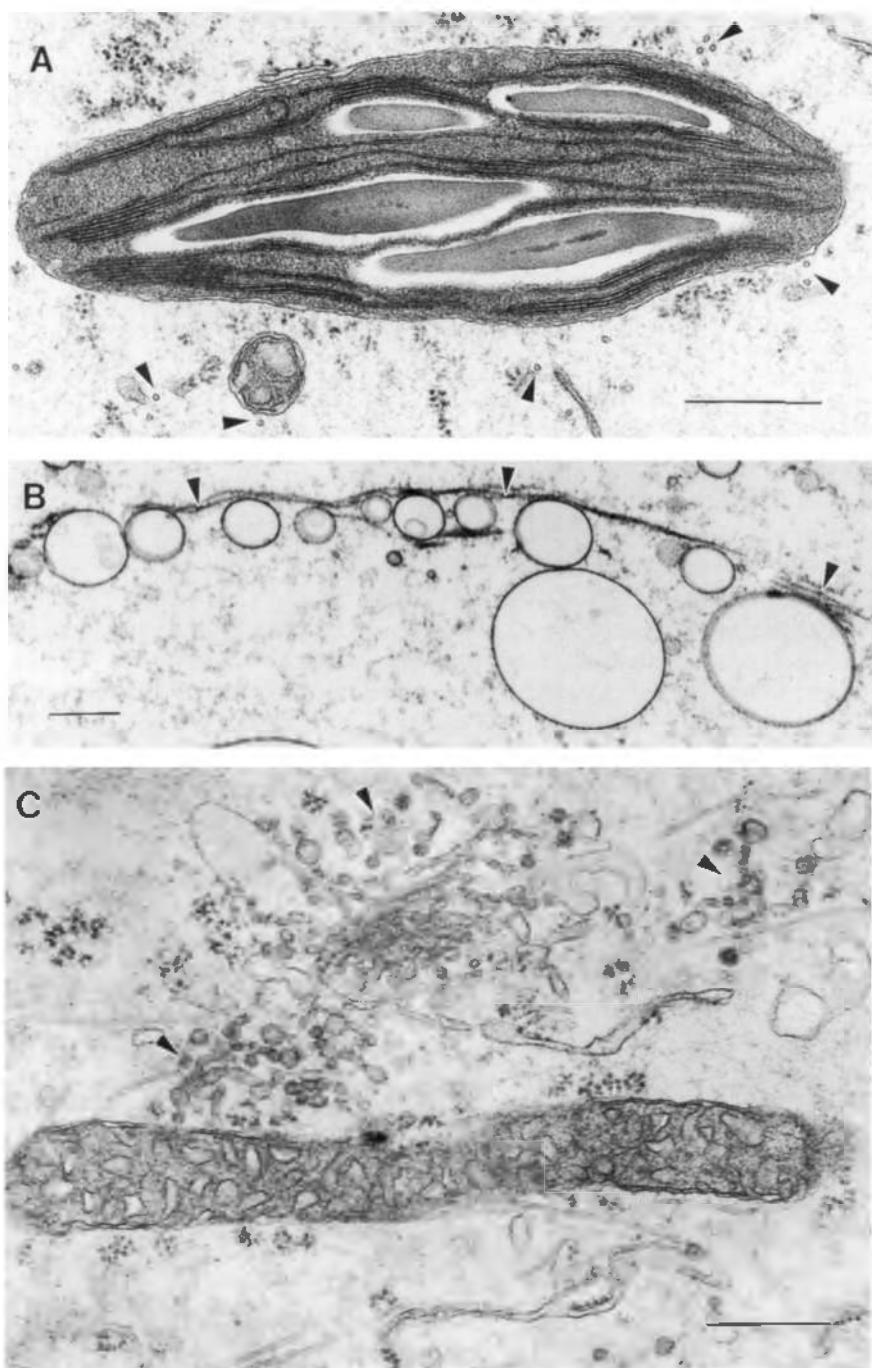


Figure 9. Details of caulinemal ultrastructure in *Funaria*. A. Transverse section showing endoplasmic microtubules (arrowed) associated with a chloroplast and a mitochondrion. B & C. Longitudinal sections. B. Microtubules (arrowed) associated with a group of vesicles. C. Microtubules associated with a mitochondrion, a Golgi body and partially coated reticulum (arrowed). Bar lines = 0.5 μm .

to the chloro/caulonemal system, rhizoids must derive their photoassimilates from the above-ground gametophores whilst mineral ions travel in the opposite direction.

We now require hard evidence to support or negate the hypothesis that caulinemata and rhizoids are principally solute-transporting systems. This should include radiolabelling experiments to establish rates and directions of movement of sugars and mineral ions, and other studies to determine the possible role of the microtubule-associated vacuole system in the process. Initial video time-lapse photography indicates the latter to be highly dynamic and that there is bidirectional movement of chloroplasts along the endoplasmic microtubules at rates of up to 20 $\mu\text{m}/\text{h}$. Curiously these are closely similar to the rates of caulinemal elongation (Russell, 1993).

Also offering much promise for the future is the use of protonemata as a model system for investigating causal relationships between nuclear and cytoplasmic differentiation (Kingham *et al.*, 1995). Whereas tracing cell lineages in the multicellular apices of higher plants may be problematic and will always be indirect, along moss protonemata nuclear morphology can be monitored directly alongside cytoplasmic differentiation. Moreover changes in protonemal nuclei (viz. endoreduplication of the genome, increase in nuclear volume and surface area, diminution in the volume of the nucleolus and separation of its granular and fibrillar components), appear to be common to differentiation in a range of plant and animal cell types. Of particular interest in this context is the demonstration that hypomethylation of the *Funaria* genome with 5-azacytidine or dihydroxypropyladenine, retards both nuclear and cytoplasmic differentiation along caulinemata (Kingham *et al.*, 1998). Unlike protonemata perturbed by growth regulators, and drugs that affect the cytoskeleton, which revert to normal morphogenesis when transferred to media lacking these agents, hypomethylated protonemata retain their abnormal morphogenesis.

15.7 SYSTEMATIC CONSIDERATIONS

Having presented a precise definition of moss protonemata in terms of gross morphology, mode of growth, cytological organization and likely functions, it is now pertinent to explore whether these differ between taxa.

First and foremost it must be underlined that one of the major differences between liverworts and mosses is the absence of well defined juvenile stages in the former. The sporelings of a few jungermannialean hepaticas (*e.g.* *Cephalozia*) are filamentous (Schuster, 1966). However, these comprise short chlorophyllose cells lacking SBIs, thus homology with moss protonemata is highly improbable. The same is almost certainly the case for liverwort rhizoids. Though the tips of these branch in contact with solid substrate (Pocock & Duckett, 1985) they remain unicellular; normal branching of multicellular moss filaments is always via SBIs (apart from drug-induced tip branching).

Unlike bryoid mosses the dominant juvenile phase in *Sphagnum* is a protonemal plate (Goode, Duckett & Stead, 1993c). Filaments may be produced immediately following spore germination or secondarily from the protonemal plates, or during regeneration from fragments of mature gametophores (Clymo & Duckett, 1986). The cross walls in these filaments are usually transverse and they never produce side branch initials in a regular manner. Their cells remain highly vacuolate. Buds are

produced on the lamina of the plates and never from the filaments. Unlike other mosses their induction is not stimulated by cytokinins. Overall this suite of major differences distances the protonemata of *Sphagnum* from those of all other mosses.

Despite repeated attempts we have not yet succeeded in culturing the protonemata of *Andreaea*. Consequently our comments on the Andreaeopsida are limited to scanty published reports (Nehira, 1976; Nishida, 1978; Murray, 1988) supplemented with observations on wild protonemata. The juvenile stages in *Andreaea* comprise somewhat ill-defined parenchymatous thalli with marginal filaments, the latter often bi- (or more) seriate at their bases and lacking SBIs. Gametophores grow directly from the thalli which remain as their only point of contact with the substratum. Rhizoids are absent from the stems of wild gametophores. Experimental attempts to induce rhizoids by subculturing gametophore fragments, even in the presence of auxin which invariably induces filament formation in all other mosses, even those lacking rhizoids in nature (e.g. *Calliergon* spp. *Pleurozium*, *Pseudoscleropodium*, *Rhytidium*, *Hylocomium*, *Scorpidium*), and including *Sphagnum*, have proved fruitless. Thus the evidence to date indicates that *Andreaea* lacks the capacity to produce rhizoids — a clear explanation as to why collections of *Andreaea* invariably separate into their individual stems.

Two conclusions may be reached concerning the 'protonema' of *Andreaea*: 1) it is impossible to equate it with the juvenile stages of other mosses; 2) its filamentous appendages are remarkably similar to the leaves of *Takakia*, perhaps suggesting that the latter genus may be likened unto a kind of neotenous *Andreaea*. Thus juvenile commonalities may be added to the already impressive list of characters linking *Takakia* and *Andreaea* and distancing them from other mosses (Renzaglia, McFarland & Smith, 1997; Garbary & Renzaglia, 1998).

Apart from *Sphagnum*, *Andreaea* and *Takakia* all other mosses manifest protonemal organization at some stage in their life cycle. In some instances (e.g. the so-called rhizoidless mosses listed above) this is normally restricted to the sporeling stage, in others such as *Tetraphis*, which has plate-like protonemata (Goode, Duckett & Stead, 1992) mainly to the rhizoids. Profound developmental differences indicate that the protonemal plates of *Tetraphis* and *Sphagnum* are analogues.

The only other mosses that produce non-filamentous protonemata are the Buxbaumiales with their massive upright funnel-like appendages (Duckett, 1994b). As in *Tetraphis* this order also produces typical caulinemata and rhizoids. Interestingly the Polytrichales also produce upright branches comprising irregular files of cells (Allsopp & Mitra, 1958). In both Buxbaumiales and the Polytrichales gametophores arise from the centre of these and not from buds at the base of side branches as in other mosses.

What then of morphological diversity in the protonemata of the Eubryidae? Typical chloro- and caulinemata occur in most orders of both the Haplolepidae and Dipolepididae from the Dicranales to the Hypnobryales. One cluster of orders stands apart; in the Orthotrichales (Goode *et al.*, 1993d), Isobryales and Thuidiales the protonemata are essentially monomorphic in terms of gross cell structure. Both upright and horizontal filaments, the latter whence buds arise, comprise short cells with transverse end walls. The former filaments have chloronemal cytology but the latter display conducting-cell organization. The rhizoids however are indistinguishable from those of other groups.

The taxonomic distribution of gemmiferous protonemata is also of interest. From the 200 or so taxa investigated to date (Duckett & Matcham, 1995b) it is estimated that between 20–30% of mosses possess gemmiferous protonemata. Their distribution however is far from uniform. To date they have not been found in the Polytrichales, Fissidentales, Grimmiales, Seligeriaceae and Ephemeralaceae and are remarkably rare in the Hypnobryales, the only examples being *Isopterygium elegans* and *Eurhynchium swartzii*. Gemma liberation mechanisms involving abscission cells are scattered throughout the Eubryidae (Ditrichaceae, some Dicranaceae, Leucobryaceae, Eustichiaceae, Calymparaceae, Encalyptaceae, Schistostegaceae, Mitteniaceae, Aulacomniaceae, Bryaceae, Funariaceae, Splachnaceae, Orthotrichaceae, Hookeriaceae and Hypnaceae) whereas separation along existing walls is far more restricted (some Dicranaceae, Pottiaceae and Mniaceae).

Although protonemal taxonomy is as yet in its infancy the foregoing discussion clearly suggests that it will prove a rich source of new data in the future. On the one hand gemma morphology, presence and absence may aid taxonomists at the species level, on the other the differences between the protonemata of *Sphagnum*, *Andreaea*, Polytrichales and Buxbaumiales, Tetraphidales and the Eubryidae have implications for understanding interrelationships between these groups.

15.8 SOME ECOLOGICAL CONSIDERATIONS

Apart from a handful of taxa with conspicuous persistent protonemata (and often small inconspicuous gametophores) such as Tetraphidales, *Pogonatum*, *Dicranella heteromalla*, *Schistostega*, *Disclerium*, *Ephemerum* and *Rhizomnium punctatum*, it is generally considered that protonemata in the vast majority of mosses are but transient progenitors of more permanent gametophores. Their principal functions being those of establishment and the production of numerous gametophores from single spores. In contrast the production of tubers by rhizoids indicates a major role in asexual reproduction. Other functions of rhizoids include attachment (Duckett, 1994b; Duckett & Ligrone, 1994) and the uptake of nutrients considered earlier in this review and by Bates & Bakken (1998). Our field observations are now beginning to reveal a much more important part played by protonemata in the overall reproductive biology of mosses than assumed hitherto.

The list of taxa that produce gemmiferous protonemata in culture now embraces over 80 species. As predicted by Whitehouse (1987), more and more of their wild counterparts are now being discovered (Duckett & Matcham, unpublished data). Field observations indicate that these wild gemmiferous protonemata are of two kinds, perennial and transient. Taxa in the former category include *Trematodon*, *Saelania*, *Epityrium*, *Isopterygium elegans* and *Eurhynchium swartzii* on soil, *Daltonia*, *Calympres*, *Octoblepharum*, *Dicranoweisia cirrata*, *Dicranum montanum*, *D. tauricum*, *Orthodontium lineare*, *Zygodon* and *Orthotrichum* on trees and *Tortula muralis*, *Syrrhopodon* and *Eustichia* on rocks. In the transient group, where the gemmae can only be found before the gametophores are fully developed, are *Ceratodon purpureus*, *Funaria hygrometrica* and a wide range of *Bryum* spp. Amongst the last taxa *B. bicolor* produces both bulbils and filamentous gemmae on the protonemata (Duckett & Ligrone, 1992). Where gemmiferous protonemata are perennial

they ensure a continuous supply of propagules throughout the year compared to the seasonal or non-existent production of spores. Moreover they almost certainly enable these mosses (Duckett & Ligrone, 1992) to survive and spread in conditions not conducive to gametophore maturation, let alone sexual reproduction. Transient production of protonemal gemmae is most often associated with maximal exploitation of transient habitats (e.g. bonfire sites, arable fields after application of fertilizers during seeding) when nutrient levels are at their peak.

At the opposite extreme to these proliferating protonemata are those which are perhaps best described, by analogy with monocarpic in angiosperms, as monogametophytic. Here in contrast to the numerous buds produced by a protonemal colony derived from a single spore as in *Funaria hygrometrica* or *Physcomitrella*, single spore protonemata produce but a single usually centrally-located gametophore. Once this gametophore matures the limited protonemal system rapidly degenerates and is very difficult to regenerate by subculturing. To date monogametophytic protonemata have been found in a handful of taxa including *Phascum* (3 species) *Acaulon muticum* and *Funaria obtusa*. All have one feature in common; the rapid production of a capsule on the gametophore.

This brief glimpse of the diverse nature of moss protonemata in nature holds much promise for future research. Several questions readily spring to mind: How does viability differ between spores, rhizoidal tubers, protonemal and gametophoric gemmae produced by the same taxon? What are the relative numbers of each propagule? What is their relative importance in the long- and short-distance establishment of new moss colonies? Why are protonemal propagules so abundant in some groups yet absent from others, e.g. Fissidentales, Grimiales. What are the relationships between the genetic heterogeneity of moss populations and their modes of reproduction? Clearly protonemata, rhizoids and their propagules now need consideration in bryophyte life strategies (During, 1979; Grime, Rincon & Wickerson, 1990).

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16. Gravitropism in moss protonemata

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16.1 SUMMARY

Protonemata of the mosses *Ceratodon*, *Physcomitrella*, *Funaria*, and *Pottia* possess apical cells which display a negative gravitropic response in the dark. Each of these taxa has amyloplasts that sediment in a broad subapical zone in horizontal apical cells. Amyloplasts also sediment along the length of vertical *Ceratodon* apical cells, and this sedimentation is restricted by microtubules. The hypothesis that these amyloplasts function in gravity sensing is supported by several lines of evidence, especially from *Ceratodon*. Other than plastids, the only structures that become laterally redistributed in horizontal cells are microtubules. The process of net upward curvature also includes two transient phases of downward curvature of the *Ceratodon* apical cell: an initial reversal, and a reversal which occurs during each mitosis. A mutant of *Ceratodon*, *wrong way response* (*wwr*) has net downward gravitropism and exhibits upward initial and mitotic reversals. Since plastid sedimentation occurs in both wild-type and *wwr* protonemata but the directions of gravitropism are opposite, it is unlikely that plastid sedimentation affects differential growth by physically redistributing Golgi stacks or exocytic vesicles. Moss protonemata appear to be the only uninucleate tip-growing cells with well-documented vigorous gravitropism and amyloplast sedimentation.

KEYWORDS: *Ceratodon*, amyloplast, gravity sensing, tip growth, mutant.

16.2 INTRODUCTION

Gravity has played a significant role in plant evolution, but we know relatively little about the effects of gravity on the growth, development and form of mosses. Clearly the upward growth (negative gravitropism) of moss stems and setae is adaptive in positioning leaves for photosynthesis and in elevating the capsule for spore dispersal. Protonemata are also negatively gravitropic, and presumably this enables filaments derived from spores buried beneath the soil to eventually reach the light. Previous research on mosses has described the phenomena of gravitropism in gametophores, sporophytes, protonemata and rhizoids, especially with respect to the possible role of starch in gravity sensing (Némec, 1906; Bischoff, 1912; Petschow, 1933; Banbury, 1962). More recent research has largely focused on gravitropism in protonemata (Jenkins, Courtice & Cove, 1986; Walker & Sack, 1990; Knight & Cove, 1991). Here

we review gravitropism in moss protonemata, especially in *Ceratodon*. For other reviews on gravitropism in tip-growing cells including mosses see Sack (1993), Sievers, Buchen & Hodick (1996), and Braun (1997).

16.3 MOSS PROTONEMATA MAY BE A UNIQUE GRAVITROPIC SYSTEM

Tip growth is characterized by the extension of a single cell at a highly confined region, the apical pole of the cell (Steer & Steer, 1989). There are many types of tip-growing cells, but the only ones with well-documented gravitropism are in algae and mosses. Gravitropic tip-growers in algae include the uninucleate rhizoids and protonemata of *Chara* which are positively and negatively gravitropic respectively, and the coenocytic negatively gravitropic rhizomes of *Caulerpa* (Jacobs, 1993; Hodick, 1994; Sievers *et al.*, 1996; Braun, 1997). In *Chara*, gravitropic sensing seems to rely upon the sedimentation of a unique membrane-bound organelle that contains barium sulphate. The organelle that sediments in moss protonemata and in the *Caulerpa* rhizome is the amyloplast, a heavy, starch-filled plastid. While some moss and liverwort rhizoids are reported to be positively gravitropic (Némec, 1906; Bischoff, 1912), this phenomenon has received little study. Thus, moss protonemata are the only uninucleate tip-growing cells with amyloplast sedimentation and well-documented vigorous gravitropism.

16.4 UPWARD GROWTH IN THE DARK

Gravitropism in moss protonemata occurs in the dark. For experimentation, protonemata are generated from spores or from fragments from light-grown cultures that are sown on agar supplemented with minerals and sugars. When *Ceratodon* cultures



Figure 1. Culture of protonemata of *Ceratodon* grown for 6 d in the dark with the surface of the agar vertical. The gravity vector is towards the bottom of the figure. Protonemata were grown on a porous membrane to prevent growth into the agar. Bar = 4 mm.

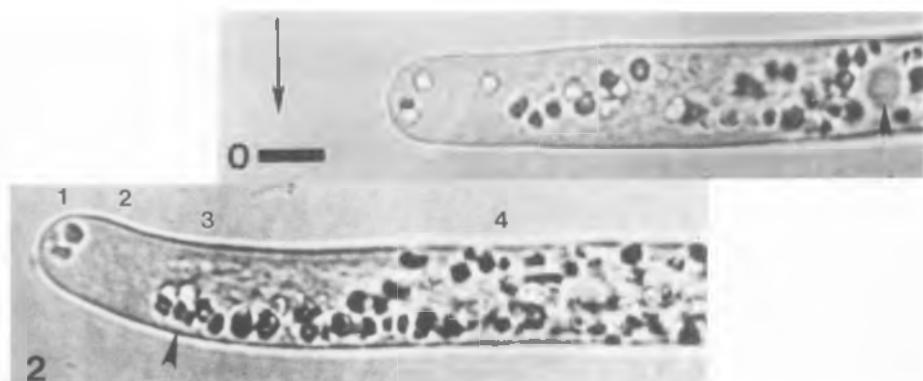


Figure 2. Micrographs from a time-lapse series of a single wild-type *Ceratodon* protonema reoriented to the horizontal (at 0 h; top micrograph). The micrographs are aligned to show the extent of growth over a two-hour period. By 2 h, upward gravitropic curvature and amyloplast sedimentation in zone 3 (arrow-head and zone number above cell) can be seen. Plastids in the apical dome (zone 1, 2 h) do not sediment and plastids are mostly absent from zone 2. Only slight plastid sedimentation (detectable quantitatively) occurs in zone 4 (labelled in 2 h micrograph). The gravity vector is indicated by the arrow (upper left, 0 h). Adapted from Young & Sack (1992). Bar = 20 μ m.

are left in the dark for five days, numerous straight, upright filaments develop (Fig. 1). When these protonemata are reoriented to the horizontal, new upward curvature usually starts after 30 min (Fig. 2), and most protonemata reach the vertical in 16 h (Walker & Sack, 1990). As in *Chara* rhizoids, moss protonemata curve gravitropically through 'bending by bowing', i.e. by a gradual curvature produced by the differential growth of opposite flanks in and near the apical dome (Sievers *et al.*, 1996; Braun, 1997).

Moss genera shown to have gravitropic protonemata are *Physcomitrella*, *Ceratodon*, *Funaria*, and *Pottia* (Cove *et al.*, 1978; Hartmann, 1984; Schwuchow, Kim & Sack, 1995; Chaban, 1996). Gravitropism is probably present in many other mosses as well, but most other genera have not been examined, or they do not grow for extended periods in the dark in axenic culture. Since gravitropism is inhibited by light, the process is studied either by reorienting dark-grown cultures and then fixing cells at various times, or by using infrared videomicroscopy (Cove *et al.*, 1978; Knight & Cove, 1991; Young & Sack, 1992; Lamparter *et al.*, 1996).

Gravitropism includes several phases. In *sensing*, gravity acts on a mass that then interacts with a receptor so that the force is *transduced* into information such as a physiological gradient. This information is then *transmitted* to the target region which *responds* with differential growth. In moss protonemata, all these processes probably occur in or close to the tip of the apical cell.

16.5 PLASTID SEDIMENTATION IS HIGHLY REGULATED

With respect to the sensing phase of gravitropism, a prominent candidate for the functional mass is the sedimenting amyloplast. These have been found in virtually all wild-type organs or plant cells that are gravitropic (Sack, 1991, 1997), including

in moss gametophores and sporophytes (Petschow, 1933; Banbury, 1962). In all cases, the location of amyloplast sedimentation is highly regulated developmentally; it occurs only in specific cells at specific stages of development. For example, although many cells in roots contain amyloplasts, their sedimentation is usually only found in the central columella cells of the rootcap. In columella cells, all the amyloplasts sediment to the bottom of the cell.

In dark-grown *Ceratodon* protonemata, amyloplast sedimentation is most obvious in horizontal cells in a specific subapical zone, zone 3 (Fig. 2; Walker & Sack, 1990). Sedimentation also occurs in vertical cells but this only becomes apparent when upright and inverted cells are compared revealing the presence of more plastids near the nucleus in upright cells (Fig. 3; Schwuchow & Sack, 1993).

Sedimentation in *Ceratodon* protonemata is similar to all other gravitropic systems in that the location of sedimentation is highly regulated in time and in space. However, protonemal sedimentation differs from, for example, columella cells in that only a subset of amyloplasts sediment and do so primarily in a specific zone. Also, the plastids that sediment in vertical protonemata do not fall completely to the bottom of the cell, whereas in columella cells sedimentation is usually complete.

Thus, in *Ceratodon*, plastid position is not only affected by mass, but by cellular elements that control which plastids fall, where they fall, and how far they fall. The application of cytoskeletal inhibitors to *Ceratodon* protonemata indicates that microtubules restrict the sedimentation of plastids along the length of the cell, and that microtubules are necessary to maintain plastid position against the force of gravity (Schwuchow & Sack, 1994). This finding also supports the general hypothesis that the cytoskeleton evolved, in part, to prevent the stratification of cell components with respect to gravity.

Sedimentation in *Ceratodon* apical cells also depends upon the proper placement of specific amyloplasts and on the properties of the sedimentation zone. Normally sedimentation occurs in zone 3, but when cells are centrifuged basipetally, amyloplasts sediment in the basal, vacuolar region of the horizontal cell (Walker & Sack,

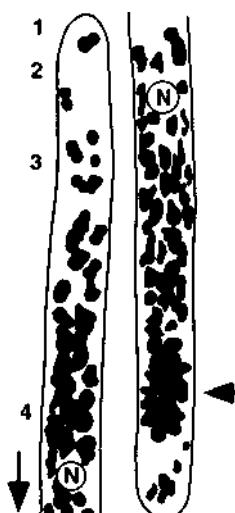


Figure 3. Diagram showing amyloplast sedimentation along the length of the cell in upright and inverted protonemata of *Ceratodon*. Plastid zones are numbered along the left. In the upright cell (left), many amyloplasts have sedimented out of zone 3 and into zone 4 where some accumulated close to the nucleus (N). The cell on the right shows a protonema that was in an inverted position for 1 h during which time many amyloplasts sedimented from zone 4 into zone 3 (arrow at right). Note that in both orientations, only some amyloplasts sediment and these do not fall all the way to the bottom of the cell. Arrow (lower left) indicates the gravity vector. Tracings of micrographs in Schwuchow & Sack (1993).

1991). After centrifugation, these amyloplasts migrate back towards the tip and rise up until they reach zone 3 when they sediment again. Thus, the amyloplasts that sediment can also fall elsewhere in the cell, but are actively positioned (presumably by the cytoskeleton) into the sedimentation zone. Furthermore, sedimentation depends not only on plastid size, but on some property of zone 3, possibly a lower cytosolic viscosity. These conclusions are reinforced by analysis of the movement of individual plastids in time-lapse video replays. In uncentrifuged cells, plastids migrate in both directions between zones 1–3. The same plastid that sediments in zone 3, rises up in zones 1 and 2 and vice versa (Young & Sack, 1992).

16.6 AMYLOPLASTS PROBABLY FUNCTION IN GRAVITROPIC SENSING

Much evidence supports the hypothesis that gravitropic sensing is plastid-based (Sack, 1991, 1997). Amyloplasts have a high density because of their high starch content, but, as indicated, this density only results in sedimentation in specific locations, suggesting that cells with sedimentation are physiologically specialized. Data supporting a role for amyloplasts in protonemal gravitropic sensing have been obtained primarily from *Ceratodon* and are summarized below.

16.6.1 Kinetics

Studies of populations (chemical fixation) or of individual protonemata of *Ceratodon* and *Funaria* (time-lapse videomicroscopy) show that amyloplast sedimentation occurs before upward curvature starts (Walker & Sack, 1990; Schwuchow *et al.*, 1995). Amyloplast sedimentation also takes place within the threshold stimulation time (presentation time) in *Ceratodon*.

16.6.2 Centrifugation

Basipetal centrifugation of *Ceratodon* protonemata removes amyloplasts from the tip half of the apical cell. When these protonemata are placed in a horizontal orientation, they grow as fast as uncentrifuged control cells, but they become gravitropic only when the amyloplasts migrate back to, and sediment in, zone 3 (Walker & Sack, 1991). The sedimentation of some amyloplasts in the bases of horizontal cells (before migration back) did not correlate with upward curvature. Thus, it appears that plastid sedimentation must occur in a specific region for upward curvature to occur.

The centrifugation data also support the hypothesis that the mass that acts in sensing is intracellular rather than involving the entire cell. After basipetal centrifugation, gravitropism is temporarily inactivated, but neither the growth rate nor the mass of the cell is affected (Walker & Sack, 1991).

16.6.3 Sedimentation zone

The four moss genera that have been shown to be gravitropic all have amyloplast sedimentation in the same zone (Walker & Sack, 1990; Schwuchow *et al.*, 1995;

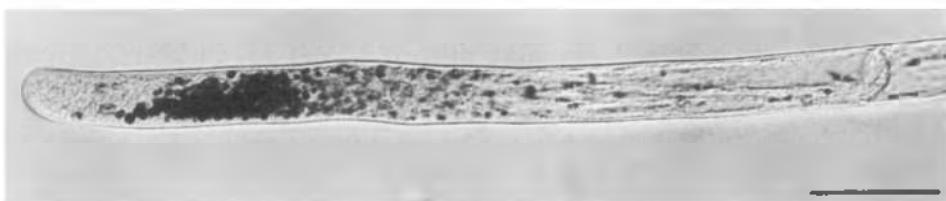


Figure 4. Start of upward gravitropic curvature in a dark-grown protonema of *Pottia intermedia*. This cell was horizontal for 2.5 h before it was chemically fixed. Note that amyloplast sedimentation takes place in a broad zone behind the tip. Bar = 50 μm .

Chaban, 1996). In *Funaria* and especially in *Physcomitrella*, sedimentation is subtler than in *Ceratodon* and *Pottia* (Fig. 4), but in all cases, it is present in a broad subapical zone in horizontal cells. Of these, only the protonema of *Ceratodon* has a cluster of non-sedimenting plastids in the apical dome (Figs 2, 4). But the fact that a sedimentation zone is conserved in all four genera supports the idea that this sedimentation serves a specialized function.

Collectively these lines of evidence support the hypothesis that specific amyloplasts provide the mass that acts in gravitropic sensing in moss protonemata. While these data are only correlations and do not establish a mechanism, the simplest interpretation is that gravitropic sensing is plastid-based in moss protonemata as well as in all plants.

16.7 APICAL CELLS HAVE A COMPLEX ZONATION

Understanding protonemal gravitropism requires knowledge of the cell biology of the regions where sensing and tip growth take place. To determine where different organelles are found in vertical *Ceratodon* protonemata, the ultrastructure of the first 35 μm of the apical cell was analyzed stereologically (Walker & Sack, 1995b). This region includes three plastid zones (Fig. 2), a cluster of plastids in the apical dome (zone 1), a plastid-free zone (zone 2), and the apical end of the sedimentation zone (zone 3).

This quantitative analysis revealed that each cell component is distributed in a characteristic pattern along the length of the tip (Fig. 5). The cisternal endoplasmic reticulum is distributed in a tip to base gradient whereas tubular endoplasmic reticulum is distributed evenly. Mitochondria are excluded from the very tip and are distributed evenly elsewhere. Small vacuoles are present in zone 3, but are excluded from the apical dome and the plastid-free zone. Golgi stacks are excluded from more of the tip than are mitochondria, and the Golgi stacks are primarily concentrated in the plastid-free zone. Golgi vesicles, identified by a characteristic staining pattern, are arranged in a tip to base gradient; they are most abundant in the very tip where presumably they fuse with the plasma membrane to contribute wall matrix materials necessary for tip growth. Thus, the longitudinal arrangement of organelles in the tip of vertical *Ceratodon* protonemata is complex, a finding which emphasizes the highly differentiated nature of tip-growing cells (Derksen *et al.*, 1995).

One of the outstanding questions in plant cell biology is how characteristic distri-

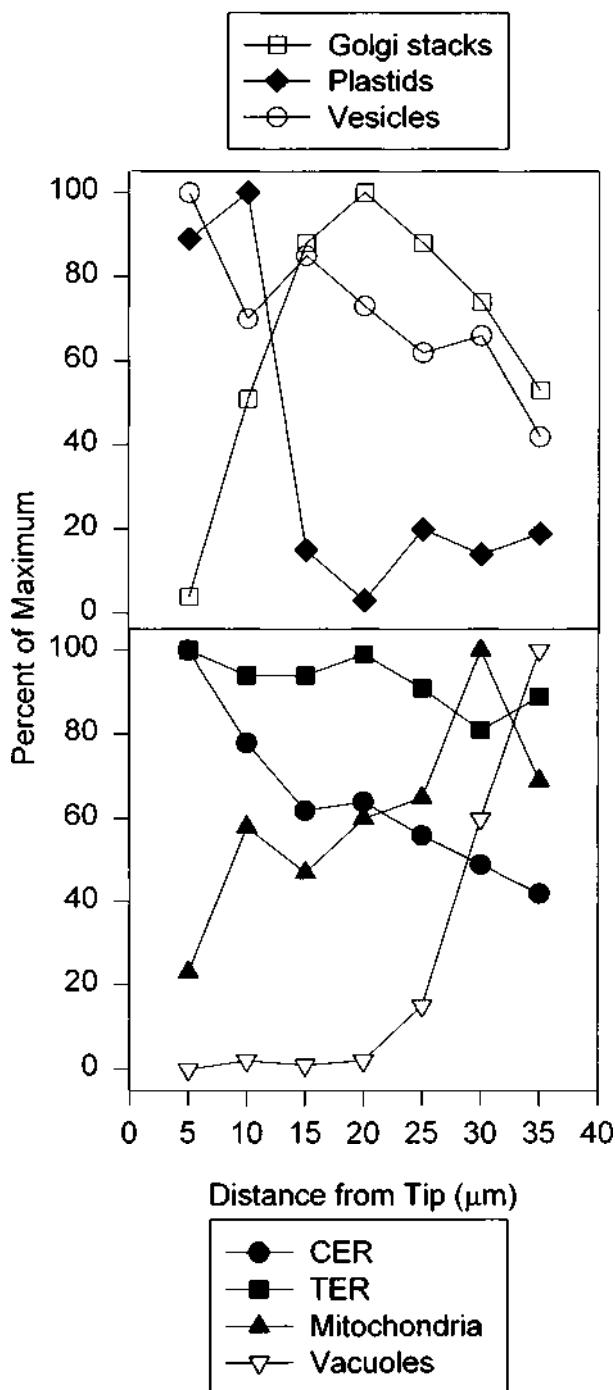


Figure 5. Distribution of different cell components in the tipmost 35 μm in vertical apical cells of *Ceratodon*. Note that each component has a different distribution, e.g. Golgi stacks are most abundant 10–30 μm from the apex (in the plastid-free zone), whereas exocytic vesicles produced by those stacks are most abundant in the first 10 μm . CER and TER = cisternal and tubular endoplasmic reticulum respectively. Data redrawn from Walker & Sack (1995b).

butions are maintained for each cell component despite organelle movement and tip growth (Young & Sack, 1992). Presumably the cytoskeleton and specific motors play a role, but all that is established to date for *Ceratodon* protonemata is that microfilaments are necessary for plastid zonation in the tip (Schwuchow & Sack, 1994).

With respect to gravitropism, these data show that the presumed site of sensing (the sedimentation zone) is separated from where wall matrix materials are synthesized in Golgi stacks (the plastid-free zone) and even further from where those materials are actually delivered to the plasma membrane (the apical dome). Hypotheses and data about possible connections between these sites are discussed later in this review.

16.8 TEMPORARY GROWTH REVERSALS OCCUR DURING GRAVITROPISM

In addition to studying sensing and cytology, an understanding of gravitropism requires careful study of the phenomenon of the growth response. Analysis of video replays shows that the direction of negative gravitropism actually reverses temporarily during two defined phases that occur during the larger process of upward curvature (Fig. 6).

The first phase is an initial reversal of curvature (IR). This is a transient downward growth response that occurs soon (5–10 min) after protonemata are reoriented from the vertical to the horizontal and before the start of upward curvature. We originally named this ‘wrong-way’ curvature, a term replaced by IR in this review to differentiate it from positive gravitropism (see below). This IR is best described for *Ceratodon*, but it also occurs in *Physcomitrella* and *Funaria* (Fig. 1 in Knight & Cove, 1991; Young & Sack, 1992; Schwuchow *et al.*, 1995).

The second phase is a mitotic reversal (MR), a temporary downward growth that

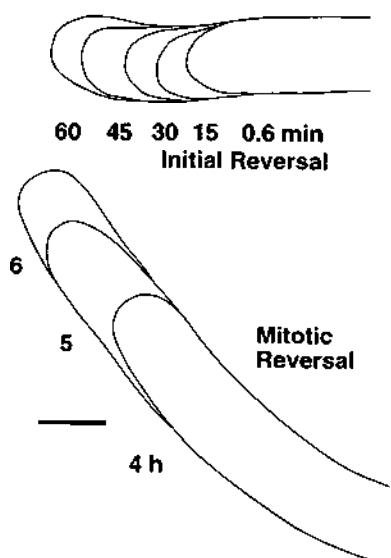


Figure 6. Diagram showing the initial reversal (IR) and mitotic reversal (MR) during negative gravitropism in wild-type *Ceratodon* protonemata. Each set of figures is traced from a different time-lapse series from Young & Sack (1992). The protonemata are aligned and superimposed to show tip extension. The numbers indicate the time elapsed since the cells were reoriented from the vertical to the horizontal. The IR (top) can be detected at 15 min by a flattening of the upper part of the apex and by a slight protuberance at the bottom. The MR (bottom) shows upward gravitropism at 4 h that is slightly reversed at 5 h but which recovers by 6 h. Bar = 20 μm .

takes place early in mitosis. Since protonemal gravitropism lasts longer than the cell cycle, mitosis occurs during upward curvature. The mitotic reversal was first described for *Physcomitrella*, but also occurs in *Ceratodon*, and *Funaria* (Knight & Cove, 1991; Young & Sack, 1992; Schwuchow *et al.*, 1995). In *Ceratodon*, the MR starts in prophase about 30 min before nuclear envelope breakdown. During the MR, the cell tip either actively grows downward or upward growth arrests. Upward curvature (negative gravitropism) resumes after cell plate formation (about 25 min after nuclear envelope breakdown). Knight & Cove (1991) present data suggesting that the degree of downward curvature is lower when a mitotic reversal occurs during the later stages of gravitropism as the protonema approaches the vertical.

The IR and the MR only affect the tip growth that occurs during these phases so that previous growth and curvature remain and are not reversed. It is not apparent how these reversals are adaptive, but their existence shows that protonemal gravitropism is a complex response. Thus, negative gravitropism is really a net growth response that includes two phases of growth of opposite polarity.

While the mechanisms underlying these reversals are completely unknown, they clearly do not result from temporary lapses in gravity sensing since the directions of the MR and IR are predictable. Previously, it was thought that the plastids in the apical dome functioned in gravity sensing for the IR since their presence correlated with that of an IR in basipetally-centrifuged *Ceratodon* protonemata (Walker & Sack, 1991). However, since *Physcomitrella* and *Funaria* protonemata were subsequently found to exhibit an IR and since they lack plastids in the dome, these plastids are not required for an IR to occur. In the case of the MR, it was hypothesized that if the nucleus functioned in gravitropic sensing, its dissolution in prophase might stop upward curvature (Knight & Cove, 1991). But, as indicated, during the MR the direction of growth is actively reversed and the MR starts before nuclear envelope breakdown.

Initial reversals also sometimes occur in the gravitropism of organs, but a mitotic reversal is unique to mosses.

16.9 WRONG WAY MUTANTS

Cove and co-workers pioneered the isolation of tropic mutants from mosses and identified three loci affecting gravitropism in *Physcomitrella* protonemata (Jenkins *et al.*, 1986; Knight & Cove, 1989). Mutations at two of these loci, *gtr A* and *gtr B*, result in weak negative gravitropism. Mutants of the *gtrC* class are positively gravitropic, i.e. they grow down towards the gravity vector. Recessive alleles were obtained at all three loci. This led to the hypotheses (1) that positive gravitropism is the default response and results from the loss of *GTRC* function, (2) that the mitotic reversal in the wild-type results from a transient inactivation of the *GTRC* gene product, and (3) that the *gtrC* mutant would therefore not show a mitotic reversal (Knight, Futers & Cove, 1991; Knight & Cove, 1991).

Recently, a positively gravitropic mutant of *Ceratodon*, termed *wrong way response* (*wwr*), was isolated (Fig. 7; Wagner, Cove & Sack, 1997). Since its gravitropism is more vigorous and faster than *gtrC*, it became technically feasible to use time-lapse videomicroscopy to determine whether *wwr* displays an IR and MR. It was found

that *wwr* protonemata show initial and mitotic reversals where they temporarily grow up. Also, protonemata of *wwr* grow down after a 90° reorientation with kinetics comparable to wild-type *Ceratodon*. It is not known whether *wwr* is recessive since somatic cell genetics have not yet been developed for *Ceratodon*.

These data suggest several interesting conclusions. Since the *wwr* mutant grows upwards during mitosis, the mitotic reversal in the wild-type probably does not result from the loss of a functional *WWR* gene product (assuming that the *wwr* mutation is recessive). Instead, since the responses of *wwr* and the wild-type mirror each other in all phases, it appears that the direction of the IR and MR are coordinated with, and opposite to, the direction of gravitropism. However, the *WWR* gene product itself is not necessary for this coordination since the *wwr* mutant has a coordinated response. Nor is the *WWR* gene product necessary for gravity sensing since *wwr* protonemata are fully capable of sensing gravity and grow down with high fidelity. Thus, the *WWR* gene product must act downstream of sensing and upstream of differential growth, and appears to be capable of interpreting some gradient containing information about cell orientation. These results with *wwr* are consistent with the hypothesis that the default response in protonemata is positive gravitropism and that the *WWR* gene product normally reverses the default in the wild-type. But they argue against the hypothesis that the IR and MR in the wild-type are default responses.

16.10 HOW MIGHT SENSING BE COUPLED TO DIFFERENTIAL GROWTH?

Assuming that amyloplast sedimentation triggers sensing, how might this event be coupled to a redirection of tip growth? The answer to this question is not known, nor are the mechanisms of either gravitropic sensing or of tip growth explained definitively for any system. But any hypothesis must account for how coupling occurs over a distance of about 30 μm , the distance between the apical end of the sedimentation zone and the apical dome.

Tip growth results from the localization of wall extension which most likely depends upon directed exocytosis of wall matrix materials and perhaps cellulose synthesis at the tip (Derksen *et al.*, 1995). Supporting data in moss protonemata include that putative cellulose synthase complexes, particle rosettes, are more abundant in the tip compared to the base of *Funaria* protonemata (Rudolph & Schnepf, 1988), and that Golgi vesicles are most abundant in the tipmost 5 μm of *Ceratodon* protonemata (Walker & Sack, 1995b).

For *Chara* rhizoids, the relatively simple 'steric block' hypothesis seems to explain existing data on gravitropism. Sedimentation close to and in the apical dome is thought to block exocytosis physically in the lower flank, thus redirecting vesicles to the upper flank and producing downward growth of the rhizoid (Sievers *et al.*, 1996). A redistribution of vesicles to the lower flank has been shown to occur in *Chara* rhizoids.

A previous model for *Ceratodon* resembled the 'steric block' hypothesis in that sedimentation was proposed to physically reposition the delivery of exocytic vesicles (Fig. 8; Sack, 1993). Microtubules, but not microfilaments, become more abundant in the lower flank of the plastid-free zone (zone 2) prior to and during upward

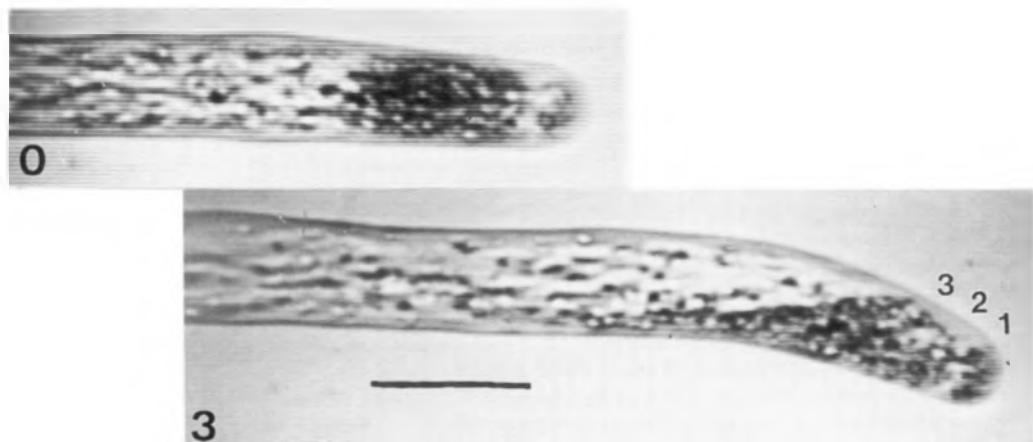


Figure 7. Time-lapse micrographs of the same *wrong way response* (*wwr*) protonema 0 and 3 h after horizontal placement. *wwr* protonemata are fully gravitropic but they grow downwards, not upwards. They also have many more plastids than wild-type protonemata, but have sedimentation in the same region and have a comparable plastid zonation (numbered in bottom micrograph). Bar = 50 μ m.

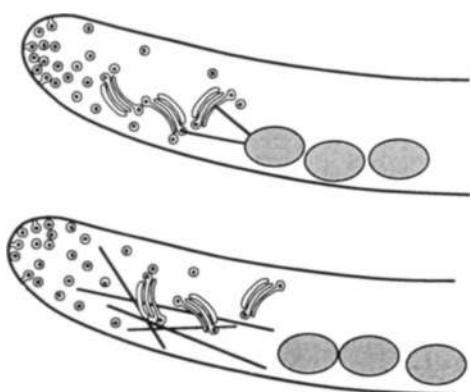


Figure 8. *Top:* Model for gravitropism in *Ceratodon* protonemata. Note the presence of more Golgi stacks and exocytic vesicles in the bottom of the cell. If amyloplast sedimentation pulled down Golgi stacks via microtubules, more vesicles might be found in the lower flank thus producing a more rapid growth of the lower cell wall and then upward growth. However stereological analysis failed to reveal a redistribution of Golgi stacks or vesicles (Walker & Sack, 1997). Thus, the *bottom* diagram summarizes cytological features that have been validated: (a) amyloplasts sediment in zone 3, (b) Golgi stacks are enriched in the plastid free zone, (c) Golgi vesicles with electron-dense contents are most abundant in the tip, and (d) microtubules are more abundant in the lower part of the plastid-free zone.

gravitropic curvature (Schwuchow, Sack & Hartmann, 1990; Walker & Sack, 1995a). If amyloplasts were coupled to microtubules — a hypothesis supported by the effects of microtubule inhibitors (Schwuchow & Sack, 1994) — then plastid sedimentation in zone 3 could passively redistribute microtubules to the lower flank of zone 2. If these microtubules were also connected to Golgi stacks, which are concentrated in zone 2 (Walker & Sack, 1995b), then sedimentation might also bring down the stacks resulting in the production of more exocytic vesicles and thus faster growth on the lower side. Thus, sedimentation would physically redirect exocytosis via cytoskeletal coupling between amyloplasts and Golgi stacks.

However, an ultrastructural stereological analysis of horizontal *Ceratodon* protonemata failed to reveal any asymmetry in the distribution of either Golgi stacks or

vesicles (Walker & Sack, 1997). It is possible that a subtle asymmetry exists but cannot be detected either because vesicle fusion is more rapid in the lower flank or because examination of a larger sample of cells (e.g. by immunofluorescence) might be required to show statistical significance.

And the phenotype of the *wrong way response* mutant of *Ceratodon* also argues against the concept that plastid sedimentation is physically coupled to exocytosis. *wwr* protonemata exhibit dramatic plastid sedimentation (Fig. 7; Wagner *et al.*, 1997). Since sedimentation leads to downward growth in *wwr* and to upward growth in the wild-type, the hypothesis of a physical redirection of growth seems untenable.

A 'current' working hypothesis is that amyloplast sedimentation is somehow coupled to the position of a growth determinant in the apical dome such as calcium channels in the plasma membrane. The apical dome in tip-growing cells such as pollen tubes is a prominent site of calcium uptake and contains a high concentration of cytosolic calcium (Derksen *et al.*, 1995; Pierson *et al.*, 1996). Active calcium channels might position growth by locally increasing the cytosolic calcium concentration which in turn may promote vesicle fusion with the plasma membrane.

Coupling might occur via extracellular currents. Transcellular current patterns exist in tip-growing cells and current patterns change when roots are reoriented to the horizontal (Weisenseel, Becker & Ehlgötz, 1992). Perhaps currents emanate from zone 3 and enter in the dome. If this positioned calcium channels, cell reorientation and plastid sedimentation might change current patterns and reposition the channels. If the *WWR* gene product were a protein that interprets this gradient, then this protein could be associated with the movement or activation of the calcium channels.

16.11 DIRECTIONS FOR FUTURE RESEARCH

Protonemal gravitropism is both intriguing and essentially unexplained. Work to date has established some probabilities and players, but much more research is needed to move beyond speculation.

One useful focus will be to identify additional gravitropic mutants and alleles and to further characterize *wwr*. The *WWR* gene is important since it functions in a crucial switch point in the gravitropic signal transduction chain. Ancillary goals are to develop the techniques for genetic analysis in *Ceratodon* and for identifying the genes disrupted by mutation in mosses.

Another important area is the analysis of the physiology of gravitropism using *in vivo* measurements such as determining whether reorientation induces changes in calcium concentrations or in current patterns. A related determination is how differential growth changes in time and space, i.e. to identify the target region for the response. To make use of fluorescent indicators feasible, it will be necessary to develop conditions or mutants that allow vigorous gravitropism in the light.

A third focus is 'moss in space'. Their small size and rich biology make moss protonemata prime candidates for spaceflight research to evaluate, for example, the effects of microgravity on cell differentiation, plastid position, tip orientation, and interactions with phototropism and light.

Moss protonemata are a unique and fertile system for investigating gravitational signal transduction and the control of cell zonation and tip growth. Since the sites

of gravitropic sensing and differential growth are located close to each other in the same cell, tip-growing systems offer much potential for reconstructing the sequence of events in gravitropism. Although most of these events necessarily differ from those in organ gravitropism, their relative accessibility should provide opportunities for experimentation not available with sensing cells located deep within a tissue.

16.12 ACKNOWLEDGEMENTS

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17. New perspectives in the biophysics and physiology of bryophytes

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17.1 SUMMARY

The paper summarizes recent advances in the biophysics of membrane transport, and the biochemistry of CO₂ fixation, in bryophytes. The fundamentals of transport of the plasmalemma and the tonoplast of bryophytes closely resemble those of their nearest relatives, i.e. charophycean algae and vascular plants. A good start has been made in relating these transport phenomena to the ecology of the organisms, and to their development (e.g. gametophyte to sporophyte solute fluxes), but further investigation is needed. A particularly interesting case is that of the 'action potentials' with a metabolic component in *Anthoceros* and *Conocephalum* which may have a role in signal transduction. An analysis of the relevant literature shows that the great majority of the bryophytes tested (mosses, liverworts, and the pyrenoid-less hornwort *Megaceros*) have C₃ physiology (diffusive CO₂ transport to RUBISCO), although two aquatic mosses (*Fissidens cf. mahalonensis*; *Fontinalis antipyretica*) show indications of the presence of a CO₂-concentrating mechanism. There is no evidence of C₄ or CAM photosynthesis in bryophytes. Those hornworts with pyrenoids (e.g. *Anthoceros*, *Phaeoceros*) have, on the basis of gas exchange and ¹³C/¹²C discrimination evidence, a well developed CO₂ concentrating mechanism. Quantitative analysis of the conductances of CO₂ in various parts of the pathway of CO₂ transport (fluid diffusion boundary layer; transport within the cell to RUBISCO; carboxylation *in situ* using ¹³C/¹²C discrimination; measurement of boundary layer conductances, measurements of biochemical capacity of photosynthesis) shows that less than half of the limitation of photosynthesis in aquatic mosses, and less than a third of that in terrestrial bryophytes, relates to CO₂ transport. In aquatic mosses in rapidly flowing water, and in some terrestrial bryophytes, there is little restriction of CO₂ fixation by CO₂ diffusion. However, there are several unexplained findings and both those bryophytes with C₃ physiology and the minority with a CO₂-concentrating mechanism need further investigation.

KEYWORDS: CO₂-concentrating mechanism, CO₂ diffusion, plasmalemma, pyrenoid, RUBISCO, tonoplast.

17.2 INTRODUCTION

Bryophytes have been used in many biophysical and physiological studies. Despite literature updates in *Journal of Bryology* and elsewhere, the results of these studies remain unfamiliar to many bryologists. In an attempt to remedy this situation, initially we review work, mostly carried out in the last 15 years, on the electrophysiology of bryophyte membranes and related phenomena, highlighting the reasons why bryophytes were used and the implications for bryology, as well as pointing out research opportunities. The rest of the paper considers work on photosynthetic inorganic carbon assimilation by bryophytes, emphasizing the transport processes involved as well as the biochemistry of CO₂ fixation and the ecophysiological implications of the findings.

17.3 MEMBRANE BIOPHYSICS AND RELATED AREAS

Raven (1989) has reviewed the work published before mid-1988 on biophysical aspects of membrane transport in bryophytes. What follows is a summary of the conclusions from that review, updated with more recent references (Fig. 1).

Measurements of the electrical potential difference (PD) across the plasmalemma of bryophytes (Anthocerotae, Hepaticae and Musci) show that the magnitude of this inside-negative PD is frequently greater than can be accounted for as the diffusion potential of any ion (Raven, 1989; Trebacz, Simonis & Schönknecht, 1994). Ion substitution and other evidence suggests that the electrogenic component of the PD (i.e. the component which is outside the range of possible PDs which could result from passive ion diffusion) results from an active efflux of H⁺ (Raven, 1989). While there does not seem to be any direct evidence in the molecular nature of this pump it is almost certainly a 'P' type ATPase catalysing the electrogenic efflux of one mole H⁺ for every mole ATP hydrolysed, as is the case for the closely related charophycean algae and vascular plants (Raven, 1989). This electrogenic H⁺ efflux pump plays a major long-term role in regulating cytoplasmic pH of bryophytes at 7.3–7.6 (Felle, 1989b; Raven, 1989; Renault *et al.* 1989). It is also responsible for energizing the influx of NH₄⁺ (*via* an electrically driven flux through channels) and of amino acids and hexoses (*via* H⁺: amino acid and H⁺: hexose cotransport using specific transporter proteins): Raven (1989); Renault *et al.* (1989; 1992); Felle & Johannes (1990) and, probably many other solutes as well (see below). The transport capacity of the H⁺ pump, as measured by voltage clamp measurements on *Riccia* rhizoids, is at least 1 μmol H⁺ (m² plasmalemma area)⁻¹ s⁻¹, while the capacities for transport of the solutes driven by the pump are, at least *individually*, less than the pump capacity: 0.2 μmol m⁻² s⁻¹ for hexoses and for neutral amino acids and 0.7 μmol m⁻² s⁻¹ for NH₄⁺ (and CH₃NH₃⁺) (Raven, 1989).

Data for other solutes in bryophytes indicate a need for active (i.e. necessarily energized by exergonic biochemical or solute transport processes) influx of K⁺, Cl⁻ and NO₃⁻ (and presumably, of SO₄²⁻ and H₂PO₄⁻) and efflux of Ca²⁺ (and presumably of Na⁺): Raven (1989); Felle (1989a); Trebacz *et al.* (1994). By analogy with charophycean algae and vascular plants active K⁺ influx presumably involves cotransport with H⁺ or Na⁺, both with exergonic influxes (Smith & Walker, 1989;

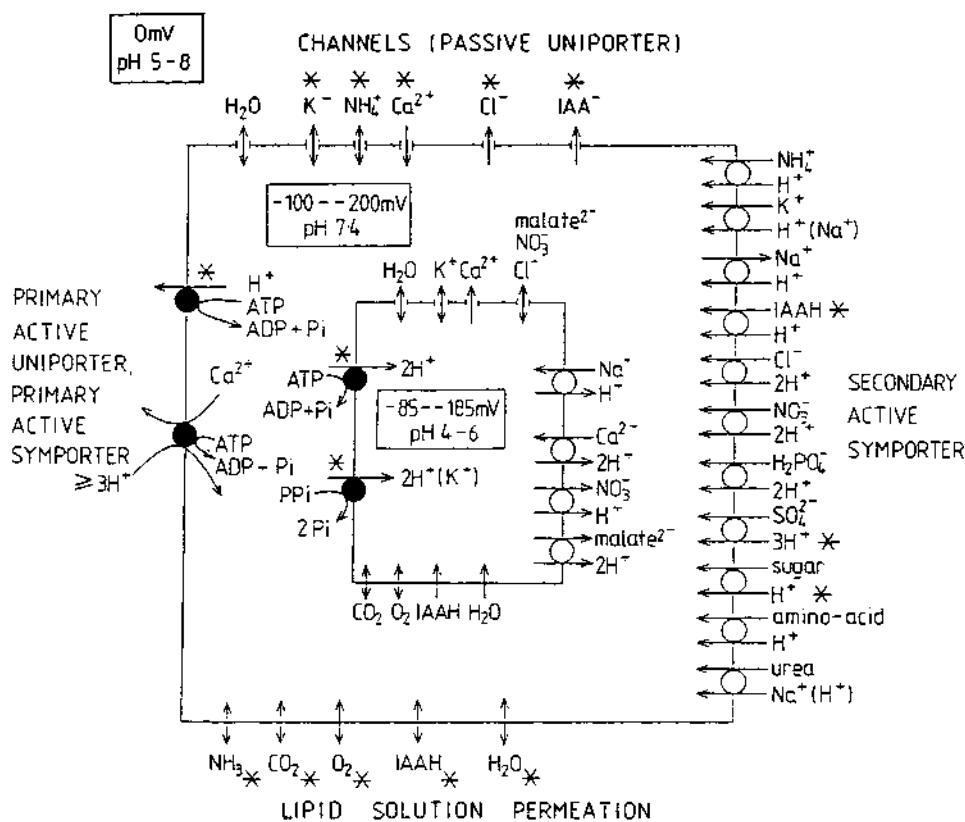


Figure 1. Transport processes at the plasmalemma and tonoplast of embryophytes, with those characterised from bryophytes indicated by an asterisk. The diagram was compiled from references in the text, and from Barkla & Pantoja (1996), Bush (1995), Lauter *et al.* (1996), Ninnemann, Jauniaux & Frommer (1995), Steudle & Henzler (1995), Tanner & Caspari (1996), Wang *et al.* (1994) and White (1996).

Maathius & Sanders, 1994; Schachtmann & Schroeder, 1994; Rubio, Gassmann & Schroeder, 1995), but this assumption needs testing on bryophytes. Again by analogy with higher plants, Ca^{2+} efflux against the very high electrochemical potential gradient (Felle, 1989a; Felle, Tretyn & Wagner, 1992; Trebacz *et al.*, 1994) presumably involves a 'P'-type ATPase catalysing the electroneutral exchange of 2 H^+ for 1 Ca^{2+} (Evans, Briars & Williams, 1991). Again, data are needed on the bryophyte transporter. The other fluxes (inorganic anion influxes; Na^+ efflux) are, again by analogy with charophyceans and vascular plants, driven by H^+ cotransport (or countertransport in the case of Na^+). No mention has been made here of active influx of inorganic C species (CO_2 and/or HCO_3^-); we shall see later that such fluxes are, apparently, only present in certain Anthocerotae and perhaps some submerged mosses.

The current vogue for patch-clamp studies of ion channels at the plasmalemma of plant cells (Hedrich & Schroeder, 1989; Tyerman, 1992) has not, apparently, so far impacted greatly on bryophytes. Ion channels catalyse the passive movement of ions, and appear to underlie the influx of (for example) NH_4^+ in bryophytes.

Modulation ('gating') of ion channel functioning is involved in (*inter alia*) signal transduction, and is exemplified in bryophytes by the action potentials which have been best characterized in *Conocephalum conicum* (Raven, 1989; Trebacz *et al.*, 1994). The action potential in *C. conicum* involves, as in other well-characterized plants, a depolarization caused by Ca^{2+} influx and Cl^- efflux followed by a repolarization related to (initially) K^+ efflux and then (presumably) H^+ efflux (Trebacz *et al.*, 1994). However, there is also a requirement for metabolism in the *C. conicum* action potential which is not completely explained by the mechanism given above (Raven, 1989; Trebacz *et al.*, 1994). *Anthoceros* shows a light-triggered electrical response resembling an action potential which is also modified by alterations in metabolism (Raven, 1989; Bulychev & Vredenberg, 1995).

Many fewer data are available on transport at the tonoplast of bryophytes than is the case for the plasmalemma (Raven, 1989). What information is available suggests great similarity to the norm for algal and vascular plant (and fungal) vacuoles with inwardly-directed H^+ active transport with the vacuole more acidic than, and electrically positive with respect to, the cytoplasm (Raven, 1989). The active H^+ transport mechanisms in the tonoplast include, as in vascular plants and charophycean and ulvophycean algae, an H^+ PPase as well as an H^+ -ATPase (V-type) (Maeshima, Mimura & Sato, 1994). As in most vascular plants, but unlike most algae, the vacuoles of bryophytes generally have a high ratio of organic to inorganic low M_r anions (Raven, 1989, 1991; cf. Raven, Rothmund & Wollenweber, 1991; Martins-Louçao, Wollenweber & Raven, 1993), possibly related to rhizosphere (including 'rhizoidosphere') acidification when organic anions are accumulated in vacuoles with implications for availability of P and Fe.

Other work on transmembrane fluxes in bryophytes includes the work on *Phaeoceros laevis* giant plastids (Raven, 1989) and on the possible role of ion fluxes across the plasmalemma in developmental processes (Raven, 1989, 1991) including indoleacetic acid transport (Rose & Bopp, 1983; Rose, Rubery & Bopp, 1983; Dibb-Fuller & Morris, 1992). In the context of the life of bryophytes, the phenomena mentioned above relate in general terms to nutrient and osmoticum transport and signal transduction in developmental processes, with more specifically bryophyte phenomena addressed in, for example, work on sugar and amino acid transfer from gametophyte to sporophyte (Renault *et al.*, 1989, 1992), and the limited range of bryophyte species which grow under saline conditions (Adam, 1976; Raven, 1989).

17.4 PHOTOSYNTHETIC CO_2 FIXATION: ENZYMES AND TRANSPORT MECHANISMS

At least 95% of the carbon in all photosynthetically grown plants has been fixed by the enzyme ribulose 1,5 bisphosphate carboxylase-oxygenase (RUBISCO); the remaining 5% or less has been fixed by other carboxylases which add C from CO_2 (or HCO_3^-) to products derived from RUBISCO activity to yield compounds essential in biosynthesis. Most of this non-RUBISCO C is fixed by an enzyme which adds $\text{CO}_2/\text{HCO}_3^-$ to a C_3 compound; in green algae and vascular plants this enzyme is almost invariably phosphoenolpyruvate carboxylase (PEPC). In a minority of vascular plants the CO_2 which is fixed by RUBISCO is first fixed by PEPC in the light

in cells which contain no RUBISCO (C_4) or in the dark in cells which contain RUBISCO (CAM).

The available evidence suggests that bryophytes are, *biochemically* at least, C_3 plants, i.e. the first carboxylation reaction for $\geq 95\%$ of CO_2 . The evidence is as follows.

(1) The ratio of *in vitro* RUBISCO carboxylase activity to *in vitro* PEPC activity in bryophytes (only mosses examined) is far higher than would be expected of C_4 or CAM plants (Rintamaki & Aro, 1985; Farmer, Maberly & Bowes, 1986; Keeley, DeNiro & Sternberg, 1986). The RUBISCO *in vitro* is not very different from the activity required *in vivo* to account for CO_2 fixation and the PEPC activity could not support the entire photosynthetic C flux (Rintamaki, Keys & Parry, 1988; Madsen, Sand-Jensen & Beer, 1993).

(2) There is no evidence of net nocturnal CO_2 fixation, or of significant increase in titratable acidity or of malic acid during the dark phase of the diel cycle, in bryophytes, thus eliminating the possibility of significant CAM-like processes (Keeley & Morton, 1992; Keeley *et al.*, 1986; Raven, MacFarlane & Griffiths, 1987; Osborne & Raven, unpublished experiments with *Marchantia polymorpha* and *Polytrichum commune*). This is another moss-dominated data set.

(3) Short-term $^{14}CO_2$ fixation experiments on *Amblystegium riparium* in the light showed the pattern expected of RUBISCO being the major initial CO_2 -fixing enzyme (Keeley *et al.*, 1986), although the 'short' time used (15 s) was rather longer than ideal to eliminate the occurrence of a preceding PEPC activity in a C_4 -like process.

Granted the moss-dominated nature of the data, we can conclude that all of the available information is consistent with bryophytes being *biochemically* C_3 plants.

Turning to the physiological (gas exchange) data, most of the evidence is consistent with C_3 biochemistry supplied with CO_2 by diffusion from the bulk external atmospheric or aquatic environment. The most widely available data relate to the CO_2 compensation concentration (the equilibrium CO_2 concentration attained in the light in a small volume of medium containing a bryophyte and initially O_2 at the air (equilibrium) value and zero to saturating CO_2). The CO_2 compensation concentration for C_3 biochemistry and diffusive CO_2 fluxes can be predicted from the kinetics (K_1 , V_{max} for CO_2 , O_2) of RUBISCO *in vitro*, with some correction for mitochondrial respiration in the light. There seem to be few data on the relevant kinetic properties of bryophyte RUBISCO (Yeoh, Badger & Watson, 1981), and what is available comes from a freshwater moss which, by comparison with data from green algae and higher plants, may have a higher K_1 than related terrestrial organisms, possibly related to the high CO_2 concentrations in many freshwaters and/or the presence of a CO_2 -concentrating mechanism (*cf.* Salvucci & Bowes, 1981). At all events the CO_2 compensation concentration of bryophytes, including values computed from pH compensation values for submerged bryophytes at various O_2 concentrations generally support C_3 physiology (Fock, Krotkov & Canvin, 1969, and references therein; Ruttner, 1947; Allen & Spence, 1981; Raven, MacFarlane & Griffiths, 1987). This agreement extends to photosynthetic rates as a function of CO_2 concentration at various O_2 concentrations (Fock *et al.*, 1969; see also Fock & Egle, 1966).

As well as these gas exchange characteristics, natural abundance level discrimination between ^{13}C and ^{12}C can be used as an indication of the means of inorganic

C entry. The $^{13}\text{C}/^{12}\text{C}$ of the organic C in the plant relative to that of the external source CO_2 is termed Δ , where

$$\Delta = \frac{\delta^{13}\text{C}_{\text{source}} - \delta^{13}\text{C}_{\text{plant}}}{1 + \delta^{13}\text{C}_{\text{plant}}} \quad (1)$$

and $\delta^{13}\text{C}$ is itself defined as

$$\delta^{13}\text{C} = \frac{^{13}\text{C}/^{12}\text{C}_{\text{sample}}}{^{13}\text{C}/^{12}\text{C}_{\text{standard}}} - 1 \quad (2)$$

where sample and standard refer to the unknown and a standard referred to the Pee Dee Belemnite (PDB). Values of Δ in excess of $\sim 20\%$ are generally indicative of diffusive CO_2 entry and C_3 physiology, although external moisture (in terrestrial bryophytes) and thick diffusion boundary layers (in aquatic bryophytes) can yield low Δ values despite diffusive CO_2 entry. Thus low Δ values can relate to C_3 physiology with a large limitation by CO_2 diffusion or to a CO_2 -concentrating mechanism (Raven *et al.*, 1994; Smith & Griffiths, 1996a, b). Relying on $^{13}\text{C}/^{12}\text{C}$ ratios from whole plants from nature suffers *inter alia* from the *integrative* nature of the plant signal. Variations in environmental factors, such as decreasing light supply (higher $\text{C}_3 \Delta$) or causing decreased diffusive conductance (lower $\text{C}_3 \Delta$), can alter the overall Δ value. Another problem (for aquatic bryophytes) is variations in water movement and hence of diffusive boundary layers. These problems, and others, can be overcome by measuring 'real time' $^{13}\text{C}/^{12}\text{C}$ discrimination values. This technique involves analysing the $^{13}\text{C}/^{12}\text{C}$ value of influent and effluent CO_2 passing over the illuminated plant material and computing the $^{13}\text{C}/^{12}\text{C}$ of the CO_2 removed by the plant. Such measurements have been made on bryophytes (Rice & Giles, 1994, 1996; Smith & Griffiths, 1996a, b; Williams & Flanagan, 1996); currently the method has only been optimized for (and applied to) bryophytes with different thallus water contents in air, not to submerged specimens, since liquid water on the surface of the thallus would impede CO_2 diffusion. The data obtained under well defined conditions of gas phase CO_2 concentration and constant gas phase CO_2 conductance, provide a better indication of whether the organism exhibits C_3 physiology, or deviates from it, than do the integrated Δ estimates from combusted specimens, although the spectre of reassimilation of respired CO_2 (depleted in ^{13}C) in contributing to the Δ values has not yet been exorcised (Smith & Griffiths, 1996a, b).

The available data on Δ values are discussed in more detail below in the context of their quantitative significance for CO_2 diffusion; for the present it suffices to say that the Δ values are consistent with C_3 physiology in most bryophytes (Raven *et al.*, 1987, 1994; Keeley & Sandgren, 1992; Rice & Giles, 1994, 1996; Smith & Griffiths, 1996a, b; and references in these papers).

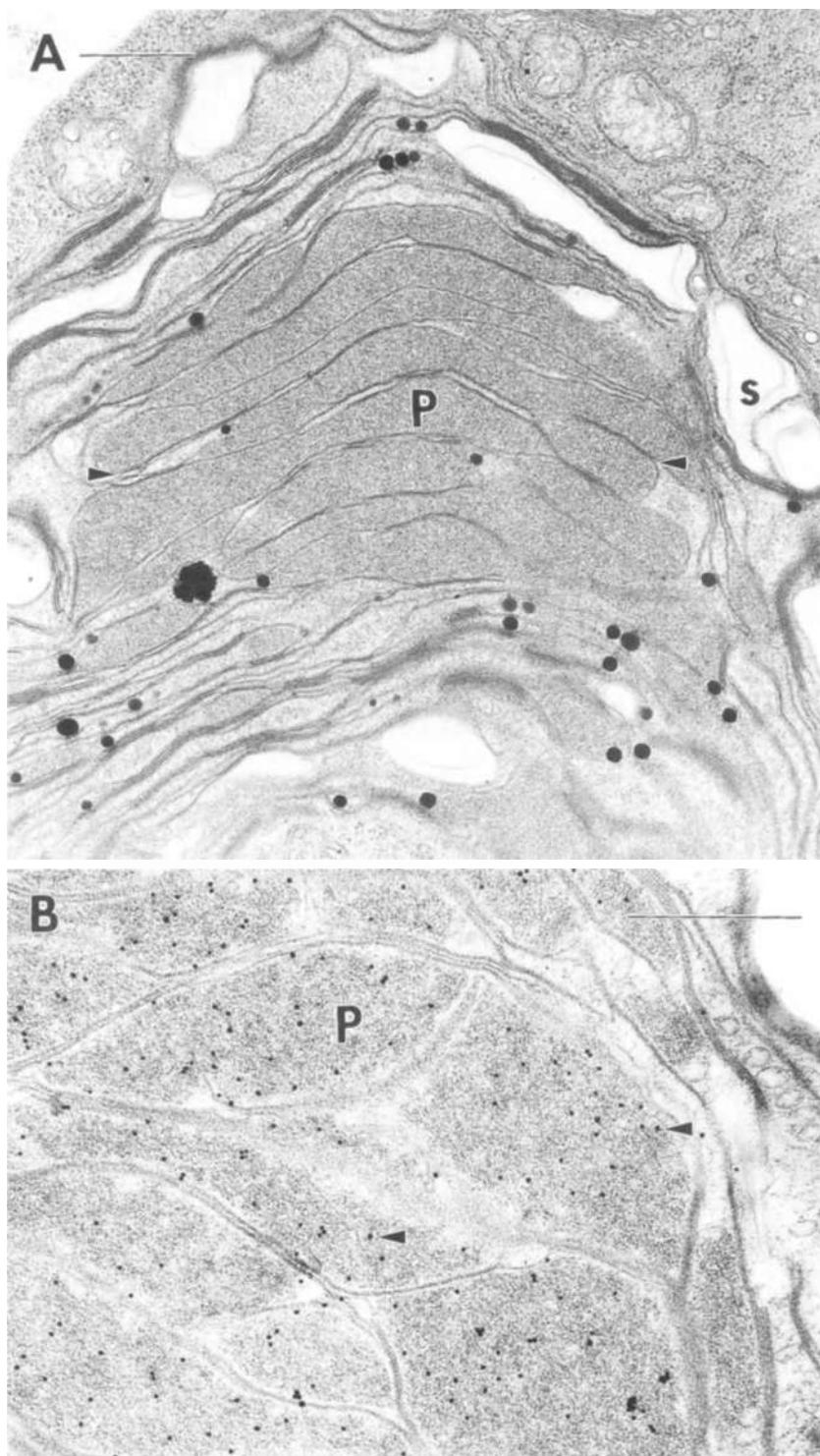
While the conclusions from these various lines of evidence discussed in the last two paragraphs are generally consistent with C_3 physiology as well as C_3 biochemistry in bryophytes there are some important exceptions (Salvucci & Bowes, 1981; Peñuelas, 1985; Smith & Griffiths, 1996a, b). Salvucci & Bowes (1981) showed that the submerged moss *Fissidens cf. mahatoensis* had a CO_2 compensation concentration consistent with C_3 physiology when cultured under 'Florida winter' conditions (12°C,

10 h daylength) but had a CO₂ compensation concentration which was much lower in 'Florida summer' conditions (30°, 14 h daylength). All the other freshwater macrophytes from Florida which were tested showed similar behaviour. The 'summer' characteristics are consistent with an inorganic entry mechanism other than CO₂ diffusion to RUBISCO which can lead to a CO₂ concentration around RUBISCO in excess of that in the medium either by C₄-like metabolism or active inorganic C transport across a membrane(s) (Salvucci & Bowes, 1981; cf. Raven, 1970).

No further information on this interesting phenomenon seems to be available for *Fissidens* cf. *mahalonensis* although much work has been done on the 'C₄-like' submerged angiosperm *Hydrilla verticillata* (Bowes & Salvucci, 1989). However, Peñuelas (1985) found that the aquatic mosses *Fissidens grandifrons* and *Fontinalis antipyretica* were able to carry out net photosynthesis at high inorganic C concentrations and high pH values which previous work suggested would yield a free CO₂ concentration below the CO₂ compensation concentration. Whether this is a result of external CO₂ production from HCO₃⁻ faster than the uncatalysed rate permits (i.e. using an external carbonic anhydrase) with subsequent CO₂ entry or direct entry of HCO₃⁻, is unclear, but the data suggest a CO₂-concentrating mechanism which could involve C₄-like metabolism or active transport of inorganic C across a membrane(s). The CO₂ compensation concentration and ¹³C/¹²C Δ values for central and Northern European populations of *Fontinalis antipyretica* (and *Fissidens rufulus*) are consistent with C₃ physiology, i.e. diffusive CO₂ entry and C₃ biochemistry (Ruttner, 1947; Osmond *et al.*, 1981; Allen & Spence, 1981; Raven *et al.*, 1987, 1994 and references therein).

The third set of data which do not fit the C₃ physiology paradigm have been more extensively investigated than the other two and relate to gas exchange and ¹³C/¹²C Δ values for those hornworts which have pyrenoids, i.e. *Anthoceros* spp. and *Phaeoceros* spp., but not *Megaceros* spp. (Smith & Griffiths, 1996a, b). As in those algae which have been investigated, the pyrenoid of anthocerotes contains most of the plastid complement of RUBISCO and RUBISCO activase (Vaughn *et al.*, 1990, 1992; Vaughn, cited by McKay & Gibbs, 1991) (Fig. 2). The pyrenoid in algae is thought to be involved in CO₂-concentrating mechanisms (references in Smith & Griffiths, 1996a, b), although pyrenoids are not present in all algae for which various lines of evidence suggest the presence of a CO₂-concentrating mechanism (Raven, 1997). Smith & Griffiths (1996a, b) therefore investigated the pyrenoid-containing Anthocerotae with respect to ¹³C/¹²C Δ values, CO₂ compensation concentrations, K_i values for CO₂ fixation, and direct evidence consistent with a CO₂-concentrating mechanism in terms of initial CO₂ uptake peaks in a dark-light transient even when inorganic C assimilation is suppressed by glycolaldehyde. Their findings are as follows.

The ¹³C/¹²C Δ values of *Anthoceros crispulus* using on-line methods, and of *A. agrestis*, *A. punctatus* (sporophyte), *Phaeoceros laevis* and *P. laevis* (sporophyte) by thallus combustion are low (7.2–11.7‰). The Δ values of *Megaceros monandreus* and *M. endivifolius*, and of the liverworts and mosses investigated by Smith & Griffiths (1996a, b) are 16.4–35.1‰, i.e. higher than for the pyrenoid-containing hornworts. These higher values for organisms in air are consistent with C₃ physiology, while the low values for pyrenoid-containing hornworts are consistent with a CO₂-concentrating mechanism.



The CO₂ compensation concentration for the only pyrenoid-containing anthocerotite (*Anthoceros crispulus*) investigated to date is 26 µmole CO₂ mole⁻¹, i.e. higher than C₄ plants and aquatic species with a CO₂-concentrating mechanism, but less than that of the typical C₃ values for liverworts and mosses (49–68 µmole mole⁻¹) reported by Smith & Griffiths (1996a, b) which are in the same range as those of earlier estimates, for liverworts and mosses.

Organisms with a CO₂-concentrating mechanism have higher affinities for external CO₂ than do those with C₃ physiology. Smith & Griffiths (1996a, b) found that the CO₂ concentration which yields half of the CO₂-saturated rate of photosynthesis (K_{½(CO₂)}) of *Anthoceros crispulus* is 167 µmole mole⁻¹, while that for mosses and liverworts is 287–348 µmole mole⁻¹, again consistent with a CO₂-concentrating mechanism in *Anthoceros crispulus* and C₃ physiology in other bryophytes (see Fock *et al.*, 1969).

The final piece of evidence relates to the occurrence and magnitude of the initial peak in CO₂ uptake after a dark-light transition which is insensitive (unlike subsequent long-term net CO₂ fixation) to glycolaldehyde which inhibits the photosynthetic carbon reduction cycle. The pool size for *Anthoceros crispulus* is 17.6 ± 1.9 µmol CO₂ g⁻¹ chlorophyll; four of the five C₃-physiology liverworts and mosses tested had no pool, while that in *Conocephalum conicum* was only 5.5. ± 1.0 µmol CO₂ g⁻¹ chlorophyll (*cf.* Fock & Egle, 1966).

In sum these data provide very convincing evidence for the presence of a CO₂-concentrating mechanism in pyrenoid-containing Anthocerotae and its absence (albeit based solely on Δ value measurements on herbarium material) for the pyrenoid-less *Megaceros* (Smith & Griffiths, 1996a, b). The presence of a CO₂-concentrating mechanism suppresses the oxygenase activity of RUBISCO *in vivo*, thus decreasing the rate of phosphoglycolate metabolism and the flux through the photo-respiratory carbon oxidation cycle. In this regard it is of interest that Frederick, Gruber & Tolbert (1973) failed to detect any glycolate-oxidizing enzymes in extracts of *Anthoceros* sp. However, no activity was detected in some of the other C₃ bryophytes tested, and a complete absence of glycolate oxidation capacity is not to be expected (Somerville, 1986); more work is needed.

Nothing seems to be known of the mechanism of active inorganic C transport or its energetics in the Anthocerotae. The evolutionary rationale for CO₂-concentrating mechanisms appears to relate to the trend (although not monotonic) of decreasing atmospheric CO₂ and increase in atmospheric O₂ since eukaryotes evolved some 2 Ga ago (Raven, 1995, 1997; Smith & Griffiths, 1996a, b). This unmasked the relatively low affinity of RUBISCO for CO₂ and has led to some changes in kinetic properties through natural selection, but there seem to be limits on the extent to

Figure 2. A. Electron micrograph of a chloroplast of *Phaeoceros laevis* through the pyrenoid-containing area of the plastid. The pyrenoid (P) is a multiple pyrenoid in that individual units of the pyrenoid are separate, intercalated by areas of non-pyrenoid stroma and thylakoids (arrowhead). Starch (S) surrounds the pyrenoid-containing area of stroma. B. Electron micrograph of a chloroplast of *Phaeoceros laevis* that has been prepared for immunocytochemical localizations. This section has been reacted with rabbit antiserum to tobacco RUBISCO and subsequently with Protein A coupled to 15 nm colloidal gold. The colloidal gold spheres (arrowheads mark two of these) are restricted to the multiple pyrenoid (P), indicating that it is the site of RUBISCO accumulation in the chloroplasts of pyrenoid-containing anthocerote species. Bars = 0.5 µm.

which the CO₂ affinity, and CO₂/O₂ selectivity ratio can be increased in plants with C₃ physiology. These kinetic attributes of RUBISCO have effects not only on the achievable rate of photosynthesis in air or air-equilibrated solution by C₃ plants, but impacts on the photon- and N-cost of growth and, for land plants, the water cost of growth (Raven, 1995, 1997; Smith & Griffiths, 1996a, b; references cited therein).

The C₄ and CAM pathways of photosynthesis represent relatively recently (tens of millions of years) evolved CO₂-concentrating mechanisms (Raven, 1995, 1997). While the pyrenoid is probably much older it is difficult to see its selective significance in the CO₂ and O₂ levels which characterized the environment of the earliest eukaryotic photosynthetic organisms, thus partially undermining the argument that it was an ancestral feature in photosynthetic eukaryotes (Raven, 1997). Furthermore, pyrenoids are not a *sine qua non* of CO₂-concentrating mechanisms in algae or in secondarily aquatic higher plants. Some molecular markers for pyrenoids whose genetic history could be traced from nucleotide sequences would be very useful in distinguishing monophyly from polyphyly of pyrenoids, and would help to clarify the evolutionary history of the pyrenoids of the hornworts. While there is currently no evidence supporting C₄ biochemistry as the means of concentrating CO₂ in organisms with pyrenoids (Raven, 1997) it would be worth checking the initial ¹⁴CO₂ fixation products and the PEPC:RUBISCO ratios in *Anthoceros* and *Phaeoceros*.

A final topic which we wish to address, that of quantitative aspects of the supply of CO₂ to RUBISCO, involves a return to considering the great majority of bryophytes with C₃ physiology and is the quantitative role of the diffusive CO₂ supply mechanism in restricting the overall rate of photosynthesis. In the formalism of flux control analysis this can be expressed as the 'control strength' of the CO₂ supply reactions. If C₃ photosynthesis is simplified to a diffusive CO₂ supply step followed by a carboxylation step (including all factors such as carboxylase content and light supply, which regulate carboxylation rate) the control strength for each of the two processes in series can, by definition, vary from 0 to 1, with the sum of the two always equal to 1 (see Woodrow & Berry, 1988; Cowan, Lange & Green, 1992). If a 10% increase in carboxylase activity causes no effect on CO₂ fixation rate then the control strengths of photosynthetic biochemistry is zero and that of CO₂ supply is 1, i.e. the only way in which the photosynthetic rate can be increased is by increasing CO₂ supply. By contrast, if a 10% increase in carboxylase activity increases the photosynthetic rate by 10% then the control strength of biochemistry (including light supply) is 1 and that of CO₂ supply is zero. In practice both CO₂ supply and subsequent reactions usually have finite (fractional) values.

We know of no data on bryophytes which can be subjected to the full rigours of flux control analysis. However, approaches can be made to examining the extent to which CO₂ supply or CO₂ consumption limit the achieved rate of photosynthesis from estimating biophysical (CO₂ supply) and biochemical (CO₂ consumption) capacities *in situ* as, for example, MacFarlane & Raven (1990) did for the freshwater red alga *Lemanea*, and from Δ values (again as performed by MacFarlane & Raven, 1990).

The Δ value approach applied to terrestrial C₃ bryophytes (e.g. data in Proctor, Raven & Rice, 1992; Williams & Flanagan, 1996; Smith & Griffiths, 1996a, b, and literature cited therein) yields limitation by CO₂ supply (=control strength of CO₂ supply) of about 0.01–0.4 based on Δ values of whole plant organic C, and lower

values (0–0.28) based on on-line Δ values at near optimal hydration. These values are in general accord with estimates based on analysis of conductances to CO_2 in various parts of the CO_2 transport and fixation sequence (Green & Snelgar, 1982; Cowan *et al.*, 1992; Green & Lange, 1994; Nobel, 1977; Raven, 1992; cf. Laisk & Loreto, 1996). The higher values of fractional limitation by CO_2 supply are similar to those found for vascular plant sporophytes with stomatal limitations on CO_2 supply added to gaseous boundary layer and aqueous phase restrictions. Williams & Flanagan (1996) have also applied on-line measurements of the $\delta^{18}\text{O}$ of CO_2 to estimate the ratio of hydration of CO_2 (catalysed by carbonic anhydrase) to carboxylation as 25 in *Pleurozium schreberi* and 30 for *Sphagnum* (section *Acutifolia*), i.e. similar to that for C_3 vascular land plants.

For aquatic bryophytes the Δ value approach involves the same formalism as used for terrestrial bryophytes, although the term for CO_2 diffusion involves a much smaller $^{13}\text{C}/^{12}\text{C}$ discrimination in water than in air (see Raven *et al.*, 1987, 1994). Application of this methodology to the much-investigated moss *Fontinalis antipyretica* (Osmond *et al.*, 1981; Raven *et al.*, 1987, 1994) gives very small limitations (0–0.15) by CO_2 supply in specimens from flowing waters. This would be expected from the reduction in boundary layer thickness associated with water flow and the CO_2 level in these waters which exceeds air-equilibrium values. However, rapid water flow can have direct inhibitory influences on photosynthesis in freshwater aquatic macrophytes, including *Fontinalis antipyretica*, independent of diffusion boundary layer effects (Madsen, Enevoldsen & Jørgense, 1993). *Fontinalis antipyretica* from several metres depth in lakes, in a much less vigorous hydrodynamic regime has lower Δ values, consistent with a greater fractional limitation by CO_2 (0.665) as a result of thicker diffusion boundary layers, despite the presence of CO_2 at concentrations in excess of air equilibrium and lower photon flux densities as a result of attenuation by a greater depth of water (Raven *et al.*, 1994; cf. Keeley & Sandgren, 1992; Maberly, 1995a, b). The Δ values of *Fontinalis antipyretica* give no direct support for the HCO_3^- use shown by Peñuelas (1985).

These estimates of limitation of *in situ* photosynthesis by CO_2 supply are in general agreement with estimates for *Fontinalis antipyretica* based on estimates of conductance in different parts of the CO_2 transport and assimilation pathway (Raven *et al.*, 1987, 1994; see also Maberly, 1995a, b). Other conductance estimates involving hydrodynamic modelling include the work of Jenkins & Proctor (1985; cf. Proctor, 1984), who examined two mat-forming leafy liverworts as well as *Fontinalis antipyretica* with its 'streamer' life form; interpretation of these data is made difficult by unexpectedly (erroneously?) high area-based rates of photosynthesis.

It should be pointed out that not all estimates of limitation by CO_2 diffusion derived from estimates of Δ agree with expectation from thallus structure and hydrodynamics. Thus, Rice & Giles (1994, 1996; cf. Rice & Schuepp, 1995) found similar Δ values for species of *Sphagnum* with superficial and with more central dispositions of chloroplasts in their leaves; the species with the more superficial chloroplasts would, other things being equal, be expected to have a higher Δ values. Similarly, *Rhynchostegium riparioides* from flowing freshwater has a lower Δ than does *Fontinalis antipyretica* of similar life form in apparently similar flow regimes (Raven, Johnston, Saville & McInroy, 1995, unpublished), although the same difference in Δ was also noted for *Lemanea* spp. (Rhodophyta) co-occurring with *Fontinalis*.

relative to those co-occurring with *Rhynchostegium* (Raven, Johnston, Saville & McInroy, 1995, unpublished). These findings show that much more detailed work is needed if we are to have a satisfactory knowledge of CO₂ supply to RUBISCO in the apparently simple case of submerged bryophytes with C₃ physiology.

17.5 CONCLUSIONS AND PROSPECTS

The last two decades have seen very important advances in the physiology and biophysics of bryophytes. Biophysical studies of transport at the plasmalemma and tonoplast of bryophytes show great similarities to phenomena in charophycean algae and vascular plants. The action potentials of *Anthoceros* and *Conocephalum* need more investigation, especially with respect to their possible function, and the solute transport measurements and their interpretation need further integration into the developmental biology and ecology of bryophytes. The very recent discovery of a CO₂-concentrating mechanism in pyrenoid-containing hornworts shows that there are still surprises in the broad-scale phenomena of CO₂ acquisition in bryophytes. Furthermore, the recent use of stable isotope measurements as an additional approach to studying CO₂ supply constraints in that majority of bryophytes which have C₃ physiology shows agreement with other estimates of CO₂ conductances in some cases. However, we still have much to learn about CO₂ transport to RUBISCO (and RUBISCO itself) in bryophytes.

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18. Ecophysiology of photosynthetic pigments in aquatic bryophytes

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18.1 SUMMARY

Aquatic bryophytes are ecologically important in river phytobenthos, especially in mountain streams, despite the adverse environmental conditions. Other hostile aquatic habitats, such as deep lakes with dramatic light starvation, are also successfully inhabited by bryophytes. Several pigment variables such as chlorophyll and carotenoid concentrations, chlorophylls/phaeopigments ratios and chlorophyll/carotenoid ratios can be used as indicators of vitality or stress, since pigment composition varies significantly as a function of both genetic and environmental (light, temperature, water and nutrient availability) factors, and its measurement is relatively easy compared to photosynthesis or growth. Also, pigment variables are very useful in the bioindication of pollution caused by turbidity, acidification, heavy metals, eutrophication, and organic matter. Some future trends in the investigation of the ecophysiology of pigments in aquatic bryophytes, such as the functioning of the xanthophyll cycle and the use of chlorophyll fluorescence as a vitality index, are outlined.

KEYWORDS: Aquatic bryophytes, photosynthetic pigments, chlorophylls, phaeopigments, carotenoids, vitality indices.

18.2 INTRODUCTION

There are only three aquatic environments dominated by bryophytes: peat bogs, lakes and mountain streams. Bryophyte domination is primarily based on the tolerance to adverse environmental factors. The key stresses and disturbances in lakes are cold water, low photon flux densities of blue-green light in the deep zones, high hydrostatic pressure, and abrasion along the shores. In streams, the adverse factors are abrasive damage, substratum movement, cold water, nutrient limitation in soft waters, CO₂ limitation in the stagnant parts of alkaline streams, high water velocity that can cause a decline in photosynthesis, seasonal desiccation, ultraviolet radiation in high-altitude streams, and diaspore difficulties in attaching to new substrates. Disturbance of bryophytes due to herbivory is considered to be negligible.

Bryophytes in lakes and rivers act as primary ecological producers and provide physical support for periphyton. They also provide a refuge, and occasionally food,

for both larvae and adults of macroinvertebrates. Aquatic bryophytes have also been used frequently as environmental tools in the assessment of water pollution.

18.3 PHOTOSYNTHETIC PIGMENTS: LOCATION AND FUNCTION

The photosynthetic pigments of aquatic bryophytes are similar to those found in green algae and cormophytes: chlorophylls (chl) a and b, as well as two dozen carotenoids, among which the pure hydrocarbons alpha- and beta-carotene and the oxygen-containing xanthophylls lutein, zeaxanthin, violaxanthin and neoxanthin are most frequent (Taylor, Thomas & Otero, 1972; Schmidt-Stohn, 1977; Czeczuga, 1980, 1985; Czeczuga, Gutkowski & Czerpak, 1982; Huneck, 1983; Farmer, Boston & Adams, 1988; Boston *et al.*, 1991). In the approximately 60 species tested, there is no outstanding qualitative difference between the carotenoids found in terrestrial and aquatic bryophytes, except for the almost exclusive auroxanthin of *Fontinalis antipyretica* (Bendz, Lööf & Martensson, 1968).

In the absence of specific data on bryophytes, the following paragraphs come from work on higher plants (Salisbury & Ross, 1992). Chlorophylls and carotenoids are embedded within the thylakoids of the chloroplasts and are attached by noncovalent bonds to protein molecules, constituting the two photosystems that co-operate in photosynthesis, PSI and PSII. The pigment composition of the photosystems is crucial to understanding the ecophysiology of pigments. Both photosystems consist of a core complex (CC) and a dedicated light-harvesting complex (LHC). The CC of PSII contains only chl-a and beta-carotene as photosynthetic pigments. It also contains the reaction centre or P680, which is a dimer of chl-a that specifically absorbs light of 680 nm wavelength. The CC of PSII is surrounded by a LHC. This antenna system contains chl-a and chl-b molecules (in an approximate proportion of two chl-a per chl-b) and numerous xanthophylls (Scheer, 1991). The reaction centre of PSI is called P700, since it is a dimer of chl-a that absorbs light of 700 nm, and the LHC is much more enriched in chl-a (proportion a:b approximately 6:1; Scheer, 1991). There is a possibility of lateral migration of LHCII from PSII to PSI, caused by an excitation excess on PSII; this permits a redistribution of the excitation energy between the two photosystems (Taiz & Zeiger, 1991).

Photosynthetic pigments absorb light somewhere in the 400–700 nm range. Chl-a and -b have a strong absorption band in the red and another stronger band in the blue region, whereas absorption is very low in the green region. Chl-a absorbs only weakly between 450 and 650 nm, and chl-b has the effect of increasing absorption within this window, at both the long- and short-wavelength ends. The carotenoids extend absorption still farther into the window, at the short-wavelength end (their maximum absorption occurs between 450 and 490 nm). The light-harvesting ability of carotenoids and, to a certain extent, of chl-b, allows for utilization of blue-green light that is poorly or not at all absorbed by chl-a; also, chl-b has a higher rate of photon absorption in the blue than chl-a, because of a concomitantly higher specific coefficient. This is especially important for aquatic species, which may receive blue-green and blue, but not red light, due to the absorptive properties of the water body (Kirk, 1994).

Together with light-harvesting functions, carotenoids have an important photo-

protective role in the photosynthetic apparatus. Under an excess of light, electron transport reactions may be blocked and the consequent accumulation of singlet chlorophyll (the 'normal' excited state) can lead to the formation of triplet chlorophyll, which readily reacts with oxygen to give rise to singlet oxygen. The xanthophyll zeaxanthin can directly deactivate singlet chlorophyll and other carotenoids can deactivate both triplet chlorophylls and singlet oxygen, dissipating energy as heat (Demmig-Adams & Adams, 1992). These mechanisms are responsible for photo-inhibition of photosynthesis under an excess of light and, at the same time, protect the photosynthetic pigments from photooxidation.

18.4 PIGMENT VARIABLES IN RELATION TO ECOPHYSIOLOGY

Photosynthetic pigment composition is highly useful in evaluating the vitality or stress state of photosynthetic organisms. The simple and rapid spectrophotometrical determination of photosynthetic pigments permits the calculation of a number of interesting pigment variables: chlorophyll concentration, chl a/b ratio, phaeophytin (a type of chlorophyll-degradation pigment) concentration, total carotenoids concentration, and some pigment indices that involve chlorophylls, phaeophytins, and carotenoids. When several pigment variables are collected in one sample, a global response of pigments to environmental changes can be revealed, and thus those results are more reliable than when a unique variable is measured. More accurate methods than spectrophotometry, like HPLC, are indispensable to analyze individually the different types of carotenoids and the numerous chlorophyll degradation products. Carotenoids and degradation pigments can be important in ecophysiological studies, but frequently HPLC is not easily applicable to routine measurements. Therefore, we will pay attention here only to the usefulness of the spectrophotometrically calculated variables.

The efficiency with which the photosynthetic organs absorb light depends on the chlorophyll concentration. A chlorophyll concentration of 300 mg m^{-2} permits 99% absorption of the incident light (Margalef, 1982), but the increase in leaf absorptance is not linearly dependent on chlorophyll content per leaf area in higher plants, since an increase in chlorophyll content per leaf area results mainly in an increased absorptance in the green and the far-red regions, with only a slight increase at wavelengths for which the chlorophyll has a high absorption coefficient (Björkman & Demmig-Adams, 1995). As a consequence, a lineal relationship between chlorophyll concentration and growth is not usually found in higher plants (Björkman, 1981) or in several *Sphagnum* or terrestrial mosses (Hodgdon & Bain, 1979; Austin & Wieder, 1987; Gaberscik & Martincic, 1987). Nevertheless, a correlation between photosynthesis and chlorophyll concentration has been found more often in situations of light deficit, both in bryophytes (Hearnshaw & Proctor, 1982; Kershaw & Webber, 1986; Gaberscik & Martincic, 1987; McCall & Martin, 1991) and higher plants, and Frost-Christensen & Sand-Jensen (1992) emphasize that chlorophyll variations directly influence both light absorption and photosynthesis in macroalgae and submerged angiosperms, whereas terrestrial plants have chlorophyll in excess. We can conclude from these data that despite its limitations, chlorophyll content is a valid indicator of vitality, also affected by adaptations and acclimations of plants to environmental

conditions. Finally, a decline in chlorophyll content is a clear symptom of damage or senescence, although non-specific because it is affected by very different stress factors.

Chlorophyll content expressed per 'leaf area' is more closely related to light interception than when expressed per plant weight, as the interference of the non-photosynthetic tissues and sediment particles is reduced. However, the measurement of 'leaf area' in bryophytes is difficult. Thus, measurements are usually expressed per unit of dry or fresh weight. Comparative values for chlorophyll concentration of photosynthetic organisms are shown in Fig. 1, based mainly on data quoted by Martínez-Abaigar, Núñez-Olivera & Sánchez-Díaz (1994). The chlorophyll concentrations of aquatic bryophytes range between 2 and 15 mg g⁻¹ dry weight and between 100 and 350 mg m⁻² (Glime, 1984; Peñuelas, 1984a; Peñuelas, Murillo & Azcón-Bieto, 1988; López & Carballeira, 1989; Martínez-Abaigar *et al.*, 1994). Chlorophyll contents of terrestrial bryophytes are comparatively lower, especially if they are compared only with obligate aquatics, because facultative aquatics and emergent species (in the sense of Vitt & Glime, 1984) have relatively lower chlorophyll contents (Martínez-Abaigar *et al.*, 1994; Rice, 1995). Aquatic cormophytes have more chlorophyll (100–600 mg m⁻²; Margalef, 1983; Frost-Christensen &

CHLOROPHYLL CONCENTRATION

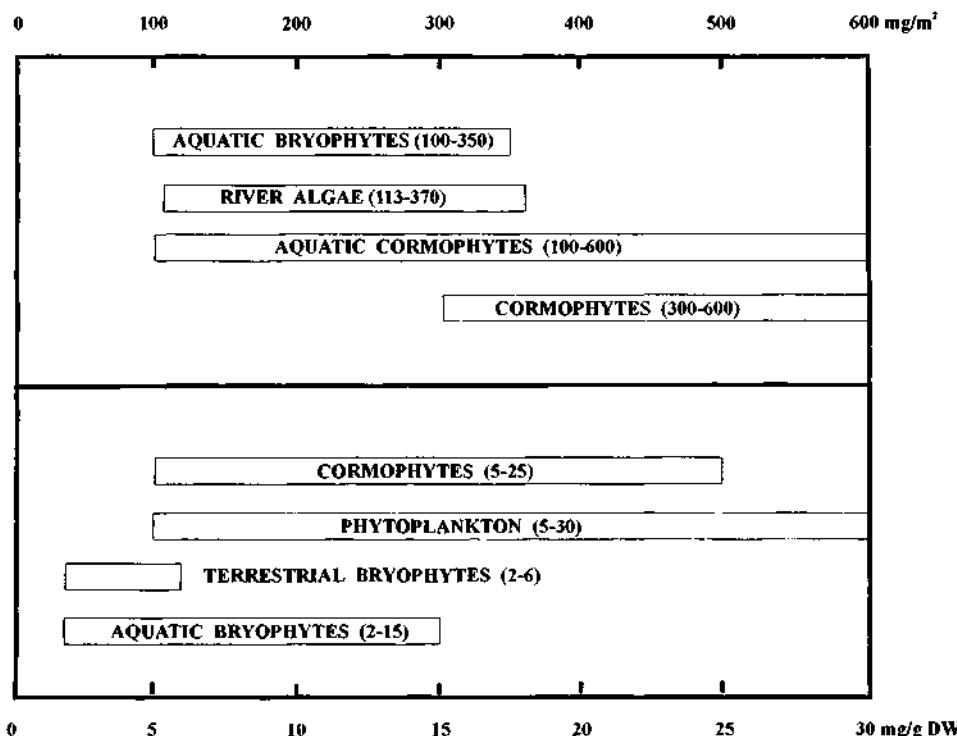


Figure 1. Comparative chlorophyll concentrations of diverse types of photosynthetic organisms expressed on an areal (above) and weight (below) basis.

Sand-Jensen, 1992) than aquatic bryophytes, due to the more complex structure of photosynthetic tissues, and phytoplankton also has more chlorophyll than bryophytes per unit dry weight, probably because of a lower mass of cell walls. Chlorophyll contents of aquatic bryophytes are thus closer to those found in river algae.

The ecophysiological relevance of the chl a/b ratio lies in the different role and abundance of the two types of chlorophyll in the core complexes (CC) and light-harvesting complexes (LHC) of the photosystems I and II (see above). The ratio increases with tissue activity, as a consequence of an enhanced synthesis of chl-a in the growing parts of the plants (Margalef, 1982). By contrast, the ratio typically decreases in plants that are experiencing senescence or under stress conditions, since the degradation of the LHCs, relatively enriched in chl-b, is slower than the degradation of the CCs, in which only chl-a occurs (Hendry, Houghton & Brown, 1987). The chl a/b ratio also decreases in plants grown under shade conditions (Martin, 1980), permitting a more intense absorption of blue-green light. However, the numerous interacting factors that can influence the chl a/b ratio bring into question its ecological significance (McCall & Martin, 1991; Post & Vesk, 1992; Rincón, 1993; Lovelock, Osmond & Seppelt, 1995).

The chl a/b ratios of aquatic bryophytes from diverse habitats range mostly between 2 and 3 (Peñuelas, 1984a, b; Peñuelas *et al.*, 1988; López & Carballera, 1989; Martínez-Abaigar *et al.*, 1994). These are normal values for bryophytes, but they are significantly lower than those of cormophytes (2.4 to 3.7; Martin & Churchill, 1982). This can be interpreted as a global adaptation of bryophytes to shade conditions (Martin, 1980), and also as a lower physiological activity in comparison to cormophytes (Martínez-Abaigar *et al.*, 1994).

The presence of chlorophyll breakdown products in plant extracts is a good tool to evaluate their physiological state. The brown-coloured phaeopigments are undoubtedly the most cited degradation pigments in ecophysiological studies. The action of dilute mineral acid upon acetone extracts of pigments transforms each chlorophyll into its respective phaeophytin, through replacement of the central magnesium by two hydrogen atoms (Lorenzen, 1967). The red maximum of phaeophytins is lower than that of chlorophylls, and the blue maximum of chlorophylls at 430–435 nm shifts to 410–415 nm in phaeophytins. The phaeophytin-a concentration can be calculated from the ratio between the absorbance at 665 nm before and after acidification, and the ratio itself is used as a vitality index. Another phaeopigment index that does not require acidification is the ratio between the absorbances at 430 and 410 nm, or between the absorbances at 435 and 415, before acidification (Moss, 1967; Ronen & Galun, 1984). All these vitality indices represent the proportion between chlorophylls and phaeopigments, and decrease under stress conditions. They must be used cautiously because of both methodological artefacts and the interference of carotenoids or phenolic compounds (Moed & Hallegraaff, 1978; Laval-Martin, 1985). Another important statement involving phaeopigments is that they do not always represent breakdown products, since functional molecules of phaeophytin-a occur in the reaction centre of PSII (Salisbury & Ross, 1992). The close correlation that has been reported in aquatic bryophytes between the spectrophotometrically-determined concentrations of chl-b and phaeophytin-a (Peñuelas, 1984b; Martínez-Abaigar *et al.*, 1994) probably derives from the characteristic presence of

both pigments in PSII (although, as it is said above, LHCII may be shared by PSI and PSII).

Low contents of phaeopigments ($0.2\text{--}1 \text{ mg g}^{-1}$ dry weight) and narrow ranges of the chlorophylls/phaeopigments indices (1.50–1.74 for 665/665 acidified, 1.26–1.38 for 430/410 and 1.27–1.35 for 435/415) have been reported in healthy aquatic bryophytes (Peñuelas, 1984a, b; Peñuelas *et al.*, 1988; López & Carballeira, 1989; Martínez-Abaigar *et al.*, 1994). These variables rarely show consistent responses to environmental changes (Bastardo, 1980), except for the influence of organic and metal pollution (Peñuelas, 1984a; López & Carballeira, 1989; Martínez-Abaigar, Núñez-Olivera & Sánchez-Díaz, 1993). This probably occurs because chlorophylls of emersed bryophytes are not degraded to phaeopigments but to other derivatives under such oxidizing conditions (Hendry *et al.*, 1987).

The most often used index that involves carotenoids is the ratio between the absorbances at 430 and 665 nm, sometimes called Margalef's index, which represents the ratio of the combined chlorophylls and carotenoids (both types of pigments absorb blue light at 430 nm) to the chlorophylls, the only pigments which absorb red light at 665 nm. Other carotenoids/chlorophylls indices are the quotient between the absorbances at 480 and 665 nm (Margalef, 1983), the quotient between the absorbances at 475 and 652 nm (Tieszen & Johnson, 1968) and the ratio between the concentrations of carotenoids and chlorophylls itself (Schmidt-Stohn, 1977). The two opposing functions of carotenoids (photoprotection and light absorption), and the diverse environmental factors that can influence these ratios, limit their ecological meaning. Healthy aquatic bryophytes show values of the ratio 430/665 between 1.72 and 2.89 (Peñuelas, 1984a, b; Peñuelas *et al.*, 1988; López & Carballeira, 1989; Martínez-Abaigar *et al.*, 1993, 1994). Values for higher plants lie more frequently within the range 1.8–2.5 (Margalef, 1982), but can reach much higher values (up to 6.8: Núñez-Olivera, Martínez-Abaigar & Escudero, 1996). This may reflect a lower photoprotective capacity by carotenoids of the shade-adapted aquatic bryophytes than in higher plants. The ratio 480/665 varies between 0.77 and 1.20 in aquatic bryophytes (Martínez-Abaigar *et al.*, 1994), and no comparative data are available.

18.5 GENETIC FACTORS INFLUENCING PHOTOSYNTHETIC PIGMENT COMPOSITION

The genetic influence on pigment composition is clearly observed in the colour of the different species, which is sometimes used as a taxonomical tool. Low chlorophyll contents are generally found in yellow-green aquatic bryophytes, while deep-green species show high chlorophyll concentrations, independently of specific environmental conditions (Martínez-Abaigar *et al.*, 1994).

Anatomical factors, such as the proportion of leaves to stems (McCall & Martin, 1991), the internal organization of thalloid liverworts (Green & Snelgar, 1982), or sclerophyllly (the proportion of non-photosynthetic tissues in leaves and stems, and/or the proportion of cell walls to protoplasts), influence pigment concentrations. Little is known about these questions, except for the inverse relationship between sclerophyllly and chlorophyll concentration per dry weight in some hydrophilous or hygrophilous species (Krupa, 1984; Martínez-Abaigar *et al.*, 1994).

The part of the bryophyte utilized in the analysis is of crucial importance, since

apical portions are more active than basal parts and tend to have higher chlorophyll contents and chl a/b ratios, as well as lower carotenoid to chlorophyll proportions (Schmidt-Stohn, 1977; García-Álvaro, Núñez-Olivera, Beaucourt & Martínez-Abaigar, unpublished results).

Pigment variations through the phenological cycle of aquatic bryophytes are currently unknown, but are probably not critical for the production of submerged species, since sporophytes appear only occasionally. External application of 0.1 mM ACC (1-amino cyclopropane-1-carboxylic acid, a precursor of ethylene) on *Fontinalis antipyretica* and *F. squamosa* causes, amongst other development alterations, qualitative chlorophyll losses in apices (Glime & Rohwer, 1983). Thus, chlorophyll degradation in senescence may be controlled not only by environmental factors, but also by internal production of ethylene.

Bryophytes can generally synthesize chlorophyll in the dark, like many lower plants including conifers (Scheer, 1991). Some work has been done on the selection of mutants incapable of synthesizing chlorophyll in the dark, for instance in *Marchantia paleacea* (Takio *et al.*, 1993). Bryophytes are a biological material of prime quality for these experiments.

18.6 ENVIRONMENTAL FACTORS INFLUENCING PHOTOSYNTHETIC PIGMENT COMPOSITION

18.6.1 Light

Aquatic bryophytes experience varying light climates. Populations from deep lakes are subjected to dramatically altered light intensity and spectral quality, since only low intensity radiation between 475 and 600 nm reaches 10 m depth (Kirk, 1994). However, in shallow waters the radiation received may be the same as for terrestrial plants (Jeffrey, 1981). Thus, for bryophytes in streams, shallow rivers, bogs and fens, light quantity and quality are influenced more by shading tree canopies and topography than by depth of water.

Bryophytes are considered shade-adapted plants, irrespective of habitat. They have a considerable acclimation ability however, both towards high and low light, and this is also evident for aquatic bryophytes. With increasing water depth, chlorophyll content of aquatic bryophytes increases, the chl a/b ratio decreases, and the concentration of the carotenoids involved in blue-green light absorption, especially lutein, increases. This has been demonstrated for *Chiloscyphus rivularis*, *Drepanocladus fluitans* and *Fontinalis antipyretica* living at 10–19 m depth (Farmer *et al.*, 1988; Boston *et al.*, 1991) and for *Scapania undulata* living at only 0.5 m (Martínez-Abaigar *et al.*, 1994). Comparable results have been obtained for isoetids (Søndergaard & Bonde, 1988) and *Chara* (Howard-Williams, Schwarz & Vincent, 1995). Similar changes can be induced by controlled shifts in the spectral quality of light. Aquatic bryophytes grown under green or yellow light have more chlorophyll and carotenoids than those grown under red or blue light (Czeczuga, 1987). This also occurs in the terrestrial liverwort *Riccia discolor* (Dagar, Ahlawat & Singh, 1980) and the alga *Chara* (Czeczuga, 1986). Earlier results for *Fontinalis antipyretica* (Bode, 1940) and

Marsupella aquatica (Bodin & Nauwerck, 1968) somewhat contradict these findings, however.

Seasonal or continuous light starvation is often experienced by aquatic bryophytes in forest streams. Their pigment responses to shade are similar to those found in deep water populations: an increase in chlorophyll content, a decrease in chl a/b ratio and a decrease in carotenoids/chlorophylls indices (Czeczuga, 1985; Patidar, Solanki & Kaul, 1986; Peñuelas & Vallcorba, 1988; Martínez-Abaigar *et al.*, 1994). Comparable responses occur in vascular macrophytes (Barko & Filbin, 1983) and terrestrial bryophytes, both under field (Miyata & Hosokawa, 1961; Martin, 1980; Martin & Churchill, 1982; Kershaw & Webber, 1986; Pande & Singh, 1987; Deora & Chaudhary, 1991; Post & Veske, 1992) and controlled (Rincón, 1993; Yang, Hsu & Shih, 1994; Bakken 1995b) conditions. Nevertheless, the effects of shade on the chl a/b ratio and carotenoids are still a matter for discussion.

By contrast, sun-exposed populations of emergent aquatic bryophytes, together with populations of submersed kinds that emerge during low water levels, show opposing pigment responses: a decrease in chlorophyll concentration, and increases in chl a/b ratio, carotenoids concentration and carotenoids/chlorophylls indices (Glime, 1984; Peñuelas, 1984b; Peñuelas & Vallcorba, 1988; Harley *et al.*, 1989; Martínez-Abaigar *et al.*, 1994). Comparable responses have been reported for terrestrial bryophytes (Alpert, 1988; Melick & Seppelt, 1994; Bakken, 1995b) and higher plants. These responses may be caused not only through an increase of photon flux density, but also at constant light levels when plants become exposed to stress factors which reduce their photosynthetic rates, such as chilling or desiccation. However, *Pohlia wahlenbergii* does not show bleaching or chlorophyll destruction following periods of high insolation exposure (Coxson & Mackey, 1990).

18.6.2 Temperature

Temperature fluctuations within the normal physiological range hardly seem to affect the integrity of chlorophylls (Hendry *et al.*, 1987). This is especially meaningful for truly aquatic, submersed bryophytes, where the surrounding water insulates against extreme cold and heat (Glime & Vitt, 1984). Hence, permanently immersed and sun-exposed bryophytes do not show significant pigment changes over an annual range of water temperatures between 3 and 15°C; however, both emergent and emersed bryophytes are exposed to more harmful temperatures and pigment changes may readily take place (Martínez-Abaigar *et al.*, 1994). Even in the submerged state, the interaction between cold stress and high-light stress might result in photoinhibition and, in extreme cases, also in pigment photooxidation.

In *Pellia epiphylla* and *Sphagnum capillifolium*, chilling causes a decrease in chlorophyll concentration (Pihakaski & Pihakaski, 1979; Gerdol, Bonora & Poli, 1994). Also, three antarctic bryophytes (*Bryum pseudotriquetrum*, *Grimmia antarctica* and *Cephalozia exiliflora*) show decreases both in chlorophyll concentration and chl a/b ratio in winter (Melick & Seppelt, 1994). However, *Grimmia antarctica* growing by a melt lake did not show pigment changes over four freezing-thawing events (Lovelock, Osmond & Seppelt, 1995). In truly aquatic bryophytes, even a moderate temperature of 15–20°C may arrest growth and cause concomitant chlorosis (Fornwall & Glime, 1982; Furness & Grime, 1982; Kelly & Whitton, 1987; Glime,

1987a, b); also, the addition of heat promotes chlorophyll loss in dried *Racomitrium aquaticum* (Hearnshaw & Proctor, 1982). Hence, resistance to cold and heat depends on both the species and the habitat considered. Particularly, the adaptive capacity of aquatic bryophytes to high temperature seems to be low, as has been pointed out for three hygrophilous thallose liverworts (Weis, Wamper & Santarius, 1986), but water temperatures of up to 42°C have been measured in *Sphagnum* tussocks (Lange, 1973). It can be concluded that much work remains to be done on the effect of temperature on pigment composition.

18.6.3 Desiccation

Photosynthetic pigments are sensitive to water availability. The chlorophyll content in a mesic growth form of *Schistidium antarcticum* was three times higher than that found in a xeric growth form (Kappen, Smith & Meyer, 1989). But the most dramatic changes in pigment composition occur on desiccation, depending on the type of pigment, the desiccation resistance of the species considered, the dehydration velocity and the interaction of light and/or temperature. Also, serious damage can occur after the desiccated samples are subsequently rehydrated, because of both lasting photoinhibition (if the samples were desiccated in the light) and the toxic effects of oxygen radicals on photosynthetic membranes (Dhindsa & Matowe, 1981; Seel, Baker & Lee, 1992; Seel, Hendry & Lee, 1992).

Chlorophylls are intensely degraded during desiccation, with a preferential degradation of chl-a (and thus chl a/b ratio declines), whereas carotenoids are more desiccation-resistant than chlorophylls (Gupta, 1978; Peñuelas, 1984b; Peñuelas & Vallcorba, 1988; Seel *et al.*, 1991; Martínez-Abaigar *et al.*, 1994). Another pigment response to desiccation is a decrease in the chlorophylls/phaeopigments indices (Peñuelas, 1984b; Martínez-Abaigar *et al.*, 1994). However, some authors have found no influence on chl a/b ratio (Streusand, Weber & Ikuma, 1986), or an increase in the chlorophylls/carotenoids ratio (Seel, Hendry & Lee, 1992), in sensitive species exposed to desiccation.

A ranking system of desiccation resistance of aquatic bryophytes may be devised based on pigment composition. Obligate aquatic bryophytes (in the sense of Vitt & Glime, 1984), such as *Scapania undulata* and *Fontinalis antipyretica*, and emergents (*Dicranella palustris*), are more sensitive to desiccation than facultative aquatics, such as *Cinclidotus fontinaloides*, *Hygrohypnum duriusculum*, *Rhynchostegium riparioides*, *Schistidium rivulare*, and hygrophilous species, such as *Rhizomnium punctatum* (Gupta, 1978; Peñuelas, 1984b; Seel *et al.*, 1991; Seel, Hendry & Lee, 1992; Martínez-Abaigar *et al.*, 1994). Non-aquatic species are even more desiccation-resistant, in terms of pigment composition (Gupta, 1978; Martin & Warner, 1984; Streusand *et al.*, 1986; Alpert, 1988; Seel *et al.*, 1991; Seel, Hendry & Lee, 1992).

Pigment degradation is enhanced by the severity of desiccation treatment and is greater at lower atmospheric relative humidity, with a higher rate of water-loss, or with additional stresses (high light, heat). Under these adverse conditions, that aquatic bryophytes frequently experience in the field, pigment responses are more rapid and clear. Some desiccation-sensitive species are affected only when a combination of stresses occurs, whereas desiccation *per se* does not affect their pigment composition (Seel, Hendry & Lee, 1992).

18.6.4 Nutrient availability

In higher plants, pigment variations (especially chlorosis) are diagnostic symptoms for several nutrient deficiencies, toxicities or antagonisms. However, the influence of mineral nutrition on the pigment composition of aquatic bryophytes is poorly known. A decrease in the chlorophyll content of *Fontinalis antipyretica* and *F. dalecarlica* was observed in conditions of copper deficiency (Glime & Keen, 1984). In *Sphagnum cuspidatum* and *Tomenthypnum nitens*, an increase in nitrogen and/or sulphur availability results in a concomitant increase in chlorophyll concentration, and sometimes also in an increase in chl a/b ratio (Rochefort & Vitt, 1988; Baxter, Emes & Lee, 1989, 1992); thus wet deposited N and S pollutants may act in some cases as a fertilizer. Also, *Fontinalis novae-angliae* cultivated in 100 ppm nitrate and stream water became much deeper green than in stream water alone (Glime & Vitt, 1984). Comparable results have been obtained for terrestrial mosses, such as *Dicranum majus* (Bakken, 1994, 1995b), although the chlorophyll content on transplantation from a low-N site to a high-N site did not increase after 16 months (Bakken, 1995a).

Aquatic bryophytes are rarely nutrient-limited in nature, because of their great accumulation capacity (Glime & Vitt, 1984). As a consequence, a high nutrient enrichment of water may cause toxicity processes on bryophyte populations. In phosphate-enriched cultures of *Jungermannia exsertifolia* subsp. *cordifolia*, the increase of internal phosphorus clearly influences pigment content: chlorophyll content slightly decreases, chl a/b ratio decreases more significantly, phaeopigment content increases, chlorophylls/phaeopigments indices (665/665 acidified and 430/410) decrease and, finally, carotenoid indices increase (Martínez-Abaigar & Núñez-Olivera, 1991). Also, *Sphagnum magellanicum* and *S. papillosum* become pale and yellow at or above 1 mM H₂PO₄⁻ (Li, Glime & Drummer, 1993). The mosses *Leskeia polycarpa*, *Amblystegium riparium*, *Fissidens crassipes* and *Fontinalis antipyretica* would not grow when phosphate exceeded 9 to 15 mg l⁻¹ (Frahm, in Glime, 1992). However, the chlorophyll concentrations of stem tips in two species of *Hygrohypnum* were three-fold higher in samples from phosphorus-fertilized riffles than in control riffles (Finlay & Bowden, 1994). Hence, the ecophysiological implications of nutrient availability in aquatic bryophytes need further study.

18.6.5 Pollution

The photosynthetic pigment composition of aquatic bryophytes has been successfully assayed in the bioindication of pollution. However, the body of basic physiological and ecological works involving photosynthetic pigments is relatively scarce, and this is an important handicap to interpretation of bioindication studies.

The different pigment variables exhibit different responses to pollution. A reduction in chlorophyll concentration of aquatic bryophytes, sometimes associated with a concomitant decrease in chl a/b ratio, has been reported as a consequence of suspended coal particles (Lewis, 1973), acidified mine water (Glime, 1992; Stephenson *et al.*, 1995), organic matter (Pefiuelas, 1984a; López & Carballeira, 1989; Martínez-Abaigar *et al.*, 1993), heavy metals (McLean & Jones, 1975; Glime & Keen, 1984; Glime, 1992), and dust (on *Sphagnum*: Farmer, 1993). Similar results have been obtained in a liquid culture of the terrestrial moss *Physcomitrella patens*.

utilized to test toxicity of diverse metals and leachates (Morgan, Wu & Swigert, 1993), and in terrestrial mosses experiencing simulated acid rain treatment (Raeymaekers & Glime, 1986, 1990). By contrast, the chlorophyll concentration was hardly affected in *Sphagnum* under simulated acid rain (Austin & Wieder, 1987; Baxter *et al.*, 1989) and in terrestrial mosses receiving dust (Adamson, Adamson & Seppelt, 1994) or metal pollution (Raeymaekers & Glime, 1986). In this last case, the chlorophyll damage may be prevented by the immobilization of metals on the cation exchange sites of the cell wall.

In environments with oxygen concentrations less than atmospheric, such as aquatic systems, chlorophylls are often degraded to phaeopigments (Hendry *et al.*, 1987). This may especially occur in waters polluted by organic matter, since metabolism of heterotrophic microorganisms can acutely deplete oxygen in water. Hence, an increase in phaeopigment concentration and a decrease in chlorophylls/phaeopigments ratios have been reported in aquatic bryophytes affected by organic pollution (Peñuelas, 1984a; López & Carballeira, 1989; Martínez-Abaigar *et al.*, 1993; Gimeno-Colera, 1996). Also, metal pollution results in similar pigment changes (McLean & Jones, 1975; López & Carballeira, 1989, 1993; López, Vázquez & Carballeira, 1994). The chl a/b ratio and carotenoid indices do not seem to be good bioindicators of water pollution (Peñuelas, 1984a; Glime, 1992; Martínez-Abaigar *et al.*, 1993). The overestimation of chl-a when phaeopigments are abundant in the extract (Laval-Martin, 1985) and the degradation of carotenoids in very advanced plant senescence are factors that may reduce the ecological significance of these ratios as health indicators in cases of severe damage.

The different species used for bioindication studies reveal a range in sensitivity to pollution, as indicated by pigment composition. Liverworts are usually more sensitive than mosses and, amongst mosses, *Fontinalis antipyretica* is very tolerant to pollution (Peñuelas, 1984a; López & Carballeira, 1989, 1993; Martínez-Abaigar *et al.*, 1993). However, *Scapania undulata* is relatively resistant to metal pollution (McLean & Jones, 1975). We can conclude that each case of pollution bioindication investigated should be studied using suitable species and ecophysiological variables, depending on the exposure period, and the type and intensity of pollution.

18.7 CONCLUDING REMARKS

Some effects of light, desiccation and pollution on the photosynthetic pigment composition of aquatic bryophytes are already clear. However, other results remain unexplained. Thus, until better information becomes available on the specific factors regulating the photosynthetic pigment composition of aquatic bryophytes in nature, pigment data will be difficult to interpret in an ecological context. Seasonal variations in photosynthetic pigment composition provide a means to analyze responses to interacting genetic and environmental factors and, to a certain extent, may reproduce the annual cycle of metabolical activity and growth. Summer desiccation, and changes in light conditions caused by canopy opening and closure, seem to be the key factors determining the seasonal pigment variations, whereas the permanently immersed species that live in forest clearings and experience rather stable environmental conditions throughout the year have attenuated seasonal pigment changes

(Martínez-Abaigar *et al.*, 1994). This may indicate a capability of those bryophytes for continuous growth, probably controlled by temperature or other interacting factors. A similar absence of seasonal variations in chlorophyll concentration has been found in *Chara* from a deep lake (Howard-Williams *et al.*, 1995), but marked seasonal pigment cycles occur in submerged benthic algae (Kirk, 1994) and seagrasses (Macauley, Clark & Price, 1988).

Diurnal variation in pigment content has been found in some seaweeds (López-Figueroa, 1992), but not in the freshwater *Chara* (Howard-Williams *et al.*, 1995). Such diurnal pigment changes have not been tested in aquatic bryophytes. Other aspects that should be investigated in the future include the role of the xanthophyll cycle in photoprotection (Schmidt-Stohn, 1977; Lovelock *et al.*, 1995), the redefinition of pigment indices through the identification of individual pigments (carotenoids, degradation pigments), the use of chl-a fluorescence as a general vitality index (Baxter *et al.*, 1989; Seel, Baker & Lee, 1992; Murray, Tenhunen & Nowak, 1993; Potter *et al.*, 1996), and the utilization of non-destructive methods of chlorophyll quantification.

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19. Nutrient retention, desiccation and redistribution in mosses

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19.1 SUMMARY

The paper reviews recent progress in our understanding of how bryophytes acquire and utilize mineral nutrients. Topics include their significance in nutrient transfers within ecosystems, sources of nutrients, the effects of desiccation on productivity and nutrient retention, and translocation and internal redistribution of nutrients. Some new data on phosphorus uptake and economy are presented for two ecologically contrasted moss species, *Brachythecium rutabulum* and *Pseudoscleropodium purum*. *B. rutabulum* exhibits higher productivity than *P. purum* when subjected to frequent nutrient additions and maintained in a constantly hydrated condition. However, *P. purum* makes opportunistic use of infrequent nutrient inputs, being capable of higher productivity than *B. rutabulum* in nutrient-free conditions after receiving a nutrient 'pulse'. Productivity and phosphate capture by *P. purum* is also less severely affected by intermittent desiccation than is the case for *B. rutabulum*. In a nutrient-poor environment, the supply of phosphorus to new growth of *P. purum* is related to the phosphorus content of the older parts of the shoot and probably occurs by a symplastic route. The greater efficiency of *P. purum* in nutrient-poor habitats may partly reflect a more effective mechanism for internal redistribution of nutrients than is found in *B. rutabulum*.

KEYWORDS: Mineral nutrition, phosphorus, nutrient cycling, desiccation, translocation, *Brachythecium rutabulum*, *Pseudoscleropodium purum*.

19.2 INTRODUCTION

The fundamental mechanism of nutrient ion absorption by bryophyte cells appears to be closely similar to that of other green plants, although there are still many instances where knowledge is incomplete (Bates, 1992; Raven *et al.*, this volume). Especially interesting are the specific nutritional problems confronted by bryophytes in different major habitats that arise through their particular life-style and morphology. Being poikilohydrous, that is undergoing cycles of desiccation and rehydration, and lacking roots and usually cuticles (see Proctor, 1979, for the occurrence of cuticles in mosses) has implications for mineral nutrition as well as for water-relations (Hébant, 1977). So too might the typical pattern of bryophyte growth and

regeneration. For example, the tendency to grow more or less continuously from an apical cell or other simple meristem whilst simultaneously abandoning older or shaded tissues that senesce and eventually decompose. As several reviews of bryophyte mineral nutrition, and the wider field of mineral and pollutant uptake, have appeared over the past 15 years (Brown, 1982, 1984; Brown & Bates, 1990; Bates, 1992), here we concentrate on selected topics where recent advances have been made.

In the examples from our own work that follow we have usually contrasted two large mosses with somewhat different ecologies. *Brachythecium rutabulum* (Hedw.) B., S. & G. is one of the more productive terrestrial bryophyte species, capable of rapid foraging growth over limited periods. The studies of Rincon (1988, 1990) and Rincon & Grime (1989) indicate that it obtains its nutrient supplies via rhizoidal attachments to decaying plant litter. This species responds to artificial nutrient applications made in field experiments by increasing growth rates (Furness & Grime, 1982). *Pseudoscleropodium purum* (Hedw.) Fleisch. ex Broth. (syn. *Scleropodium purum* (Hedw.) Limpr.) is a carpet-forming moss with more modest but steady productivity through the year. It lacks rhizoidal attachments and usually does not show a growth response to nutrients applied in the field (e.g. Bates, 1987).

19.3 NUTRIENT TRANSFERS IN ECOSYSTEMS

The importance of bryophytes in the nutrient economies of ecosystems seems to be firmly established in different types of mossy forest, where they intercept nutrients in throughfall and may deprive the underlying tree roots (e.g. Oechel & Van Cleve, 1986). Bryophytes are also of primary importance in many mire communities and various types of polar ecosystem (e.g. Longton, 1988; Greenfield, 1992).

In forests, significant proportions of the total nutrient capital may reside in the moss layer. The mossy coverings of tree branches in upland tropical forests are undoubtedly important in their hydrology (Pócs, 1982) and may be similarly important in nutrient dynamics (e.g. Veneklaas, 1990) but this needs detailed study. In terms of nutrient transfers between ecosystem components, some tantalizing evidence of moss-fungal interconnections and translocation (Chapin *et al.*, 1987), although doubted by Brown & Bates (1990) and Bates (1992), is reiterated in more recent work. In contrast to liverworts which show a range of symbioses with fungi (Duckett, Renzaglia & Pell, 1991; Duckett & Read, 1995), no bona fide moss-fungus symbioses have yet been demonstrated. Wells & Boddy (1995) demonstrated translocation of ^{32}P from a distant source — pieces of wood inoculated with fungus and immersed in the leaf litter — via natural connections made to the moribund parts of *Hypnum cupressiforme*. The fungus in this instance was a saprotrophic basidiomycete, *Phanerochaete velutina*. The ^{32}P was observed to move into the living apices of *H. cupressiforme*. This is the opposite direction of phosphorus transport from that reported in earlier studies. Such connections could offer a preferential pathway for transfer of phosphorus from leaf litter to the moss carpet and might explain the source of this element which is scarce in precipitation. However, the nature of this moss-fungus relationship needs further critical study before we can be confident about the existence of this uptake (or loss) pathway in nature.

19.4 NUTRIENT CAPTURE

It should not be overlooked that gametophores start life as microscopic spores and protonema, or else as small fragments or propagula. These will all achieve intimate contact with the substratum, or its surface moisture film, and significant stocks of nutrients may be accumulated at this stage. This may also be the predominant stage at which soil type acts in selecting calcicoles and calcifuges, or other chemical 'specialists'. Later, some life forms become separated from the underlying substratum by accumulations of their own unhumified litter and/or unincorporated humus. It is thus generally assumed that the main source of mineral elements available to these bryophytes is in precipitation and from other atmospheric sources. This idea particularly derives from Tamm's (1953) study of *Hylocomium splendens*. This hypothesis is supported for ombrotrophic *Sphagnum* species by the study of Woodin, Press & Lee (1985) showing close coupling of nitrate reductase activity in *Sphagnum* to nitrate supplied in precipitation. However, there is less evidence for other elements such as phosphorus which may instead derive predominantly from leachates of vascular plant leaves or from soil. In a study of nutrient uptake by the moss *Calliergonella cuspidata* in Chalk grassland, Van Tooren, Van Dam & During (1990) concluded that only the ammonium ion was utilized amongst various nutrients arriving in precipitation. Other nutrients were presumed to derive from soil sources. In the case of calcium, usually considered to be a macronutrient in green plants (Burström, 1968), Bates & Farmer (1990) showed considerable uptake into *Pleurozium schreberi* from a calcium carbonate layer on the soil. In later work involving transplants of *Rhytidadelphus triquetrus* (Bates, 1993) with different underlying soil types a more complex picture emerged. The calcium-status of the transplant-receiving site, rather than that of the soil immediately underlying *R. triquetrus*, had the greater influence on moss chemistry. Possibly, leachates from the overlying tree canopy were the major source of Ca rather than the soil directly. Leaf leachates would be expected to reflect the Ca content of the soil within the ranges of the tree root systems rather than over the much smaller areas of the individual experimental plots beneath the moss transplants (Bates, 1993).

Species dependent on irregular nutrient supplies in precipitation or from other sources would be expected to be adept at capturing 'pulses' of nutrients and retaining them for use when conditions were most favourable for growth. Bates (1994) tested this hypothesis using the two mosses *Brachythecium rutabulum* and *Pseudoscleropodium purum*. As *B. rutabulum* becomes attached by rhizoids to litter (Rincon, 1988, 1990) it would be expected to depend on a nearly constant nutrient source and, indeed, it exhibited a significantly higher relative growth rate (RGR) than *P. purum* in experiments where a high nutrient regime was supplied (Rincon, 1988). *P. purum*, a more typical 'feather-moss' of grassland, scrub and forest that often accumulates a thick litter layer, might be expected to be more opportunistic in its acquisition and use of nutrients arriving in precipitation and throughfall. Shoots of both species were given a 'pulse' of the three major nutrients nitrogen, phosphorus and potassium by spraying to saturation with a solution containing 5 mM potassium orthophosphate and 5 mM ammonium nitrate on each of 8 consecutive days. Following this pretreatment the shoots were cultivated in a growth cabinet in nutrient deficient conditions at full hydration for 10 weeks (Bates, 1994).

In terms of growth, *B. rutabulum* benefited much less from the nutrient pulse than

P. purum (Fig. 1a, b). This also applied when the results were expressed as relative growth rates (Bates, 1994). Normally, when nutrients are added we expect *B. rutabulum* to out-perform *P. purum*. These results are partly explicable with reference to information on phosphorus content of the shoots (Fig. 1c, d). *P. purum* experienced greater net uptake of P than *B. rutabulum* during the nutrient 'pulse' and Bates (1994) concluded that this had sustained its greater subsequent growth. In the early stages of the experiment the shoots of both species were inadvertently desiccated on one occasion. From later work (see below), we now suspect that this may have seriously inhibited the ability of *B. rutabulum* to absorb nutrients, whereas *P. purum* appears to be less sensitive to drying. Interestingly, the shoots of both species became surprisingly phosphorus-deficient, apparently by growth dilution, when treated with distilled water only (Fig. 1c, d). As the graphs show total P content, this cannot be simply growth dilution but represents true loss of P from the shoots. A possible explanation (Bates, 1994) is that lowered cell P content, as a result of growth dilution, limits phospholipid synthesis to such an extent that it inhibits cell membrane synthesis. This would increase leakage of the remaining pool of soluble phosphorus compounds to the external medium (distilled water).

Overall, these results support the view that *P. purum*, a species believed to be primarily dependent on atmospheric nutrient inputs, is better able to capture opportunistically and retain and deploy the nutrients in later nutrient-poor conditions, than the 'litter species', *B. rutabulum*.

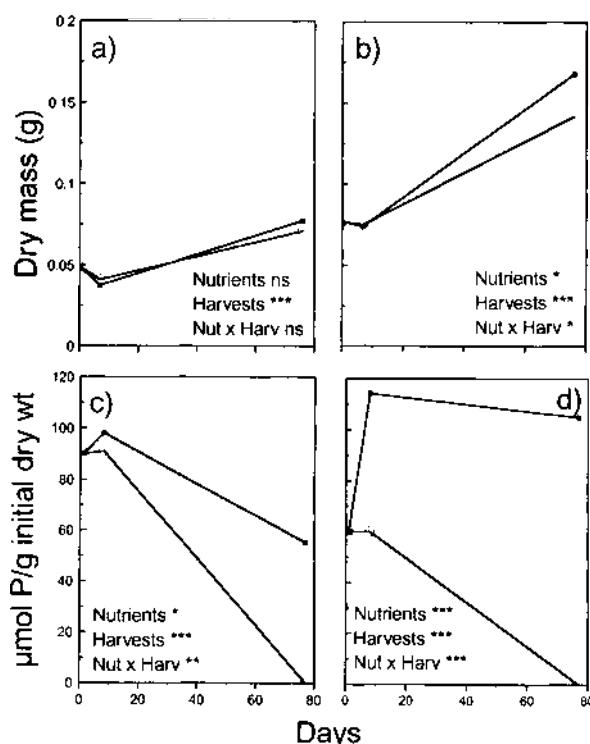


Figure 1. Biomass and phosphorus content per sample of 20 shoots (initially cut to 2 cm) of *Brachythecium rutabulum* (a, c) and *Pseudoscleropodium purum* (b, d) before and after receiving a nutrient (NPK) pulse, and after cultivation for 10 weeks under distilled water mist, untreated control, NPK-treated. Probabilities for factorial anova: ns, not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.

19.5 DESICCATION, UPTAKE AND LEAKAGE

Desiccation is a regular feature of the lives of many terrestrial bryophytes (Proctor, 1990; Proctor & Smith, 1995). Bryophytes characteristically leak significant quantities of cell solutes including electrolytes to the rehydration water (Gupta, 1976, 1977, 1981; Brown & Buck, 1979; Schonbeck & Bewley, 1981; Dhindsa, 1991; Oliver, Mishler & Quisenberry, 1993). This process continues for tens of minutes or hours depending on the ability of the species to repair damaged membranes, and there is often reabsorption of the lost solutes. However, almost all studies have been undertaken in sealed laboratory flasks. The study of Coxon, McIntyre and Vogel (1992) in tropical montane rainforest is unique in showing that a pulse of release of organic solutes occurs to the throughfall on rehydration of the epiphytic liverwort mat. Significant losses of electrolytes including mineral nutrients (N, P) accompany the organic solutes (Coxon, 1991). Several recent studies (Bates, 1987, 1989a, b; Van Tooren *et al.*, 1990; Bakken, 1994) attributed rather weak growth responses of bryophytes to nutrient additions, in field conditions, to limitations imposed by natural desiccation. Could bryophytes generally have a weak capacity for retaining absorbed nutrients owing to rehydration leakage?

This possibility has been considered in further comparative work with *Brachythecium rutabulum* and *Pseudoscleropodium purum* (Bates, 1997). Shoots of the two species were cultivated for 7 weeks under the nutrient and moisture regimes shown in Table 1. Plants were either kept continuously moist — indicated by the two treatments with a small 'w' or subjected to a 24 h desiccation (equilibrated at 42% RH) once per week. Superimposed upon this was a single weekly application of an NPK solution, except in the case of the untreated controls. Furthermore, for the plants receiving intermittent desiccation, the NPK was applied at either the start, middle or end of the hydrated period.

The mean biomass increments for each species are presented in Fig. 2 as shoot dry mass and relative growth rate (RGR). The higher productivity of *P. purum* was largely explicable by the greater initial mass of its shoots and this effect is removed by expressing the data as RGR. In these circumstances RGR is higher in *B. rutabulum*, but only in the most benign conditions — in the wNPK treatment where there is continuous moisture and regular applications of nutrients. The most striking result

Table 1. Weekly programme of maintenance of *Brachythecium rutabulum* and *Pseudoscleropodium purum* in an investigation of the effects of intermittent desiccation on nutrient uptake by the two mosses (Bates, 1997). Daily treatments: W, sprayed once to saturation with distilled water; NPK, sprayed once to saturation with a solution containing 5 mM KH_2PO_4 and 5 mM NH_4NO_3 ; D, allowed to dry at 42% RH for 24 h.

Treatment	Day						
	1	2	3	4	5	6	7
dCON	W	W	W	W	W	W	D
sNPK	NPK	W	W	W	W	W	D
mNPK	W	W	W	NPK	W	W	D
eNPK	W	W	W	W	W	NPK	D
wCON	W	W	W	W	W	W	W
wNPK	NPK	W	W	W	W	W	W

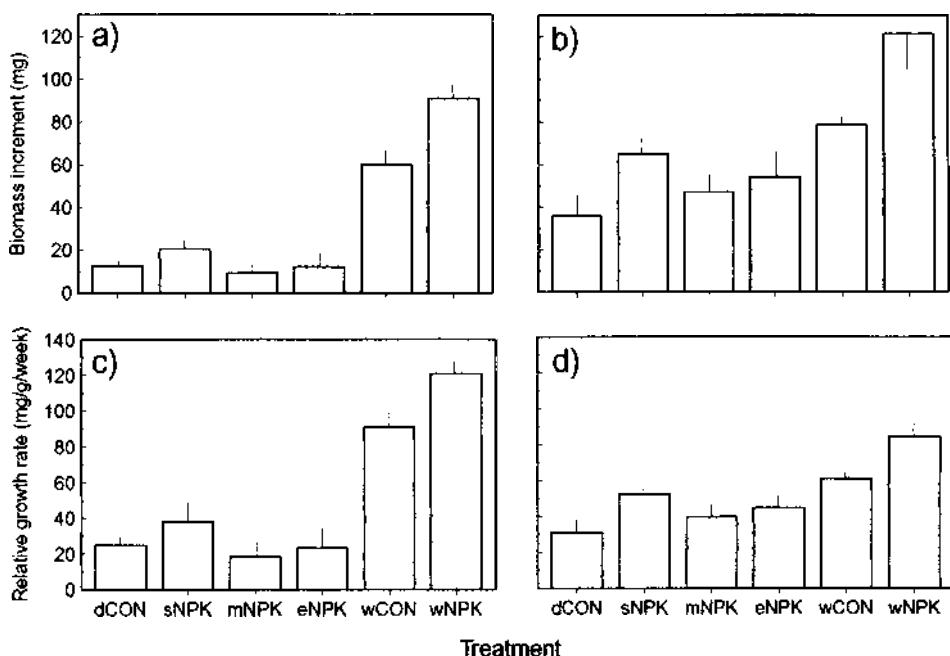


Figure 2. Biomass increment and relative growth rates of samples of 20 shoots (initially cut to 2 cm) of *Brachythecium rutabulum* (a, c) and *Pseudoscleropodium purum* (b, d) subjected to weekly nutrient (NPK) additions with and without intermittent desiccation over 7 weeks. See Table 1 for explanation of treatments. Vertical bars show 1 standard deviation, see Bates (1996) for full statistical analysis.

was in the effect of intermittent desiccation. In *B. rutabulum* there was very little growth under the intermittent desiccation regime (i.e. the dCON treatment), and NPK applications caused virtually no growth stimulation when combined with intermittent desiccation (sNPK, mNPK, eNPK treatments). In *P. purum* there was slightly more growth under intermittent desiccation; moreover, the NPK applications had a more noticeable stimulatory effect. In both species there was better growth with continuous hydration, and here NPK caused a more marked stimulation, especially in *B. rutabulum* (Fig. 2).

Total phosphorus contents of the shoots at the beginning and end of the experiment are shown in Fig. 3. Initially, P content of *P. purum* was only 63% of that of *B. rutabulum* despite a higher dry weight which may be explicable by a greater cell wall thickness of the former. *P. purum* showed the more efficient net uptake in the intermittent desiccation treatments: to 3.5 (sNPK), 3.2 (nNPK) and 3.2 (eNPK) times the initial P content. P uptake in *P. purum* under continuous hydration (wNPK) was only marginally better at 3.7 times the initial content. Net phosphorus uptake in the intermittently desiccated *B. rutabulum* was lower: respectively, to 3.1, 2.4 and 2.6 times the initial P content. However, it was markedly higher in continuously hydrated plants (wNPK): to 7.3 times the initial content. When net uptake of P in the intermittent desiccation treatments is considered as a proportion of uptake in the wet-NPK treatments it is clear that *B. rutabulum* is considerably more inconvenienced by drought than is *P. purum*. In summary, this work shows that occasional

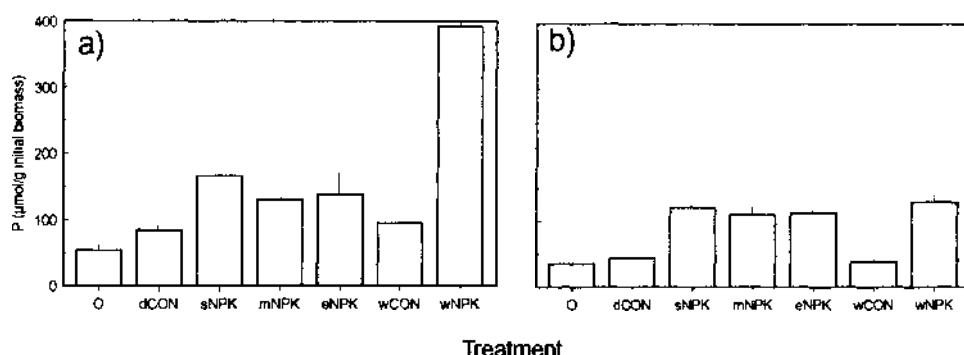


Figure 3. Phosphorus content of samples of 20 shoots (initially cut to 2 cm) of *Brachythecium rutabulum* (a) and *Pseudoscleropodium purum* (b) subjected to weekly nutrient (NPK) additions with and without intermittent desiccation over 7 weeks. See Table 1 for explanation of treatments. Vertical bars show 1 standard deviation, see Bates (1996) for full statistical analysis.

Table 2. Rank orders of the intermittent desiccation-with-NPK treatments by the magnitudes of the responses of several positively affected variables in *Brachythecium rutabulum* and *Pseudoscleropodium purum*. NPK was applied at the start (S), middle (M), or end (E) of the hydrated period (see Table 1; Bates, 1997).

Variable	<i>B. rutabulum</i>	<i>P. purum</i>
Biomass increase	S > E > M	S > E > M
Nitrogen content	S > E > M	E > S > M
Phosphorus content	S > E > M	S > E > M
Total K ⁺ content	S > E > M	S > E > M

short bouts of desiccation have a major effect in limiting productivity of these relatively mesic bryophytes. Also, desiccation severely restricts the abilities of bryophytes to utilize nutrient supplies. This appears to be especially important to *B. rutabulum*, a species that exhibits a 'high productivity' life strategy. Such a strategy appears to require long periods of continuous hydration for successful nutrient uptake and deployment. The less productive *P. purum* appears to be generally more desiccation tolerant and has a lower capacity to absorb large quantities of nutrients but, as described above, it is more efficient in their use.

Bates (1997) also considered time of application of NPK in the hydrated period. The results did not show very large differences (Figs 2, 3) but when the rank orders of the stimulatory effects are examined there is a consistency (Table 2). In seven out of eight cases, application at the start of the hydrated period was most stimulatory and, in all cases, application at the middle of the hydrated period was least stimulatory. A possible explanation is that the rate of uptake of nutrients is higher during the early stages of rehydration. We have explored this possibility by investigating net uptake of phosphorus at various times after rehydration but conclude that, even when *B. rutabulum* and *P. purum* are rehydrated in dilute phosphate solution, there is net loss of phosphorus. No evidence has been obtained for accentuated P absorption during the early phases of rehydration (S. Bakken & J. W. Bates, unpublished data).

19.6 TRANSLOCATION AND REDISTRIBUTION IN GAMETOPHORES

Central in any consideration of bryophyte mineral nutrient relations is the possession (or not) of a mechanism for translocating nutrients around the shoot or thallus. Apart from Polytrichaceae, some other endohydric mosses and a very few liverworts (*Haplomitrium*, some Metzgeriales; see Hébant, 1977 for references), the conventional view has been that bryophytes lack recognizable conducting tissues and therefore do not transport materials internally. This view now seems less supportable. The evident occurrence of apical dominance in many species suggests that there is movement of hormones from cell to cell by an unknown mechanism. Rydin & Clymo (1989) presented clear evidence of translocation of carbon and phosphorus from older to younger tissues in *Sphagnum recurvum*. This seems to occur by the symplast pathway and probably via abundant plasmodesmata that connect adjacent stem cells. Similarly, Alpert (1989) demonstrated translocation of photoassimilate from leaves of the moss *Grimmia laevigata* to stem bases and underground axes. Neither of the above species shows differentiated vascular tissues. Furthermore, Wells & Boddy (1995) showed movement of ^{32}P from the bases of *Hypnum cupressiforme* to the stem apices, although the means of delivery involved fungal hyphae attached to the stem base only, again suggesting efficient acropetal transport. The food conducting cells described by Ligrone & Duckett (1994, 1996) are possibly of significance in these cases. These unusual, polarized conducting cells appear to be widespread in mosses including those, like *Neckera crispa*, that apparently lack differentiated conducting tissue. Ligrone & Duckett have hypothesized that relatively large volumes of solutes may be conducted through cells within vesicles that move along microtubules aligned with the cell's long axis. Cell to cell transport is presumably via the plasmodesmata that are particularly numerous in these cells. We do not know yet whether such a mechanism would transport electrolytes as opposed to organic solutes but it is potentially of great significance in understanding bryophyte mineral relations.

Where there is a transport system there exists the possibility of internal redistribution of mineral nutrients from old senescing tissues to younger growth. If bryophytes should prove to be efficient at such internal redistribution this would largely isolate them from reliance on external supplies (Brown & Bates, 1990; Bates, 1992). Wells & Brown (1996) presented evidence that nutrient cations are redistributed from older tissues to new growth in the moss *Rhytidadelphus squarrosus*. However, unlike some of the earlier work indicating symplast transport, these authors favoured an apoplast pathway for nutrient movement.

Recently, we have investigated the potential for redistribution of mineral nutrients in *Brachythecium rutabulum* and *Pseudoscleropodium purum* employing experimental treatments that may help us to identify the type of pathway. We compared the nutrient pools available to new growth provided by four different kinds of manipulation of standard shoots involving cutting and steam-killing of segments (Fig. 4). The starting unit was a healthy green 4 cm long stem-with-apex. This was treated as consisting of a basal and an apical 2 cm segment, and new apical growth occurring during the experiment was treated as a third segment during the analyses of nutrient content. The various segment lengths and steamed zones resulted in a range of different-sized nutrient pools available by either the symplast (living) or apoplast

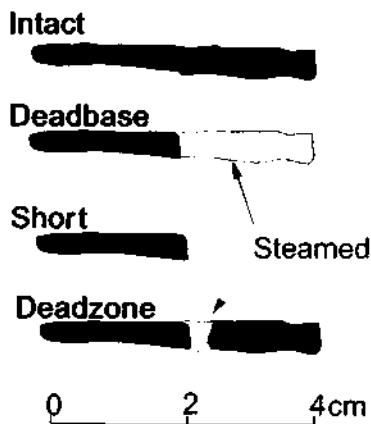


Figure 4. Experimental manipulations applied to healthy green apical portions of *Brachythecium rutabulum* and *Pseudoscleropodium purum* to obtain contrasted nutrient pools for later growth in a nutrient deficient environment.

(living and dead) pathways. Throughout the seven weeks of this experiment the plants were cultivated under distilled water mist and so had to depend on nutrients already in the shoots for their new growth.

As in the earlier work (Bates, 1994; Fig. 1), *P. purum* grew more rapidly than *B. rutabulum* in a nutrient-deficient environment (Fig. 5a, b). *B. rutabulum* exhibited no significant differences in growth between the shoot nutrient-pool treatments. In contrast, *P. purum* grew best from the large 'living' nutrient pool in the intact shoots, however, growth in the other treatments was somewhat greater than might be

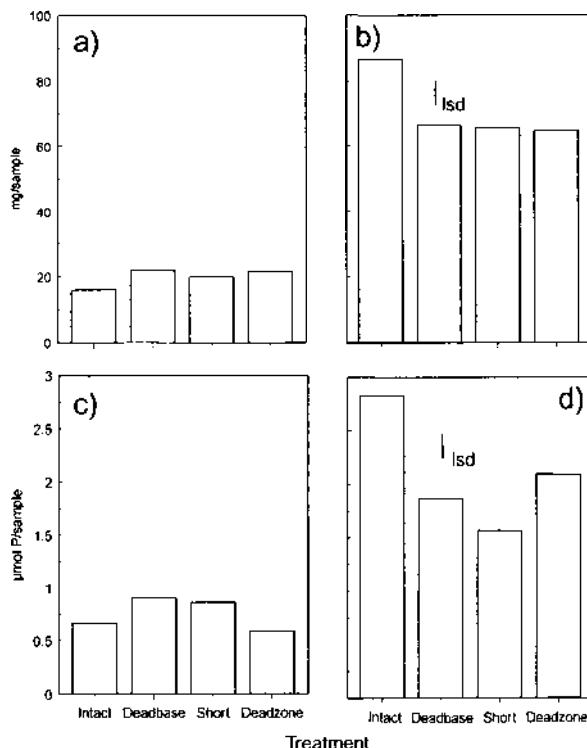


Figure 5. New apical growth increment and its phosphorus content obtained from samples of 20 shoots of *Brachythecium rutabulum* (a, c) and *Pseudoscleropodium purum* (b, d), prepared as in Fig. 4, and cultivated under distilled water mist for 7 weeks. The vertical bar shows the least significant difference ($p < 0.05$) in those instances where a one-way anova revealed a significant treatment effect.

expected from the reduced nutrient pool available. A similar pattern is apparent in the phosphorus contents of the new growth (Fig. 5c, d). This contained very little phosphorus in *B. rutabulum* and there were no significant differences between treatments as with the biomass data. In *P. purum*, P content of the new growth was highest in the intact treatment and there was only about half this quantity in the deadbase and short treatments. These results favour the symplast route for phosphorus redistribution in *P. purum*. The deadzone results are somewhat intermediate but do not seriously conflict with this interpretation.

A picture is thus emerging of some significant physiological differences between the two moss species we have compared that correlate with rather different life styles. In this latter instance there is a distinct possibility that efficient use of nutrient resources by *Pseudoscleropodium purum* is coupled with a more effective, and less desiccation-disturbed, mechanism for internal (symplast) transport of nutrient resources. However, it should not be overlooked that biomass increase and phosphorus content were not closely quantitatively related in this experiment. In fact, there is evidence from our work and the paper of Wells & Brown (1996) that bryophytes may grow for extended periods with very low tissue contents of what we would normally consider to be 'essential' mineral nutrients. This aspect, and the basis of the species differences, clearly require further investigation.

19.7 CONCLUDING REMARKS

Although some very significant steps have been made in our knowledge of bryophyte mineral nutrient relations during recent years, we should remember that it is based mostly on a few large and easily manipulated moss species. It has also been located mainly in temperate broad-leaved and coniferous forests, and mires, although work performed in the Antarctic (see Longton, 1988) has given a further dimension. We need to tackle other biomes and especially the bryophyte-rich types of tropical forest where nutrient cycling via bryophytes, epiphytes as well as ground-dwelling types, may be more important than in other communities. The use of manipulative experiments involving removal of mosses from specific microhabitats may be particularly revealing in unravelling ecosystem nutrient pathways (*cf.* Knops, Nash & Schlesinger, 1996). The role of bryophyte-saprotroph connections could be especially important in tropical forest nutrient dynamics but appears never to have been studied. We should not forget that there are many fundamentally different gametophyte morphologies, growth-forms and life-forms. The mineral nutrition of a diminutive *Seligeria* growing on a loose stone where it is frequently immersed within the stone's surface water film may provide different perspectives to the more familiar ankle-deep carpets of *Hylocomium splendens* and *Sphagnum*. Perhaps too, the persistent protonemal systems of some mosses, such as *Rhizomnium punctatum* (Duckett & Ligrone, 1994) and *Dicranella heteromalla* (Duckett & Matcham, 1995), deserve attention as potential nutrient capturing structures. It is quite possible that there may yet be more to learn about the subtleties of nutrient uptake, translocation and utilization in some of these unexplored situations than we already know.

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20. Quantifying bryophyte-environment relationships

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20.1 SUMMARY

Although some bryophytes form a major part of several vegetation types in northern and western areas, the majority of bryophytes are not common and have a narrow habitat specificity. Little is known about their environmental niches or the responses of bryophytes to environmental change. Detailed quantitative studies of fossil mosses preserved in lake sediments in western Norway show how rapidly mosses responded to a major environmental change 11,300 years ago at the late-glacial/post-glacial transition. Recent developments in applied statistics, in particular generalized linear and generalized additive modelling, permit the quantification of the realized environmental niche of bryophyte species and the examination of the interaction between environmental variables on the occurrence or abundance of species. This modelling approach is illustrated by recent studies on *Andreaea* and *Racomitrium* in western Norway. Predicting the response of bryophytes to future environmental change requires direct field-manipulative experimentation on bryophyte responses to warming, snow-lie, moisture, nutrients, etc.

KEYWORDS: *Andreaea*, *Racomitrium*, fossil mosses, response surfaces, environmental niche, western Norway.

20.2 INTRODUCTION

Although bryophytes form a major part of several vegetation types and ecosystems in northern and western Europe and in northern North America, relatively few bryophyte species are ecologically abundant or dominant. Few species are ever present in great abundance, occupy large geographical ranges, and occur in a wide range of habitats. The few species that show all these features are, in the terminology of Rabinowitz (1981) and Rabinowitz, Cairns & Dillon (1986), 'common' species. The majority of bryophytes are, in Rabinowitz's terminology, 'rare' species or at least 'not common' species, in that they do not occur in great abundance, do not have large geographical ranges, and/or do not occur in a wide range of habitats.

Preliminary classifications of the British liverwort and moss floras as mapped by Hill, Preston & Smith (1991, 1992, 1994) into the seven categories of rarity and the one category of common species as defined by Rabinowitz (1981) give very consistent

results for both groups. The eight categories are defined by the eight possible combinations of large or small geographical range (here confined to Eurasia), wide or narrow habitat specificity, and large populations somewhere or consistently small populations. Within the 293 liverwort taxa considered, 5.8% are 'common', whereas in the 743 moss taxa considered, 6.7% fall in the 'common' category. The best represented category of rarity is the category defined by the combination of large geographical range, narrow habitat specificity, and small population size (42.7% hepatics, 42.8% mosses). Narrow habitat specificity includes 79.5% of the hepatics and 77.2% of the mosses, whereas the category of consistently small population size includes 80.6% of the liverworts and 81% of the mosses. These broad categories are not mutually exclusive, as species can have narrow habitat specificity but have large or small populations. This preliminary analysis highlights that most bryophytes are not common and many have a narrow habitat specificity. Such species may thus be potential environmental indicators because of their narrow habitat specificity.

Each species occupies a particular ecological niche, habitat, or portion of the available environment defined by a multitude of climatic, chemical, and physical variables, so-called 'environmental space'. A species' fundamental niche is defined by Hutchinson (1957) as that hypervolume defined by the environmental dimensions within which the species can survive and reproduce. A species may, however, be excluded from parts of its fundamental niche because of historical factors, competition, and other biotic interactions. This reduced hypervolume is termed the realized niche by Hutchinson (1957). To avoid confusion with other meanings given to the term niche, we use here the unambiguous term environmental niche of Austin, Nicholls & Margules (1990) and equate it with the terms realized niche of Hutchinson (1957) and the realized habitat of Whittaker, Levin & Root (1973).

An experienced field bryologist develops a 'feel' for the habitats of different species and can often say with a fair degree of confidence that a particular habitat is 'just the place for' a particular species (Proctor, 1980). This ability to identify a habitat as 'just the place' for certain species is based on extensive field experience and a qualitative and largely subconscious assessment of many features of the habitat (Proctor, 1980) such as geography, landscape topography, geology and soils, light, slope, aspect, moisture, land-use, and associated species. Clearly if bryophytes are to be used as environmental indicators and as monitors of environmental and habitat change in the past, it is necessary to quantify the environmental niches of different species today as reliably as possible.

There are at least four important reasons for attempting to quantify the environmental niches and environmental relationships of bryophytes. First, in monitoring environmental change today and in reconstructing environmental change in the past we need information on the ecological response of different species in terms of their environmental optima and tolerances, and of the variation in their occurrence or abundance along different environmental gradients. Second, insights into modern bryophyte-environment relationships are required if we are to predict changes in the occurrence or abundance of species in the future, given a likely environmental change or to use observed changes in bryophyte occurrences or abundances to infer subtle environmental changes. Third, if habitat parameters of a species are to be used as characters in systematics and cladistic analysis (*e.g.* Hedenäs, 1989, 1990; Hedenäs & Kooijman, 1996) or for comparing taxa within a genus (*e.g.* Kooijman & Hedenäs,

1991), the habitat parameters must be estimated as reliably as possible over the whole geographical range. Fourth, the understanding of the relationships between structure and ecological adaptation in bryophytes (e.g. Longton, 1980; Proctor, 1979, 1984) requires characterization of the environmental preferences (optima and tolerances) of the species of interest.

Despite the importance of quantifying the environmental niche of bryophytes, such quantification is not easy for several reasons. There is the problem of how to characterize and measure the multitude of environmental variables that are potentially important in influencing the occurrence and growth of different species. There is the problem of what spatial scale to study bryophyte responses to the environment. There is also the problem of sampling bryophytes and their environment so as to provide unbiased estimates of habitat parameters (Alpert, 1991). The problem of what scale to study was elegantly highlighted by Forman (1964) in his analysis of the factors influencing the growth of *Tetraphis pellucida* at scales varying from a single clump to its world geographical range.

Approaches to characterizing some aspects of the environmental niche of bryophytes have included (1) hemispherical photography to quantify the radiation climate experienced by different species (Proctor, 1980), (2) qualitative ranked indicator values for central European species for variables such as pH, light, and moisture (Düll, 1991) derived from various criteria but mainly general field observations, (3) measures of soil or water chemical variables or other environmental variables and graphical representation of species occurrences or abundances (e.g. Shaw, 1981; Gignac *et al.*, 1991a, b) or estimation of niche breadth and overlap indices (e.g. Horton, 1988; Slack, 1990; Slack & Glime, 1985; Vitt & Slack, 1984; Gignac, 1992), and (4) multivariate analysis of the occurrences of selected species in relation to environmental variables (e.g. Hedderson & Brassard, 1990). The approach we discuss here is more quantitative and statistical and builds on the approach pioneered by Austin *et al.* (1983, 1984, 1990) for eucalypt trees and the related but non-statistical response-surface approach used by Gignac *et al.* (1991a, b) with mire bryophytes in North America. Our approach involves generalized linear models and related generalized additive models. Before considering this approach for modelling bryophyte environmental niches, it is important to know if bryophytes are capable of responding to major environmental changes. For this, we can use the rapid and large climatic warming that occurred at the late-glacial/post-glacial transition about 11,300 years ago. In this 'natural experiment', we can study the results by analysing the fossil mosses preserved in sediments formed during this transition.

20.3 BRYOPHYTE RESPONSES TO PAST ENVIRONMENTAL CHANGES

Jonsgard & Birks (1995) have published a detailed stratigraphical analysis of the moss remains preserved in lake sediments at Kråkenes, a small lake on the west coast of Norway at 62°02'N latitude and 5°00'E longitude (see Birks *et al.*, 1996 for details of the site). The changes in the composition and relative abundance of the fossil mosses (Fig. 1) provide clear evidence for the rapid response of bryophytes to the major climatic warming that occurred at the late-glacial/post-glacial transition 11,300 calendar years ago (Jonsgard & Birks, 1995). The relative abundances of

KRÅKENES Fossil Mosses

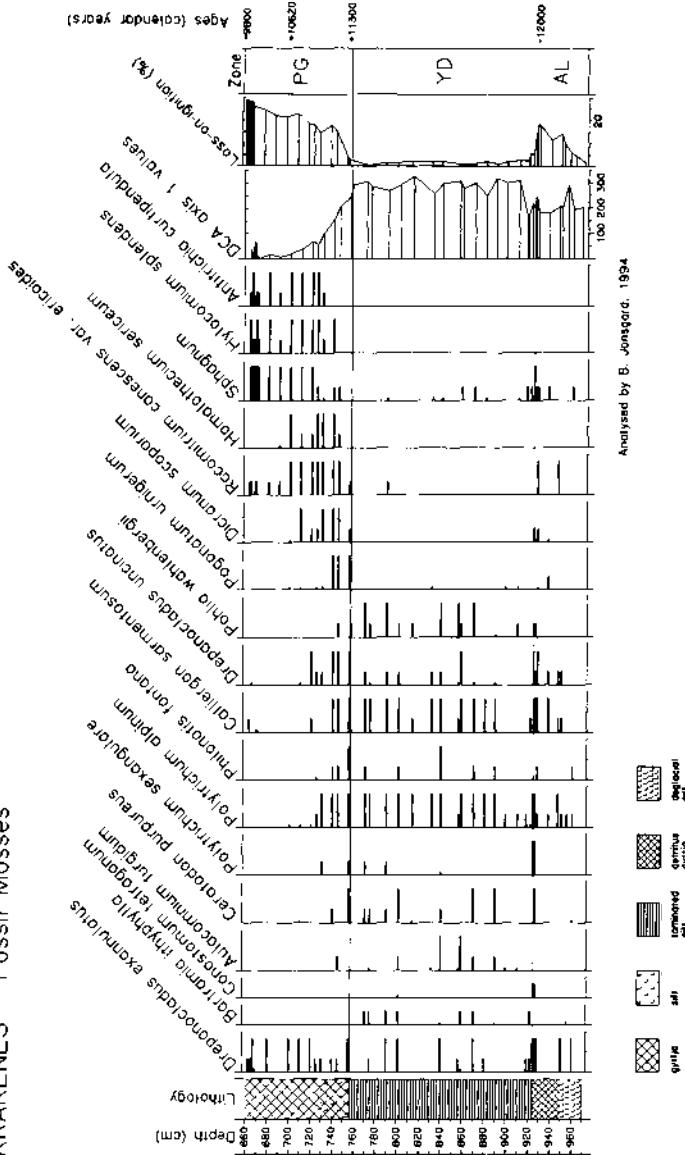


Figure 1. Stratigraphical diagram of the relative abundances of selected moss taxa found fossil in lake sediments at Kråkenes, western Norway. Taxa are arranged according to their approximate time of appearance. The relative abundances are shown as three classes (rare, occasional, abundant) by bars of different lengths. The sample scores ($\times 10^2$) on axis 1 of a detrended correspondence analysis (DCA) of the full data set are plotted stratigraphically and are in standard deviation units ($\times 10^2$) of compositional turnover (Hill & Gauch, 1980). Sample loss-on-ignition (%) at 550°C is also shown as a reflection of sediment organic content and hence of landscape soils and stability. AL = Allerød interstadial, YD = Younger Dryas stadial (both part of the late-glacial), PG = post-glacial. The boundary between the late- and post-glacial is shown by a solid line. The approximate ages (based on AMS ^{14}C -dating) of selected levels are shown in calendar years before present. See Jonsåsgård & Birks (1995) for further details.

selected taxa are plotted against depth in Fig. 1. At or near the transition between the Younger Dryas (YD on Fig. 1) cold period at the end of the late-glacial and the warm early post-glacial (PG on Fig. 1) there is the loss of some taxa (e.g. *Bartramia ithyphylla*, *Aulacomnium turgidum*, *Polytrichum sexangulare*), the expansion of some taxa (e.g. *Racomitrium canescens* var. *ericoides*, *Sphagnum*), and the arrival and subsequent expansion of other taxa (e.g. *Hylocomium splendens*, *Antitrichia curtipendula*). There is hardly a taxon in the whole assemblage (Jonsgård & Birks, 1995) that does not change in its relative abundance across the late-glacial/post-glacial transition. The one exception is *Drepanocladus exannulatus* (Fig. 1).

The environment of the Younger Dryas resembled the cold and wet mid- and high-alpine zones of the west Norwegian mountains today. Within 500 years of the major climatic warming at the beginning of the post-glacial, the environment resembled the temperate birch forest of the area today. Each phase had its own characteristic range of habitats, and these appear to have been occupied by bryophytes as soon as they developed and became available. The fossil sequence at Kråkenes demonstrates the remarkable speed that bryophytes of all major habitat types can migrate and colonize newly created habitats.

The major overall patterns in the stratigraphical changes in the relative abundances of the 71 taxa found fossil at Kråkenes can be summarized as the first axis of a detrended correspondence analysis (Hill & Gauch, 1980) of the fossil bryophyte data. The first axis is large and captures 23.2% of the total variance in the data. When plotted stratigraphically (Fig. 1), it provides a summary of the major patterns of floristic change. It shows that the fossil bryophyte assemblage began to change in composition immediately at the late-glacial/post-glacial transition and that the assemblage underwent major changes during the first 300–500 years of the post-glacial. The assemblages showed 50% turnover or compositional change (Hill & Gauch, 1980) within 500 years since the onset of the post-glacial.

Such a stratigraphical record shows the remarkably rapid response of bryophytes to major environmental changes. The detailed interpretation of such changes requires information on the relationships between bryophytes and the environment today.

20.4 STATISTICAL MODELLING OF BRYOPHYTE-ENVIRONMENT RELATIONSHIPS

The approach we have adopted to model statistically the environmental niches of bryophyte species takes advantage of recent developments in applied statistics, in particular the development of generalized linear models (GLM) (McCullagh & Nelder, 1989) and generalized additive models (GAM) (Hastie & Tibshirani, 1990). These developments provide very flexible and extremely powerful statistical tools for modelling the response of a species to one or more environmental variables in the form of a 'regression-type' model.

The basic idea is, given a large, consistent, and representative data-set, to model statistically the probability of occurrence, the number of occurrences (frequency), or the relative abundance of a species in relation to gradients of individual environmental variables such as soil pH, moisture, or radiation index. The aim is to derive the simplest statistically significant model (*i.e.* with the largest number of degrees of freedom in the random or residual component) with the maximum explanatory

power (*i.e.* with the smallest random component) of the species' response to an environmental variable. GLM and GAM take account of important properties of the data, in particular the type of data (presence-absence, counts, proportions, etc.) and the underlying error distribution (normal, Poisson, binomial, etc.). They provide flexibility that is unavailable in standard regression analysis. A response function or curve can be derived to show the predicted response of the species in relation to the environmental variable for the selected statistical model. This provides a convenient graphical representation of the habitat requirements or realized environmental niche of the species and of the variation of the species' abundance along an environmental gradient. It is also possible to consider several environmental variables together and to examine whether different variables interact significantly to influence the response of the species. Response surfaces can be constructed to show how the species' response varies in relation to two environmental gradients.

Useful introductions to GLM and to model specification, simplification, and assessment are provided by Crosbie & Hinch (1985), Nicholls (1991), Oksanen, Läärä & Zobel (1991), and Crawley (1993) and to GAM by Yee & Mitchell (1991) and Trexler & Travis (1993).

The GLM/GAM approach is an attempt, admittedly rather crude conceptually but statistically flexible and robust, to quantify what an experienced field bryologist does subconsciously when thinking 'just the place for' a particular species. We will illustrate the approach with examples for some *Andreaea* and *Racomitrium* taxa in western Norway.

Heegaard (1996, 1997) studied the comparative ecology of 12 *Andreaea* taxa in western Norway in relation to 21 environmental variables in 568 10 × 10 cm plots. Some representative results for *A. nivalis* are shown in Fig. 2 as response functions for the number of occurrences of *A. nivalis* in relation to individual environmental variables and as response surfaces for the number of occurrences in relation to radiation index, flushing, and snow-cover. Details of methods are given by Heegaard (1996, 1997). All the statistical models shown in Fig. 2 are statistically significant ($p < 0.001$). They show that *A. nivalis* favours high altitudes (Fig. 2a) (the optimal altitude was not sampled), gentle slopes (10–40° — Fig. 2b), moderately high rock-pH (Fig. 2c) but low rock-Ca (Fig. 2d), intermediate water-pH (Fig. 2e), and sites with a high radiation index (Fig. 2f) that are flushed by seepage water or by streams (Fig. 2g), often from nearby snow-beds (Fig. 2h), although it avoids extremely late snow-beds (Fig. 2h).

The same approach can be used to compare the environmental niches of closely related taxa, for example *A. rothii* ssp. *rothii* and *A. rothii* ssp. *falcata* (*sensu* Murray, 1988) (Fig. 3). The modelled responses for these two taxa are generally very similar (Heegaard, 1996, 1997), except that ssp. *rothii* occurs more frequently on sites with very low soil-pH (Fig. 3a) than ssp. *falcata* (Fig. 3b), and that ssp. *falcata* favours sites flushed after rain (flushing level 2, Fig. 3d) in contrast to ssp. *rothii* which is more frequent at sites flushed only during rain (flushing level 1, Fig. 3c).

Heegaard (1996, 1997) did comparable analyses for the 12 *Andreaea* taxa growing in western Norway and compared the modelled responses of these taxa in relation to the same environmental variable as a means of studying niche differentiation between the taxa. He showed that all taxa favour sites with a high potential radiation index. There is a positive correlation between this environmental parameter and the

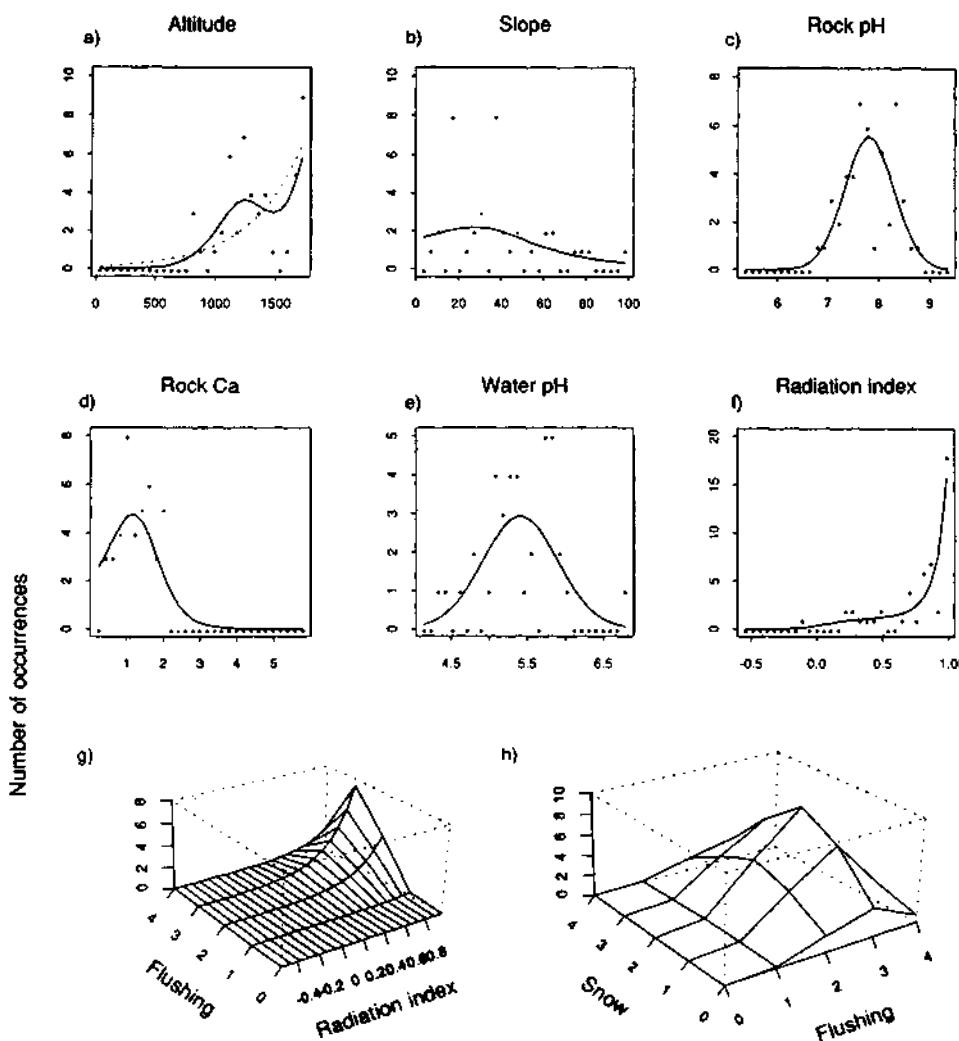


Figure 2. The modelled response functions for the number of occurrences of *Andreaea nivalis* in relation to (a) altitude (m), (b) slope (degrees), (c) rock pH, (d) rock Ca ($\ln (\text{mg})/100 \text{ mg}$), (e) water pH, and (f) radiation index. The modelled response surfaces of *A. nivalis* in relation to (g) flushing (5 ranks) + radiation index and (h) snow-lie (5 ranks) + flushing. Continuous environmental variables were transformed into 30 equal segments prior to modelling and the number of occurrences of the species within each segment was used as the response variable.

degree of flushing, number of cracks, and shelter for most of the taxa. These environmental variables are best regarded together as reflecting the moisture levels experienced by the different taxa (see Alpert, 1991). There is, however, considerable niche differentiation in relation to altitude, with the most frequent taxa falling into three groups occurring at low (*A. megistospora* ssp. *megistospora*, *A. rothii* ssp. *rothii*, *A. rothii* ssp. *falcata*, *A. alpina*), intermediate (*A. frigida*), and high altitudes (*A. obovata*, *A. nivalis*, *A. alpestris*, *A. blyttii*, *A. rupestris* var. *papillosa*). *A. rupestris*

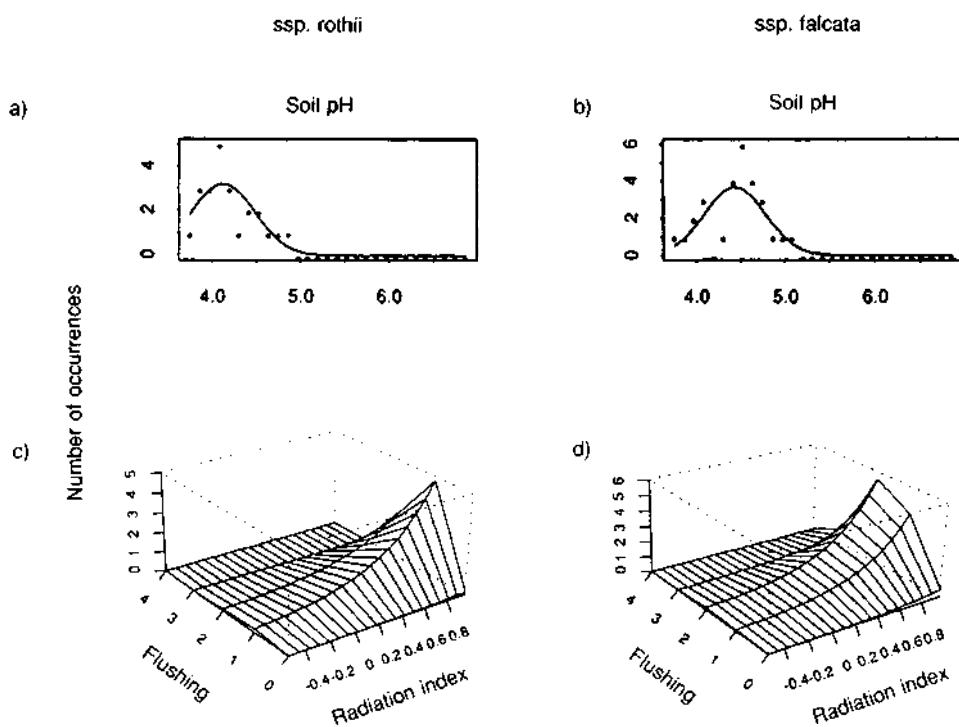


Figure 3. The modelled response functions for the number of occurrences of *Andreaea rothii* ssp. *rothii* and *A. rothii* ssp. *falcata* in relation to soil pH (a, b) and the modelled response surfaces in relation to flushing + radiation index (c, d).

var. rupestris is the only taxon that occurs over the whole altitudinal range sampled (0–1750 m). Altitude is, of course, an indirect environmental gradient (*sensu* Austin *et al.*, 1984) and is a complex of temperature, moisture, and snow-lie. In general the most moisture-demanding taxa occur at high altitudes, with the exception of *A. alpina*. In Heegaard's (1996, 1997) study *A. alpina* occurred between 90 and 490 m whereas in other parts of Norway it occurs up to 1200 m (Størmer, 1969) and in the British Isles it is predominantly above 500 m, although it has an altitudinal range there of 50–1300 m (Murray, 1988).

Although all *Andreaea* taxa grow in sites with a generally low soil pH (3.7–6.9), there is some differentiation between taxa in relation to soil pH (Fig. 4). There is a ranking of optima from low to intermediate soil pH of *A. rothii* ssp. *rothii*, *A. rothii* ssp. *falcata*, *A. obovata*, *A. rupestris* var. *rupestris*, and *A. blyttii*. *A. rothii* ssp. *rothii* and *A. rothii* ssp. *falcata* have soil-pH optima significantly different from the other taxa and clearly favour the most acid soils.

A similar statistical modelling has been done of the number of occurrences of the commonest four *Racomitrium* taxa along the Sognefjord in western Norway (Jonsgård & Birks, 1993) in relation to annual precipitation and mean January temperature (Fig. 5). There is a gradient along the 160 km long fjord from the coastal, oceanic climate to the continental inner-fjord climate. All four taxa (Fig. 5) have their optimal number of occurrences in areas of high annual precipitation and

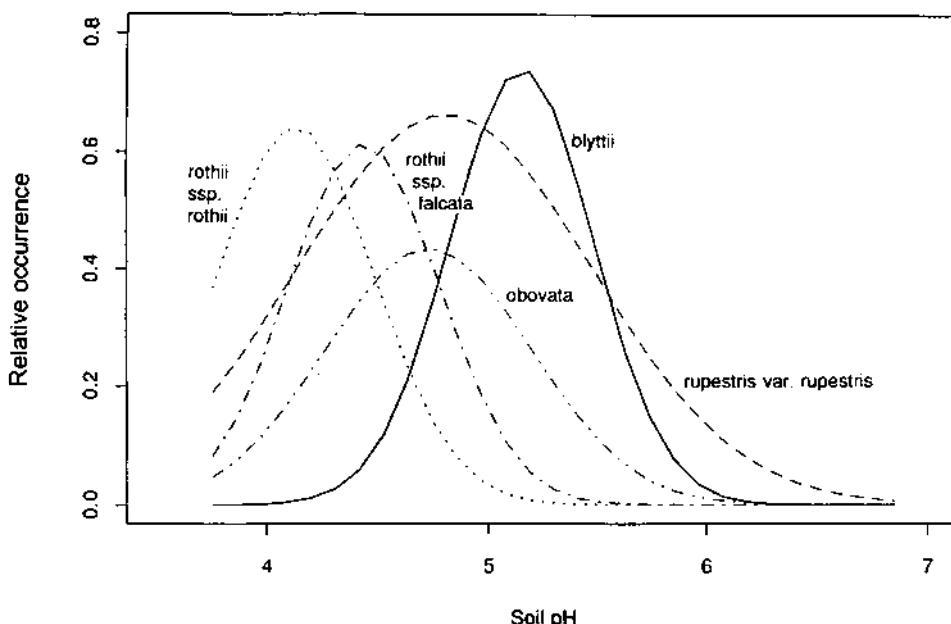


Figure 4. The modelled response functions for the relative occurrences of five *Andreaea* taxa in relation to soil pH.

mild January temperatures. There is, however, some differentiation in the mean January temperature optima, with *R. sudeticum* (*sensu* Frisvoll, 1988) with an optimum of at least 2°C, *R. lanuginosum* and *R. heterostichum* (*sensu* Frisvoll, 1988) with optima of 1–2°C, and *R. elongatum* (*sensu* Frisvoll, 1983) with an optimum of 0.5–1.5°C.

This type of statistical modelling involving GLM and GAM can provide some insights into the environmental optima and tolerances of bryophytes and into how species' responses vary along environment gradients. By considering the optima and tolerances of different species it is possible to discover what environmental variables certain bryophyte species may be potentially responsive to and hence indicative of. Such information is needed if bryophytes are to be used as monitors of environmental change today and in the future as well as a means of reconstructing past environmental change from fossil bryophyte assemblages.

In addition to these GLM/GAM modelling techniques, other numerical techniques are appropriate if all the species of interest are to be considered simultaneously. In GLM/GAM each species is analysed individually in relation to different environmental gradients. A simultaneous analysis of all species of interest that incorporates biological factors and environmental gradients in one analysis of the realized species niches (Heegaard, 1996, 1997) can be implemented by the constrained ordination technique of canonical correspondence analysis (ter Braak & Verdonschot, 1995). In this type of niche analysis the optima of the species are estimated for a synthetic environmental space that is formed by linear combinations of the measured environmental variables.

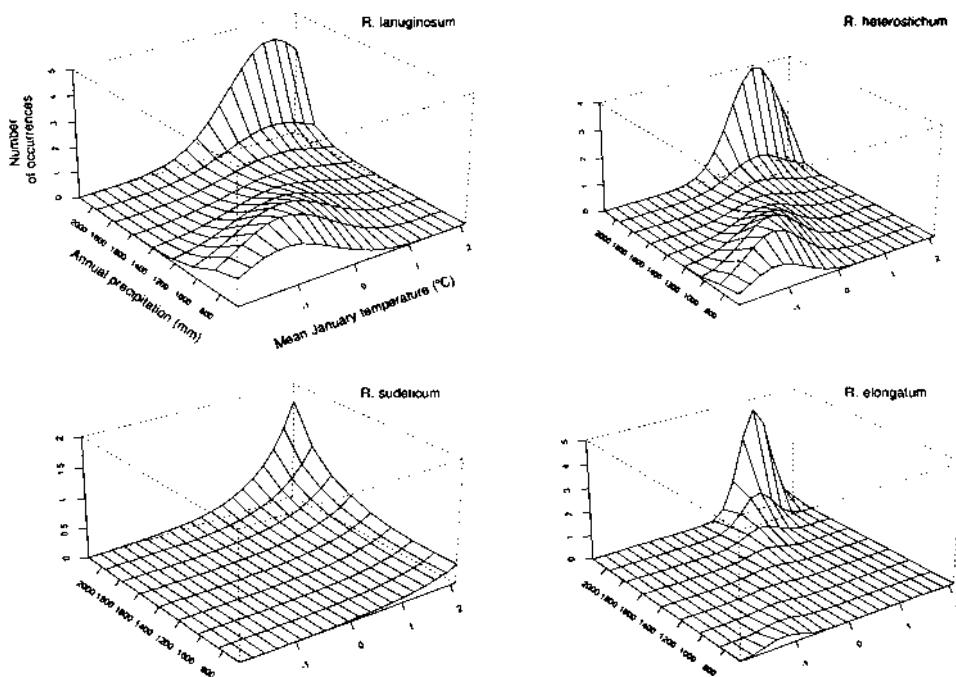


Figure 5. The modelled response for the number of occurrences of *Racomitrium lanuginosum*, *R. heterostichum*, *R. sudeticum*, and *R. elongatum* in relation to annual precipitation (mm) + mean January temperature (°C). The environmental variables were transformed into 30 equal segments prior to modelling and the number of occurrences of the taxa within each segment was used as the response variable.

20.5 PREDICTING BRYOPHYTE RESPONSES TO FUTURE ENVIRONMENTAL CHANGES

One reason for attempting to quantify bryophyte-environment relationships is to provide a means of predicting how bryophytes might respond to environmental changes in the future. Our environment is currently changing and there is a strong likelihood of substantial climatic changes in the near future because of increasing anthropogenic emissions of so-called greenhouse gases such as CO₂. The question arises how will bryophytes respond to global warming associated with a doubling of pre-industrial CO₂ concentrations?

One rather obvious approach of deriving an answer to this question builds on the approach of modelling species-environment relationships, namely to map present-day distributions of species in modern 'climate space' defined by several different climatic parameters. Given predictions about future climate from general circulation model (GCM) simulations and modelled species-climate relationships today, it is possible to predict the future geographical distribution of the species of interest by finding where in geographical space today the predicted future climate occurs that corresponds to the present-day climatic demands of the species. This indirect correlative approach to prediction has not, as far as we know, been applied to bryophytes (but see Gignac & Vitt, 1994). It has, however, been applied by Huntley *et al.* (1995) (see also Huntley, 1995) to *Hymenophyllum wilsonii*, a filmy fern that commonly

grows together with several Atlantic bryophytes along parts of the western seaboard of Europe.

Huntley *et al.* (1995) compiled climatic data for 'mean' elevation in each 50×50 km grid square across Europe. They used a weighted smoothing procedure to derive a species-climate response surface that graphically displays the relation between the current species' distribution and present-day climate. Simulated climate values for two alternative GCMs for $2 \times \text{CO}_2$ levels at a spatial resolution of $5^\circ \times 7.5^\circ$ and $4^\circ \times 5^\circ$ were used to predict potential species' distributions in the future in equilibrium with these future climates. In the case of *H. wilsonii*, Huntley *et al.* (1995) predict major changes in its geographical distribution, with a marked displacement northwards. Climate values from one GCM predict its expansion in Iceland and into northern Norway, its extinction in the Azores, Ireland, England, and Wales, and its decline in Scotland. The other GCM values result in predictions of its extinction from the Azores, the British Isles, and western Norway, of a major shift into the Scandinavian mountains, the Kola peninsula, and Iceland, and of scattered occurrences in the Mediterranean and northern and central Italy. If these predictions are correct, they suggest major changes will also occur in the Atlantic bryophyte flora of the British Isles. Many Atlantic bryophytes have distributions and ecologies similar to *H. wilsonii* today (Ratcliffe, 1968). If these bryophytes were to respond in a similar way to the predicted responses of *H. wilsonii*, there would be extinctions of many species from the British Isles.

The predictions of Huntley *et al.* (1995) for *H. wilsonii* and for many of the other species that they studied are for very large and rather surprising changes in geographical range. Their approach does not, however, consider the scale that environmental factors affect plants. The modern climate data used are for 'mean' elevation in 50×50 km grids and the predicted climate values are for $5^\circ \times 7.5^\circ$ or $4^\circ \times 5^\circ$ blocks. Clearly these data relate to a very broad spatial scale, whereas many bryophytes and filmy ferns occupy subtle microhabitats. It is difficult, if not impossible, to model the realized environmental niche of a species at the spatial scale of the actual micro-habitat for the entire geographical range of the species. As Forman (1964) showed, different environmental variables may be important at different spatial scales and simple scaling up or down of an environmental variable can be unreliable in predicting the distribution of species such as *Tetraphis pellucida*.

A potentially more useful and ecologically more realistic approach to predicting the response of bryophytes to environmental change is the direct experimental approach in which the growth responses of bryophytes to changes in temperature, moisture, nutrients, etc. are studied in the field using manipulative experiments. Work in northern Sweden by Potter *et al.* (1995) on *Hylocomium splendens* and *Polytrichum commune* shows increased air temperatures reduce the annual growth of *H. splendens* whereas the growth of *P. commune* is enhanced. These experimental results therefore predict a decrease in *H. splendens* if temperatures rise and there is no increase in precipitation, whereas the growth of *P. commune* is predicted to increase under a warmer and/or wetter climate. Jägerbrand (1996), also working in northern Sweden, has similarly shown that the growth of *H. splendens* as well as of *Tomenthypnum nitens* is decreased by increased temperatures, whereas the growth of *Sphagnum teres* and *Aulacomnium turgidum* is enhanced by increased temperatures. Kennedy (1996) has elegantly combined field manipulations, microcosm observations, and laboratory

experiments to predict bryophyte responses to future climate change in a maritime Antarctic fell-field. He emphasizes that this 'tripartite approach ... renders the conclusions more robust than any single study considered in isolation'.

20.6 CONCLUSIONS

In reviewing current approaches to quantifying bryophyte-environment relationships and to the modelling of the response of bryophytes to environmental change, there are four main conclusions.

First, fine-resolution palaeoecological studies of fossil bryophytes preserved in lake sediments show that bryophytes responded rapidly to major environmental changes in the past. These observations, coupled with the fact that very many bryophytes show a narrow habitat specificity, indicate the potential sensitivity of bryophytes to environmental change.

Second, it is possible to quantify the realized environmental niche of bryophyte species using GLM and GAM approaches. Such statistical modelling does, however, require large, consistent data-sets. There are problems in compiling such data-sets related to the problems of measuring ecologically relevant environmental variables, of scale of study, and of representative, unbiased sampling.

There are particular problems related to fine-scale environmental measurements. The estimated response *per se* may be masked by other variables operative at the same or broader scales, and thus any analysis should incorporate combinations of environmental factors. For example, the high potential radiation index found for all *Andreaea* taxa is positively correlated with moisture-conserving environmental variables. This correlation indicates that in the most desiccated and exposed habitats, as estimated by the radiation index, there is a constraint on *Andreaea* taxa to occur in moist microsites. Another problem related to fine-scale estimation of the environment experienced by bryophytes is the difficulty of obtaining a representative coverage of the natural gradient. If the entire gradient is not included in the analysis, some bias can occur in the modelling results.

These potential problems must be considered in the sampling strategy used and borne in mind in discussions of the results obtained. In general, fine-scale environmental sampling is to be recommended as this will better estimate the actual environment in which bryophytes naturally respond (Alpert, 1985, 1991; Hedderson & Brassard, 1990; Pentecost, 1980; Heegaard, 1996, 1997). A potential solution worthy of further study is to sample the environment at more than one scale, as suggested by Armstrong (1974).

Most of the studies concerned with estimating and quantifying the realized environmental niches of bryophytes have only considered adult individuals in a static estimation of the environment. The responses of bryophytes in the establishment stage (Li & Vitt, 1994, 1995) should also be studied if we are to improve our understanding of bryophyte-environment relationships and our ability to predict bryophyte responses to environmental change. Moreover the response to annual and inter-annual environmental fluctuations is important in understanding the dynamics of the realized environmental niche. This is evident as individuals can change their physiological responses and tolerances with age and at different size classes (Proctor, 1984).

Third, the modelled environmental niches can be used to aid interpretation of stratigraphical changes of fossil bryophytes in terms of environmental change, to identify potential environmental monitors within the bryophytes, and to suggest potentially important environmental variables for manipulative experimental studies.

Fourth, field manipulative experiments are required to predict how bryophytes might respond to future environmental change. Prediction remains a major challenge in all ecology (Peters, 1991), not only in bryophyte ecology. Such predictions about responses to future climate change are critically important for future conservation. Bryophyte conservation is particularly important in the British Isles because of their remarkable bryological diversity and because several species that are rare when viewed at a European scale are locally frequent in western areas of the British Isles. Prediction of bryophyte responses to environmental change will be a major challenge for bryology in the second century of the British Bryological Society.

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21. Modelling the dynamics of bryophyte populations

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21.1 SUMMARY

Bryophyte habitats are patchy in time and space. The population dynamics of such systems operate at two levels, within-patches and between-patches. Simulation models are powerful tools to aid understanding of the dynamics and to make predictions about future development of populations. The models must, however, be built on the knowledge of both population and habitat parameters. In this paper, the use of metapopulation models is discussed and a few examples where such models may be useful to predict the outcome of, for example, environmental changes are given.

KEYWORDS: Metapopulation, diaspores, dispersal, establishment probability, patch dynamics, habitat fragmentation, climate change.

21.2 INTRODUCTION

Bryophyte habitats are patchy, both in time and space (Herben, 1994; Söderström & Herben, 1997). This is because within the landscape, each species is able to grow only in certain spatially limited regions (habitat patches), and growth is impossible elsewhere. In most cases, the habitat patches are of limited duration, although life-spans may vary from several months to millennia. Such 'habitat islands' are well known in ecological theory and much work has been done to describe their dynamics. It is essential to realize that the dynamics of these systems are governed both by population dynamics of the species involved, and by the dynamics of the habitat patches. Dynamics involving a set of patches in the landscape is termed metapopulation dynamics (*cf.* Hanski, 1991) in contrast to within-patch dynamics, which is the more widely known case of population dynamics.

The population dynamics of any species within a system of habitat patches operates at two levels, at the within-patch level and at the between-patch level. Whereas the former is governed by the well-established rules of growth, reproduction and mortality, the latter is determined by the ability of the species to cross the barriers between the patches (Levins, 1969). This has important implications for the dynamics of the populations involved. These two spatial scales of population growth and persistence may have different rates. One has to think of two different types of turnover. As two contrasting examples, one may consider a high-turnover species which easily crosses barriers between habitat patches but which has a low-turnover

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rate within habitat patches (e.g. *Polygonatum urnigerum*), and a species that does not cross patch barriers easily but has a high turnover rate within a habitat (e.g. *Phascum*, *Riccia*). The overall dynamics of the system are determined by the interaction between these two temporal scales which may be far from trivial (Hanski, 1983).

In the simplest case, one can assume that the dynamics within habitat patches are rapid enough to be ignored if the between-patch dynamics are being studied. Then the proportion of available patches colonized is dependent on the relation between immigration and extinction only. Survival of a species in such a system requires that the extinctions of the species in different patches are not synchronized but rather, stochastic events. This system may occur on isolated islands where immigration is rare, but not so rare that evolutionary forces (i.e. speciation) play a significant role. Often, however, the dynamics of immigration/extinction and of within-patch growth occur on similar time-scales so that the interplay between them cannot be ignored (Gilpin & Hanski, 1991). Persistence then depends on population processes both within and between the patches (Table 1, Hanski, 1982; Verboom *et al.*, 1991).

Parameters of both within- and between-patch processes contribute to the stability and survival of a species within a system of habitat patches, though their importance may differ according to the species in question and the structure of the habitat patch system. On the other hand, this linking between the levels may produce correlated behaviour between both levels (e.g. core-satellite types of distribution *sensu* Hanski, 1982). Determining the type of interaction between these two levels is not straightforward. Study of the dynamics of such systems must be either by analytical or simulation models.

The existence and magnitude of the dynamics of bryophyte metapopulations is determined by two basic conditions: the ability of the species to disperse, and the particular structure of the environment, usually a landscape of non-contiguous habitat patches. The analytical approach needs to consider two sets of parameters, (1) population parameters, such as local population growth rate, local extinction rate, diaspore production, dispersal ability, diaspore survival and establishment ability, and (2) habitat parameters such as habitat duration, patch favourability, patch size, patch distance, spatial pattern of habitats and number of habitat patches. Some of these parameters operate within patches while others operate between patches (Table 1).

Parameters of bryophyte population biology have been collected over several decades. Although more data are available on the processes within habitat patches (Herben, 1994), dispersal and establishment parameters are also available for several

Table 1. *Species and habitat parameters operating between and within patches.*

	Within patch	Between patch
Species parameters	<ul style="list-style-type: none"> ● Local population growth rate ● Diaspore production ● Local diaspore dispersal ● Establishment rate 	<ul style="list-style-type: none"> ● Diaspore production ● Distance dispersal ability ● Establishment rate
Habitat parameters	<ul style="list-style-type: none"> ● Habitat patch size ● Habitat patch favourability 	<ul style="list-style-type: none"> ● Habitat duration ● Habitat patch distance

species (Miles & Longton, 1990, 1992; see also Söderström & Herben, 1997). Recently, there has been a marked interest in comparing the bryophyte population dynamics of many species (e.g. Hedderson & Longton, 1995). Such data can provide insights into the parameters of both within-patch and between-patch processes. Compared to the parameters of species biology, habitat parameters have been studied much less intensively and the information has often been derived from non-bryological sources. Invariably there is insufficient knowledge about the dynamics of the habitats. This knowledge is often in the form of a naturalist's 'folk-lore' (e.g. peat bogs are stable, whereas pond bottoms are not), and quantification is rare. Söderström & Herben (1997) give a review of the available data on both populations and habitats, and their importance for within-patch and between-patch dynamics.

The occurrence of metapopulation dynamics in the field has to be demonstrated by careful observations. Two approaches are possible here: (1) a deductive approach using models, or (2) long-term direct observation of the process. The latter is difficult due to the often long turnover times and we are not aware of any examples. The former approach has been used in a more systematic fashion in bryophytes (Herben, Rydin & Söderström, 1991; Marino, 1991). However, as with any model, results obtained in this way are always dependent on assumptions built into the model and the results have always to be interpreted with caution. The quality of predictions increases when the parameters are taken from field data.

21.3 DISPERSAL-LIMITED VS HABITAT-LIMITED SYSTEMS

Most population models assume that dispersal between habitat patches is important, but the effects of dispersal on habitat occupation may vary. Dispersal may be sufficiently efficient that the species essentially occupies every available habitat patch, i.e. its frequency at the landscape scale is determined by the number of available habitats. Alternatively, dispersal may be slow relative to the duration of a habitat patch, and many habitat patches then remain unoccupied. In this case frequency is determined by dispersal constraints. A central question then is — under what circumstances is dispersal ability limiting and when is habitat patch availability limiting? Herben *et al.* (1991; see also Herben & Söderström, 1992, and Söderström & Herben, 1997) built a model to investigate this question for dynamic habitats (i.e. habitats with a limited duration), based on the population and habitat parameters mentioned above (local growth rate, diaspore production, diaspore transport, establishment probability, habitat size, distance between habitats, habitat configuration). Since establishment ability was the least known parameter, all simulations were done at a range of values for this parameter. The basic model showed that if the species was able to survive at all, it was able to colonize almost all available patches (i.e. it was habitat limited) except under a rather narrow set of conditions where the system was dispersal-limited. The interval when the system was dispersal-limited increased considerably when variation in establishment ability increased, and with increased variation in mean distance between patches (i.e. with habitat patches clumped; Fig. 1; see also Fahrig & Paloheimo, 1988; Hansson, 1991; Adler & Nuernberger, 1994). The regions of true dispersal limitation are those where the metapopulation processes determine regional abundance of the species.

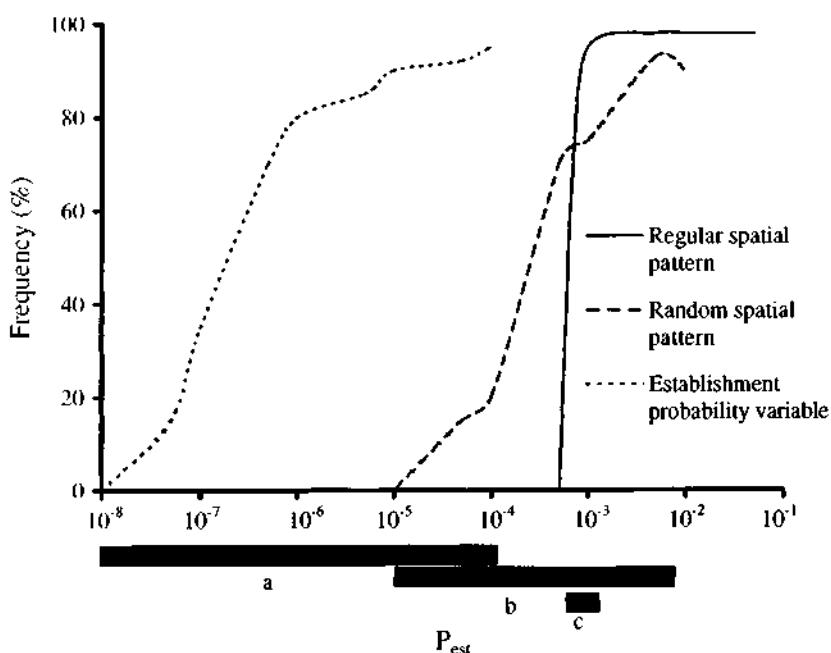


Figure 1. Frequency of available patches occupied under different establishment probabilities (P_{est} , probability of establishment for a spore reaching the new locality). The black bars below indicate the region for dispersal limitations when a) P_{est} varies, b) spatial pattern of habitat patches is random, and c) spatial pattern of habitat patches is regular.

Sensitivity analysis showed that the distance between habitat patches, and species dispersal ability, were the parameters where the smallest changes, in terms of the variation of these parameters in the field and/or potential measurement error, had the largest effects. These are the parameters that determine dispersal success. These parameters also play a crucial role in determining whether the changes in other parameters would affect the metapopulation dynamics of a species and thus its regional abundance. Further analysis (Herben & Söderström, 1992) showed that long-lived species could survive in dispersal-limited systems much more often than short-lived species. If establishment probability varies between years, long-lived species can afford to 'sit and wait' for the right conditions to appear, whereas suitable conditions may never occur within the life span of a short-lived species. Different population parameters will have different effects on species with different strategies. Parameters affecting between-patch dynamics (distance between localities) affects colonists and perennials (*sensu* During 1992) more than shuttle species (Söderström & Herben, 1997). Shuttle species are much more sensitive to factors affecting within-patch dynamics (habitat duration and initial number of colonies).

21.4 USE OF MODELS

One of the great advantages of models is (if they are tested and proven reliable) that they can be used to predict what will happen in the future if one or more parameters

are changed. I will give four examples of how dynamic models can be used to predict outcome of environmental changes.

21.4.1 Fragmentation of habitats (forests)

One of the obvious changes of the modern landscape is the fragmentation of many habitats that earlier covered vast areas, e.g. boreal forests. The simulation model of Herben *et al.* (1991) shows, for species living in patchy dynamic habitats, that the proportion of occupied sites decreases if the area of local populations decreases, spore production decreases, local extinction rate (disturbance rate) increases or the distances between patches increases. A fragmentation of habitats means that: 1) the area of each locality decreases, which decreases the local populations and thus increases the risk for local extinctions; 2) the local population sizes decrease, which decreases total diaspore production; 3) the distance between the localities increases so that individual diaspores must travel longer before they reach new localities.

These factors reduce the possibilities for developing large and stable populations. Based on values of variables for a short-lived, fugitive species (like e.g. *Orthodontium lineare*) and a long-lived, resident species (like e.g. *Ptilidium pulcherrimum*) from Herben & Söderström (1992), an increase of average distance between localities to 200% the original distance has to be balanced with an increase in establishment probability of two orders of magnitude for the short-lived species to survive, while a long-lived species is not so much affected (Fig. 2). A decrease of locality size to 5% of its original size, or decreasing the number of patches by 75% give similar results (Fig. 2). In these examples, only one of the parameters is changed at a time. With any combination of decreased patch sizes, increased distances between patches and decreased number of patches, the effect will be even more severe. This simple simulation example tells us three things. First, since the distance between localities is the habitat parameter most sensitive to small changes, the effects of habitat fragmentation will be less severe if the remnant fragments are not separated by large distances (*cf.* Herben *et al.*, 1991). Secondly, long-lived species with large between-year fluctuations in colonization ability suffer less than short-lived species that need to recolonize often (*cf.* Herben & Söderström, 1992). Third, species where within-patch dynamics are more important than between-patch dynamics (e.g. shuttle species *sensu* During, 1992) are less affected than colonists and perennials (*cf.* Söderström & Herben, 1997), and mostly affected by a reduction of patch size. Thus it is predicted that short-lived species with low dispersal ability will be the most severely affected by habitat fragmentation.

21.4.2 Reduced reproduction

It is known that sexual reproduction fails in some bryophyte populations or over larger areas, although the gametophyte persists and grows well. For instance, some populations of *Dicranum majus* in southern Norway lack, or very rarely have, sporophytes, while most populations of *D. majus* in Norway produce many sporophytes (Sagmo Solli, 1996). Pollution may reduce the reproduction of bryophytes (e.g. Raeymaekers & Glime, 1986; Winner & Bewley, 1978) without affecting persistence and growth. The non-reproducing populations of *D. majus* in Norway lack dwarf

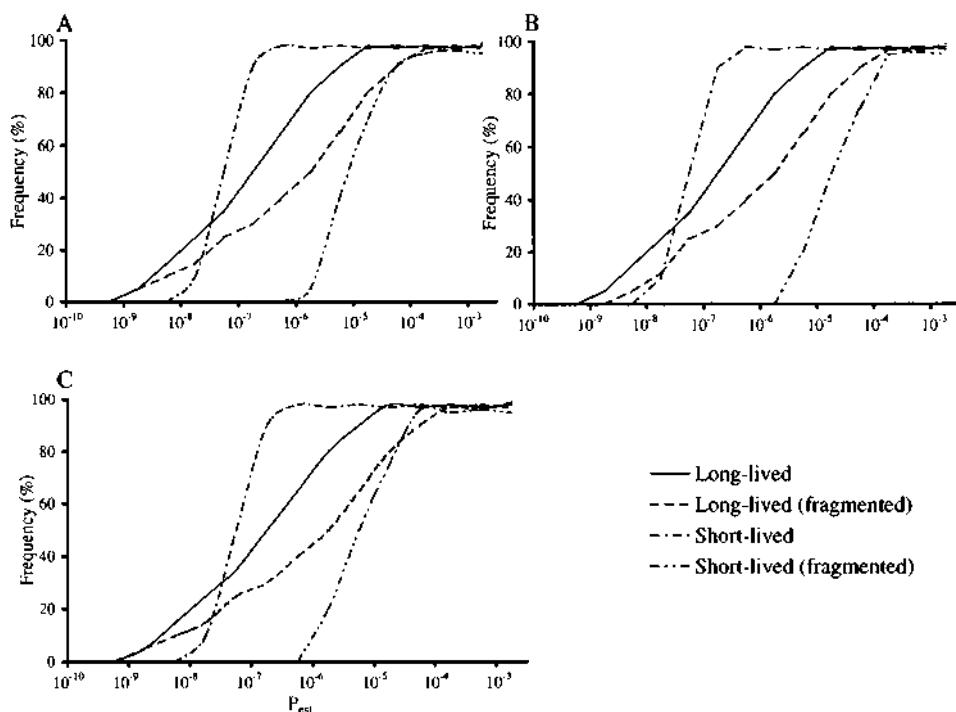


Figure 2. Simulation of effects of habitat fragmentation on the population survival ability. Each variable is simulated individually and no simulation has been made for combination effects. A) Distance increased by 200%. B) Locality size decreased by 95%. C) Number of habitat patches reduced by 75%. Simulations have been made separately for a short-lived and a long-lived species (cf. Herben *et al.*, 1991). P_{est} = Probability of establishment when a diaspore has reached a new locality. Frequency = Frequency of available localities occupied.

males (Sagmo Solli, 1996). A possible explanation is that acid precipitation has selectively killed the dwarf males with little or no impact on the normal sized females. These populations are unable to participate in between-patch dynamics. What effect might this have on survival of *D. majus* in the region? Using the model of Herben *et al.* (1991), the outcome for bryophyte species with different life history strategies may be postulated. For species relying mostly on within-patch dynamics, the effect will be small provided that local survival is not dependent on local spore production. For species where the between-patch dynamics is important (e.g. for species living on temporal substrates) and where the production of diaspores is essential, this will lead to regional extinction if all populations become sterile.

There are three possible ways in which reproduction may be affected, all of which it is possible to investigate in the simulation model of Herben *et al.* (1991). 1) If the absence of reproduction is irreversible and all sub-populations are affected, the extinction time of populations may be estimated by calculating the longevity of populations, i.e. the average time needed for a population to be replaced by a newly recruited individual. 2) If the effects are temporary, the effects on different species with different life spans will be possible to estimate. 3) If reproduction is not com-

pletely absent, but only reduced, it may be possible to calculate the long-term effects on the whole metapopulation by using a lower value of reproduction in the metapopulation model. If the total spore production per patch is lowered to one third for *Orthodontium lineare*, the establishment probability must be one order of magnitude larger to balance it, and if the spore production is only 4% of the original, establishment probability must be two or three orders of magnitude larger to achieve balance (Fig. 3).

21.4.3 Climate change

Climate change is one of the most discussed global environmental changes postulated for the near future. Since all species are dependent on their macro- and microclimate, such changes will have an impact on the ability to survive. When the responses of a species to climate variables are known, the outcome of a change in microclimate can be postulated. Huntley *et al.* (1995) postulated the change in distribution area of eight vascular plant species using three different proposed scenarios of climate change. Few bryophytes have been investigated in such detail that it is possible to use them in models of this kind. However, Heegaard's (1995, 1997) studies of 12 species of *Andreaea* and Jonsgaard & Birks' (1993; see also Birks *et al.*, 1998) study of four *Racomitrium* species may be detailed enough.

For each scenario of climate change, individual species may respond in one of three different ways. First, a species may be able to adapt to the new conditions appearing at its pre-existing locality. Secondly, a species may not adapt to the new conditions but it may be able to disperse to new areas where suitable conditions appear. Third, if it is unable to adapt or disperse effectively it will go extinct. All

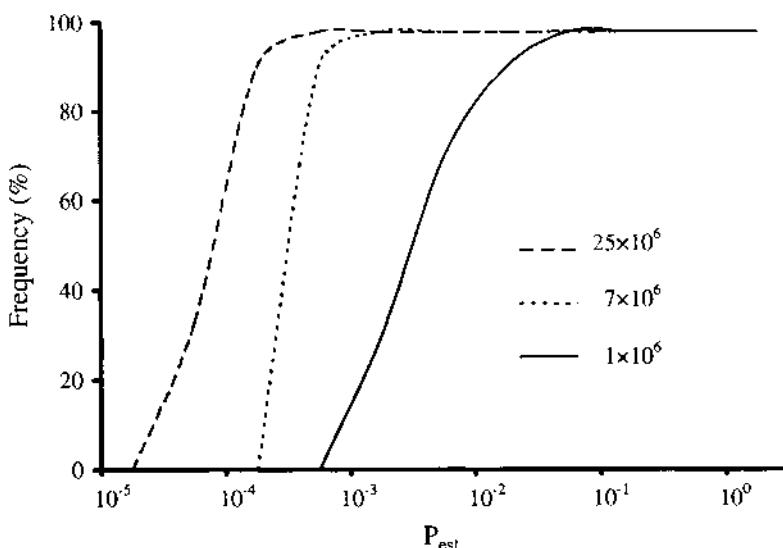


Figure 3. Frequency of occupation of available patches with different spore production in *Orthodontium lineare*. P_{est} = Probability of establishment when a diaspore has reached a new locality.

models used to predict changes in distribution of the climate changes have so far assumed that species will move to new areas where a suitable environment occurs. This is probably true for most species, especially if the changes are slow and the ability to disperse to, and establish on new localities is good. If we know the dispersal ability (i.e. diaspore production, diaspore survival, diaspore transport ability, establishment rate), together with the changes in spatial arrangement of localities and the time-scale on which this occurs we are able to model the probability that a species will be able to find new suitable localities using a model of the type employed by Herben *et al.* (1991).

The process of colonizing newly created localities by an established species is basically the same as the process with which an introduced species is spreading among the 'newly available' localities. Studies of *Orthodontium lineare* (Hedenäs *et al.*, 1989) have shown that the density of available localities is important for the spread, i.e. the number of localities and the distance between them. Therefore, we should expect that the spatial rearrangement of localities under new climatic conditions is important, which is exactly what the Herben *et al.* model predicts.

21.4.4 Survival within habitat patches

The foregoing examples deal mainly with the dynamics between patches and their effect on regional survival. Models have also been used to investigate the dynamics within patches and local survival. One example is During's (1997) study of the survival of species in frequently disturbed habitats in relation to the diaspore bank.

Many bryophyte species are adapted to grow in habitat patches that are regularly disturbed. Disturbance allows early succession stages to reoccur. Species adapted to early stages (shuttle species) often survive unfavourable situations by producing diaspores that will develop new gametophores when the situation becomes suitable again (see During, 1997, for a review on the bryophyte diaspore bank).

The composition of the diaspore bank is determined by the interplay between many processes (During, 1997). Inputs are the result of production, dispersal and incorporation of the diaspore in the diaspore bank. Outputs are germination and mortality. During's model includes values on the chance of gap formation, recruitment chance, diaspore production, fraction of produced diaspores incorporated in the diaspore bank, export of diaspores and mortality (both density dependent and density-independent) of diaspores.

With this model it is possible to simulate the chances of survival in a specific habitat patch as a function of available patches and varying the population parameters for each species and the survival ability of the diaspores (the last factor is still almost completely unknown, cf. During, 1997). It is also possible to predict the effect on different species of an alternate land-use or disturbance regime by altering the parameters determining gap formation and mortality of diaspores. This simulation model is a more important tool than the spatial simulation model of Herben *et al.* (1991) when estimating the effects of environmental changes on species where within-patch dynamics are more important than between-patch dynamics, as in shuttle species.

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22. Effects of ozone and atmospheric nitrogen deposition on bryophytes

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22.1 SUMMARY

The changing nature of the pollution climate in the second half of the 20th Century has resulted in the increased importance of ozone and nitrogen pollutants and a decreased importance of sulphur dioxide. However, there have been few studies of the effects of any dry deposited gas on individual bryophyte species or on bryophyte-rich plant communities. In those studies which have been made, there is evidence that well hydrated bryophytes may not be particularly sensitive to ozone or NO_x at realistic atmospheric concentrations. However, species differ in their sensitivity to both acute and chronic ozone concentrations.

Ombrotrophic bryophytes are largely dependent on wet deposition as a source of nitrogen. In polluted districts several species have been shown to have markedly raised tissue nitrogen concentrations and little or no induction of nitrate reductase activity by nitrate. Current atmospheric nitrogen deposition is thus in excess of demand by bryophyte growth in these regions. The ecological significance of this in terms of bryophyte growth, decomposition and competitive ability is largely unknown, but experiments suggest that increased nitrogen deposition can markedly decrease the growth of at least some species.

Future controlled environment investigations might be employed to study interactions between O₃ and NO_x on bryophyte growth. There is also the need to assess interactions among O₃, drought, and frost episodes. Much more emphasis should be placed on *in situ* studies of bryophyte communities, preferably utilizing free air enrichment technology, and on the use of stable isotopes to investigate the fate of atmospheric nitrogen deposition on moss carpets.

KEYWORDS: Bryophytes, pollution climates, ozone, nitrogen deposition, ecological responses.

22.2 INTRODUCTION

The potential sensitivity of cryptogams to atmospheric pollution was first recognized during the 19th Century when their disappearance from the immediate vicinities of industrial towns and cities was first correlated with the presence of coal smoke (e.g.

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Grindon, 1859). The burning of coal, leading to the release of SO₂ and particulates, resulted in cryptogam deserts in and around these urban centres. Where these centres abutted semi-natural ecosystems, then large scale modification of the cryptogamic flora occurred. A good example of this is the virtual disappearance of *Sphagnum* from ca 50,000 hectares of the southern Pennine blanket peats during the 19th and early 20th Centuries (Tallis, 1964). Experimental evidence to support the view that these changes were the result of the major phytotoxic pollutant in coal smoke (SO₂) came much later (e.g. Ferguson, Lee & Bell, 1978) when in many parts of Western Europe at least this form of pollution was in decline. The reason for such delay was the difficulty in devising appropriate experimental techniques to mimic the 'natural' deposition of individual pollutants.

Pollutants reach vegetation in one of three ways: as dry deposition of gases and particulates; as wet deposition in precipitation; and as occult deposition in wind driven fog and cloud. The occult form is particularly important in upland regions where it can represent ca 25% of total pollutant input (see UKRGAR, 1990). Techniques for the study of dry deposited gases have been improved considerably during the last five decades. Early studies involved passing polluted air over potted plants in modified glasshouses (e.g. Bleasdale, 1952). Later, controlled concentrations of individual gases were employed in closed chambers out of doors. These chambers were largely superseded by open-top chambers because the latter more closely mimic the thermal and hydrological regimes of natural environments. A further advantage of open-top systems is that they can be superimposed on semi-natural vegetation, and thus plant responses to individual pollutant gases can be studied *in situ*. However, all chamber systems modify the environment to some extent, and none is entirely suitable for the study of bryophytes because all involve the forced movement of air through vegetation with increased risk of desiccation. Free air release systems were designed to study crop responses to SO₂ and other gases (McLeod, 1988) without the confounding effects of chambers. One such system was used to study mixtures of SO₂ and O₃ on cryptogams as an incidental part of a fumigation study of forest crops (Bates, McNee & McLeod, 1996). Free air release systems are unusually expensive to build and run, and are now largely used to study the effects of raised concentrations of CO₂. Concomitant with the changes in out-of-doors exposure systems, controlled environment chambers have been developed (e.g. Caporn & Wood, 1990) in which the effects of known concentrations of gases can be studied under defined light, temperature and humidity conditions. The usefulness of these chambers for the study of bryophytes is limited by the need to minimize boundary layer resistance to deposition by maintaining air flow around the plants, and to operate at generally low relative humidities when studying the effects of freely soluble gases (e.g. SO₂). The net effect of the expense of producing facilities and the difficulty of adapting them to study bryophytes, and the perceived unimportance of bryophytes (compared with crops) means that few experimental studies of the effects of dry deposited gases have been undertaken on these plants when compared to arable and forest crops.

Studies of wet deposition are equally problematic, but are in general cheaper than those involving gaseous pollutants. In laboratory studies there is the difficulty of mimicking the duration and nature (droplet size and intensity) of natural deposition events. In field studies these problems also occur, and have led to the development

of elaborate roof systems in which precipitation collected above vegetation is sprayed back, either modified following deionization or unmodified, onto the vegetation (e.g. Wright, Lotse & Semb, 1988). More usually field studies have involved supplementation of natural deposition by periodic (typically between monthly and weekly) spraying of pollutants on to vegetation over short periods (typically < 5 minutes). Such studies alter, at least to some extent, the hydrological regime, and also involve large near-instantaneous increases in pollutants which may not mimic natural deposition at all well.

22.3 THE CHANGING POLLUTION CLIMATE

During the second half of the 20th Century SO₂ has declined in importance as an atmospheric pollutant in at least Western Europe and parts of North America. Changes in fuel usage, pollutant scrubbing and combustion technology together with the growth in the use of motor vehicles have resulted in decreases in SO₂ and increases in O₃ and NO_x pollution. The intensification of agriculture in some regions has also resulted in large increases in NH_x pollution. The latter is largely a rural source of pollution, and the formation in sunlight of ozone in air masses from its precursors (NO_x and low molecular weight hydrocarbons) means that high concentrations of nitrogenous and O₃ pollution can occur remote from urban areas. Indeed, there is evidence to suggest that O₃ pollution may potentially be more important in some rural regions than in the vicinity of urban areas. Typically in the latter, O₃ is produced during the day, reaches peak concentration in the late afternoon, and declines during the evening and night. In upland regions, however, O₃ may persist during the night because conditions for its decay are absent (e.g. high NO concentrations). Thus O₃ episodes may occur over at least several diurnal cycles (e.g. UK PORG, 1993) during which bryophytes, because of their continuous exposure to the pollutant, may be particularly at risk.

Although O₃ is an important phytotoxic pollutant, it is naturally present in unpolluted atmospheres at appreciable concentrations (20–50 ppb). In this it differs from nitrogenous pollutants which are generally present at very low gaseous concentrations (except e.g. NH₃ close to large seabird colonies). This natural background concentration of O₃ is usually accounted for in pollution studies by the use of the AOT40 measure: the accumulated exposure above 40 ppb for daytime hours during the months of April–September. This is the concentration above which ozone is potentially phytotoxic (e.g. PORG, 1993; UN-ECE, 1994). The use of 40 ppb as the cut-off concentration has been shown to provide a good linear relationship between ozone exposure and plant response for a number of higher plant species (PORG, 1993; UN-ECE, 1994). Critical levels have been set for crops and forest species, but no separate guidelines have been established for bryophyte-rich semi-natural vegetation.

The solution of acidic gases in precipitation results in acidic wet deposition. The decline in SO₂ and increases in NO_x emissions have resulted in changes in the contribution of these gases to the overall acidity of precipitation, but not necessarily in any marked change in acidity. A further complication is that NH₃ dissolved in rainwater provides a major neutralization mechanism of dissolved acidity. Thus there

may have been appreciable changes in the nitrogen and sulphur composition of precipitation in recent decades without marked changes in acidity. However, these changes in chemical composition may be particularly important for bryophyte communities. In the relatively unpolluted environment of sub-Arctic Sweden Woodin & Lee (1987) demonstrated that *Sphagnum fuscum* carpets represented a near perfect sink for NO_3^- and NH_4^+ . This is a reflection of the importance of the atmospheric supply as the major source of nitrogen for ombrotrophic bryophytes. Changes in this supply may have far reaching effects on the growth and physiology of the bryophytes, and potentially on ecosystem function (Woodin & Lee, 1987).

22.4 RESPONSES OF BRYOPHYTES TO OZONE AND NITROGEN

22.4.1 Dry deposition

The sensitivity of bryophytes to pollutant gases may be assessed at high (acute) concentrations over short time periods (hours or days) or at lower (chronic) concentrations, more typical of polluted environments (weeks or months). The former assessment method may not relate well to the latter, but has been widely used in screening studies and in demonstrating potential differential sensitivities among species.

There have been relatively few studies of the effects of ozone on bryophytes. In an acute fumigation exposure only four out of 22 species (Table 1) studied showed any adverse physiological response to 150 ppb O_3 under controlled conditions (6 hours at 5°C and $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR for 7 days) (Foot, 1996). Comparison with one higher plant study suggests that many bryophytes may not be particularly sensitive to O_3 since Farage *et al.* (1991) reported that the rate of light saturated CO_2 assimilation in wheat was not affected after 16 hours exposure to 200 ppb O_3 . Potter *et al.* (1996) showed that there were differences in the response of *Sphagnum* species to acute O_3 concentrations including effects on both photosynthesis and membrane leakage. The most sensitive of the four species tested was *S. recurvum*, which is the most tolerant of SO_2 and its solution products (Ferguson *et al.*, 1978), raising the prospect that despite the apparent lack of sensitivity of many species to acute episodes, O_3 may further modify bryophyte communities recovering from SO_2 damage. Differential effects of chronic exposure of *Sphagnum* species to O_3 exposure have also been reported (Gagnon & Karnosky, 1992). Besides effects on photosynthesis of *Sphagnum* species, these workers also demonstrated reduction in growth at higher concentrations of O_3 in open top chambers. However, in none of these studies did bryophytes die after exposure to O_3 , and rapid recovery of physiological activity was often observed when fumigation ceased (Potter *et al.*, 1996).

There have been very few studies of the effects of NO and NO_2 on bryophytes. In a series of investigations, Morgan, Lee & Ashenden (1992) showed that three weeks' exposure of four bryophyte species (*Ctenidium molluscum* (Hedw.) Mitt., *Homalothecium sericeum* (Hedw.) Br. Eur., *Hylocomium splendens* (Hedw.) Br. Eur. and *Pleurozium schreberi* (Brid.) Mitt.) to chronic concentrations of NO and NO_2 (35 ppb) did not markedly disrupt the metabolism of the mosses. However, NO_2 induced nitrate reductase activity 24 hours after the start of the fumigation period whereas NO rapidly inhibited the activity of the enzyme. After 21 days' exposure to

Table 1. Results of screening programme where bryophyte species collected from areas in the UK, remote from major sources of pollution, were exposed to acute ozone concentrations of 150 ppb for 6 hours at 5°C and a PAR flux of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Species	Sensitivity to acute ozone
<i>Breutelia chrysocoma</i> (Hedw.) Lindb.	ns
<i>Hylocomium splendens</i> (Hedw.) Br. Eur.	ns
<i>Hypnum jutlandicum</i> Holmen & Warneke	ns
<i>Isothecium myosuroides</i> Brid.	ns
<i>Leucobryum glaucum</i> (Hedw.) Ångstr.	ns
<i>Mnium hornum</i> Hedw.	ns
<i>Plagiomnium undulatum</i> (Hedw.) Kop.	ns
<i>Plagiothecium denticulatum</i> (Hedw.) Br. Eur.	ns
<i>Plagiothecium nemorale</i> (Mitt.) Jaeg.	ns
<i>Pleurozium schreberi</i> (Brid.) Mitt.	+ (2 days)
<i>Polytrichum commune</i> Hedw.	+ (4 days)
<i>Polytrichum formosum</i> Hedw.	ns
<i>Polytrichum juniperum</i> Hedw.	ns
<i>Racomitrium lanuginosum</i> (Hedw.) Brid.	+ (2 days)
<i>Rhytidadelphus loreus</i> (Hedw.) Warnst.	ns
<i>Rhytidadelphus squarrosus</i> (Hedw.) Warnst.	ns
<i>Sphagnum capillifolium</i> (Ehrh.) Hedw.	ns
<i>Sphagnum cuspidatum</i> Hoffm.	ns
<i>Sphagnum papillosum</i> Lindb.	ns
<i>Sphagnum recurvum</i> P. Beauv.	+ (1 day)
<i>Sphagnum squarrosum</i> Crome	ns
<i>Thuidium tamariscinum</i> (Hedw.) Br. Eur.	ns

ns indicates no measured effects following ozone exposure after 7 successive exposures.

+ denotes a measured effect (reduction of photosynthesis and/or an increase in membrane leakage or both) following acute ozone exposure.

NO the inducibility of nitrate reductase by 10 mM NO_3^- was lost, but there was little effect on photosynthesis. However, after a further 21 days in clean air with no additional nitrogen supply the NO_3^- inducibility of nitrate reductase was restored. These experiments support the view that dry deposition of NO and NO_2 in rural regions is unlikely to markedly influence bryophytes. Annual mean concentrations of NO and NO_2 recorded throughout the United Kingdom over the period 1972 to 1987 were 9 and 15 ppb (UK PORG, 1990).

Ammonia is potentially a very phytotoxic pollutant. However, it is typically present in the atmosphere at concentrations of <10 ppb. At this concentration it is difficult to maintain steady concentrations in fumigation systems because of the high solubility of the gas. There is currently no evidence to suggest that NH_3 causes acute toxicity at concentrations as low as 10 ppb, but even at these concentrations, dry deposition of ammonia may make a major contribution to total atmospheric nitrogen deposition, e.g. on wet acidic mires dominated by *Sphagnum* species.

22.4.2 Wet deposition

The responses of bryophytes to wet atmospheric nitrogen deposition can be examined most readily through studies of nitrate assimilation in near pristine environments.

Woodin, Press & Lee (1985) showed in a short-term spray experiment that in an ombrotrophic mire in northern Sweden the activity of nitrate reductase in *Sphagnum fuscum* was proportional to nitrate supply. Further, these workers demonstrated that the enzyme was induced by nitrate in each of several natural wet deposition events. In polluted environments, this close coupling of nitrate reductase activity and wet deposition in *Sphagnum* species is absent (Lee *et al.*, 1987), and is rapidly lost when *Sphagnum* plants are transplanted from regions of low to high atmospheric nitrogen deposition.

Spray experiments with other bryophyte species under controlled conditions generally show a rapid (within 2 hours) induction of nitrate reductase activity in response to nitrate supply. Under field conditions, the peak nitrate reductase activity in shoot tips may occur as much as 1 to 2 days after precipitation has ceased, probably as a result of nitrate transport within moss cushions as desiccation ensues. *Racomitrium lanuginosum* can show this response (Fig. 1). In a study of the long term response of this moss to atmospheric deposition, Baddeley (1991) showed that nitrate reductase activity responded to episodes of nitrate deposition at a site in northern Sweden. However, pre-treating the moss with nitrogen sprays daily for several days, prior to studying responses to natural deposition alone, resulted in reduction of enzyme activity, and the loss of the moss's ability to respond to the natural atmospheric nitrate supply (Fig. 1). This loss of inducibility (which is reversible) presumably results from end product inhibition of enzyme synthesis or activity as a

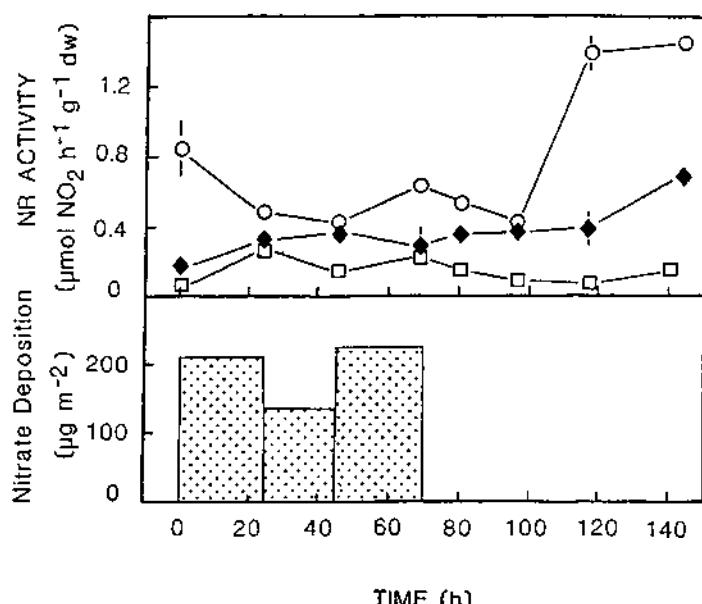


Figure 1. Changes in nitrate deposition ($\mu\text{g m}^{-2}$) and nitrate reductase activity on a dry mass basis ($\mu\text{mol g}^{-1} \text{h}^{-1}$) of *Racomitrium lanuginosum* stem tips (1 cm) in late June 1989 near Abisko, North Sweden. Moss was subjected to natural deposition only (○) or pre-treated with short (>5 min) daily sprays (4.3 mm precipitation equivalent) containing either 1 mM NaNO_3 (◆) or 0.5 mM $\text{NaNO}_3 + 0.5 \text{ mM NH}_4\text{Cl}$ (□) for 10 days. Vertical bars are $\pm 1 \text{ SE}$, $n=4$.

mechanism to limit nitrogen assimilation at times of excess supply. Loss of nitrate reductase inducibility has been shown for several other bryophytes, e.g. *Rhytidadelphus squarrosus* (Morecroft, Sellars & Lee, 1994), and is at present the most readily determined biochemical 'marker' of enhanced atmosphere nitrogen supply.

Another marker of enhanced atmospheric nitrogen supply is the total tissue nitrogen concentration. Several workers have correlated current and past atmospheric deposition with the total tissue nitrogen concentration of mosses (Baddeley, Thompson & Lee, 1994, Pitcairn, Fowler & Grace, 1995, Woolgrove & Woodin, 1996). If nitrogen supply and uptake increases and does not stimulate growth, then the tissue content will increase. Since many bryophytes are adapted to utilize the normally low atmospheric nitrogen supply for growth, it is not unreasonable to expect that they will have evolved very efficient and effective mechanisms for nitrogen acquisition. This expectation has been demonstrated at least in the case of *Sphagnum* species. For example, Woodin & Lee (1987) showed in a lysimeter study that *Sphagnum fuscum* provided an almost perfect sink for the atmospheric nitrogen supply. These workers also demonstrated, using small artificial bog pools, that *Sphagnum cuspidatum* from low nitrogen deposition environments efficiently assimilated the atmospheric supply. Ombrotrophic bryophytes in near pristine sub-arctic and arctic environments typically have very low tissue nitrogen concentrations (*ca* 2–5 mg g⁻¹ dry wt). Herbarium studies suggest that this was formerly the case at much lower latitudes. Thus Baddeley *et al.* (1994) showed that *Racomitrium lanuginosum* in Britain had much lower tissue nitrogen concentrations during the 19th than in the late 20th Centuries which presumably reflects the marked increase in atmospheric nitrogen deposition. The response of total tissue nitrogen concentration to the atmospheric supply can also be readily demonstrated in transplantation studies from low to high nitrogen deposition environments (e.g. Baddeley *et al.* 1994).

The effects of changes in the atmospheric nitrogen supply on growth can also be demonstrated. Work by Aerts, Wallén & Malmer (1992) suggests that ombrotrophic Sphagna in sub-arctic Europe are still limited in growth by the atmospheric nitrogen supply whereas at lower latitudes and higher nitrogen deposition this limitation has been lost. Studies involving the experimental raising of atmospheric nitrogen supply other than in arctic regions have generally shown either no response or an inhibition of growth (e.g. Aerts *et al.*, 1992). Fig. 2 shows an example of one such experiment on acidic grassland in Derbyshire, England. Details of this experiment are described in Morecroft *et al.* (1994) which involves spraying the vegetation monthly with a range of ammonium nitrate concentrations and a single ammonium sulphate treatment. After more than five years of experimentation, there was a marked reduction in the shoot density of *Rhytidadelphus squarrosus* (and also that of *Pleurozium schreberi*, data not shown) in the higher nitrogen deposition treatments. Experiments of this kind must always be artificial (see above), but the question that they raise is this: if the present nitrogen supply is not limiting moss growth, are increases in atmospheric nitrogen likely to be detrimental for bryophyte communities in the long term?

22.5 FUTURE DIRECTIONS

The major questions remain, first, to what extent have bryophyte communities been adversely affected by nitrogen pollutants and ozone so far, and, secondly, how will

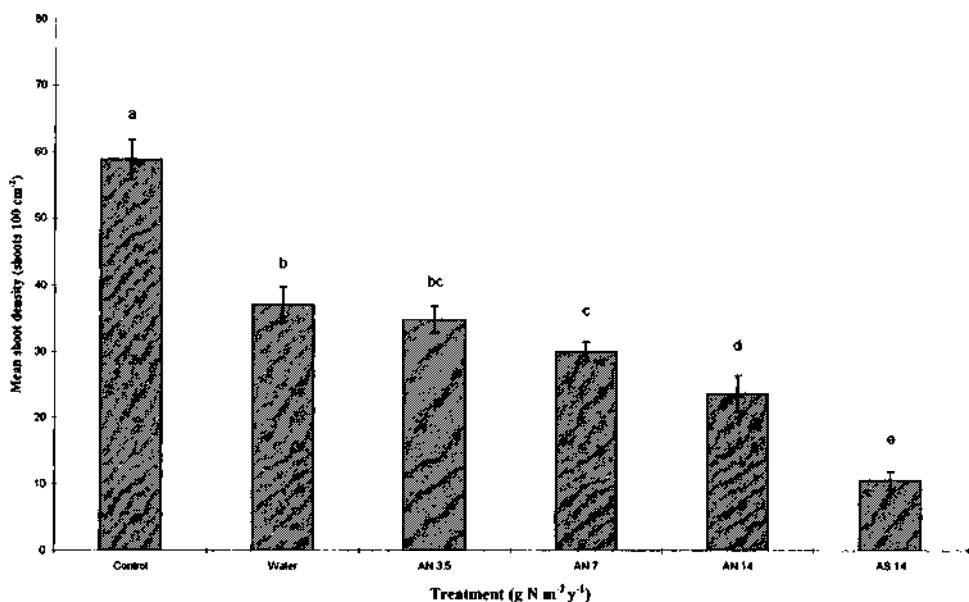


Figure 2. Mean *Rhytidadelphus squarrosus* shoot density (shoots 100 cm^{-2}) in plots receiving sprays of distilled water, N (as ammonium nitrate) ($3.5, 7$ and $14\text{ g m}^{-2}\text{ y}^{-1}$) and N (as ammonium sulphate) ($14\text{ g m}^{-2}\text{ y}^{-1}$) at Wardlow, Derbyshire. Shoot densities were determined in January 1996 following $5\frac{1}{2}$ years of approximately monthly spray treatments. For details of experimental design see Morecroft, Sellars & Lee (1994). Columns not bearing the same letter are significantly different (two way ANOVA, LSD $p < 0.05$).

they be influenced in the future. Although the first question can be answered to some extent, the answer is very much constrained by the small number of studies. Only very few species have been subject to experimentation, and generalizations are difficult. The answer to the second question depends to a large extent on trends in pollutant concentrations and deposition, but it also depends on changes already induced by current pollution loads but which are not yet manifest.

A good example of the potential latent problem is the increase in total tissue nitrogen concentration in ombrotrophic bryophytes. There is ample evidence to suggest that this is an inevitable and widespread response to enhanced atmospheric nitrogen deposition. Even if there are no effects of the deposition on bryophyte growth, are there long term ecological effects? Are there small but significant effects on processes such as herbivory and decomposition? The prospect of higher tissue nitrogen concentrations resulting in more rapid decomposition has been proposed previously (e.g. Lee, Parsons & Baxter, 1993), but there are at present too few data to assess its importance. Certainly slow decomposition and the existence of a large proportion of standing dead play a major part in the success of bryophytes in ombrotrophic mires and *Racomitrium* heaths, and anything which reduces the amount of standing dead plant matter is likely to increase the competitive ability of vascular plants in these ecosystems. It is important to establish whether there are effects of increased tissue nitrogen concentration on decomposition, because even

with immediate pollution abatement, there may be long-lasting ecological changes already in train.

A promising approach which may allow us to identify some of the important ecological effects of enhanced atmospheric nitrogen deposition is through stable isotope technology. Recent work by J. Pearson & A. Soares (personal communication) has used $\delta^{15}\text{N}$ in an attempt to distinguish between NO_x and NH_x deposition to mosses on the assumption that $\delta^{15}\text{N}$ in $\text{NH}_4\text{-N}$ is more negative than that in $\text{NO}_3\text{-N}$ ($ca -8\text{\textperthousand}$ as against $ca 0$). Mosses growing near urban roadsides where NO_x concentrations are generally high did have much more positive $\delta^{15}\text{N}$ values than in rural areas where NH_x deposition is greater. However, we need to know much more about the constraints on the interpretation of $\delta^{15}\text{N}$ signatures in moss cushions and how they may be influenced by decomposition processes before the promise of this technique can be assessed.

The most important bryophyte communities generally occur in regions where gaseous nitrogen pollutants are in low concentrations. The presence of only low basal activities of nitrate reductase during dry periods, and the rapid induction of the enzyme during or immediately following precipitation points to the fact that dry deposition of NO_x is unimportant in many bryophyte communities. The same may not be true for NH_3 deposition, even at concentrations of a few ppb. Assessment of the importance of NH_3 deposition to bryophytes at low concentrations, and whether it differs between calcicole and calcifuge communities, urgently requires assessment.

There are all too few fumigation studies of bryophytes with both ozone and nitrogenous pollutants singly, and even fewer that have studied interactions between O_3 and NO_x . Ozone, because it can be present at appreciable concentrations in regions with bryophyte-rich plant communities, demands much further study. Although the evidence so far suggests that bryophytes may not be particularly susceptible to ozone, we need further *in situ* experiments. Ideally such experimentation would involve free air systems thus minimizing 'chamber' effects. However, such investigations may have to await exhaustion of the current enthusiasm for elevated CO_2 experimentation. Appropriately designed *in situ* experiments would provide the best opportunity to assess the importance of differential species sensitivities, e.g. in *Sphagnum* lawns.

The fact that ozone does affect membrane leakage in susceptible bryophytes suggests that another profitable line of enquiry is the effect of ozone on drought and frost sensitivity. Many bryophytes undergo repeated wetting and drying cycles, and their ability to withstand repeated dehydration and rehydration might be affected by ozone. Similarly, despite the fact that bryophytes show no physiological activity when dry, ozone susceptibility may be enhanced at low water contents because of a much reduced diffusive resistance.

There is great scope for experiments involving the interaction between ozone and other environmental variables, not least other phytotoxic gases. The pity is that when such experiments have been attempted in the past, it has been usually because bryophytes have happened 'accidentally' to be present in the system or space for bryophytes can be found in fumigation chambers designed for work with crop species. It would be nice to think that more effort would be made to undertake experiments designed specifically for bryophytes. Despite their generally small size and difficulties in providing appropriate environmental conditions, bryophytes are important com-

ponents of ecosystems and are amenable to physiological, biochemical and molecular studies.

22.6 ACKNOWLEDGEMENTS

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23. Effects of increased carbon dioxide and nitrogen supply on mosses

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23.1 SUMMARY

In photosynthesis of mosses, available CO₂ interacts with other abiotic factors such as water content, radiation flux and temperature. In *Sphagnum*, the optimum rate of net photosynthesis in raised CO₂ shifts gradually to higher water contents with increasing CO₂ and the typical decrease in the response at higher water contents, found at lower CO₂ concentrations, disappears. The rate of net photosynthesis increases in raised CO₂ with increasing temperature and radiation flux so that the temperature/light optimum is shifted to a higher value. Raised CO₂ can compensate for the effects of low radiation in photosynthesis. Increase in CO₂ concentration can cause temporary decrease in the light compensation point and can delay dormancy at the end of the growing season. Prolonged exposure to raised CO₂ concentrations causes CO₂ acclimation. Production estimates on a dry mass basis in increased CO₂ are the result of either or both positive and negative responses to prevailing CO₂ concentration on moss densities, dry masses and length increments.

The effects of nitrogen deposition on ombrotrophic *Sphagnum* have been well studied and the responses of mosses in unpolluted sites often differ from those in N-polluted sites. *Sphagnum* can immediately use even occasionally available high NO₃⁻ pulses, although the capability is usually lost on prolonged exposure. Accumulation of NH₄⁺-N in tissues lowers the ability to reduce nitrate. Both increased NH₄⁺- and NO₃⁻-nitrogen deposition increase tissue N-concentration. *Sphagnum* exposed to increased N deposition may exhibit a change in chlorophyll concentration and the concentrations of amino acids such as arginine, asparagine and glutamic acid increase. Photosynthesis and growth in mosses exposed to increased N deposition are species-dependent. However, duration of N treatment, deposition rate and form of the supplied nitrogen also affect species' responses. Both positive and negative feedback may be demonstrated in changes in plant densities, dry mass, length increment or species vitality under increased N input. According to the few published results, simultaneously increased N deposition and CO₂ have only small effects on bryophytes.

KEYWORDS: Bryophytes, global change, pollution, photosynthesis, physiology, production, *Sphagnum*.

23.2 INTRODUCTION

During recent decades, there has been considerable interest in the influence of human activities on ecosystems. The concentration of CO₂ in the atmosphere has increased and is expected to continue increasing until the next century. Increasing the concentration of CO₂ in the air has been shown to increase the rate of photosynthesis especially in C₃ plants. Bryophytes often grow close to the ground, forming a tight cover over it. If the substratum of mosses is formed from decaying litter, CO₂ is released by below-ground respiratory processes and therefore mosses may be exposed to CO₂ concentrations above those in bulk air a great deal of the time, regardless of the general increase in the atmospheric concentration (Silvola, 1985; Sonesson, Gherke & Tjus, 1992; Tarnawski *et al.*, 1992; Hogg, 1993). This could mean that the increase in atmospheric CO₂ concentration would have a lesser effect on bryophytes than on vascular plants.

In the northern hemisphere, the amount of nitrogen deposition has increased markedly due to industrialization. Nitrogen is a limiting nutrient in many ecosystems, but it can also be regarded as a pollutant when the supply exceeds the plants' requirements for a prolonged time. The first effects on mosses grown at supra-optimal nitrogen deposition were detected in ombrotrophic *Sphagnum* which have adapted to grow under low N-input rates (Press & Lee, 1982; Woodin, Press & Lee, 1985; Press, Woodin & Lee, 1986; Lee *et al.*, 1987; Lee, Baxter & Emes, 1990). *Sphagnum* is the main component in peat deposits in most boreal mire ecosystems and is an important CO₂ sink via peat formation. This is why the genus has been studied quite intensively while other bryophytes have received less attention.

In this review, we deal first with the effects of increased carbon dioxide on bryophytes. Then we deal with the effects of increased N deposition on mosses and the combined effects of CO₂ and N. We also have some views on what should be studied in the future concerning the reaction of bryophytes to increased CO₂ and N concentrations.

23.3 CARBON DIOXIDE AND PHOTOSYNTHESIS

The presence of a moss species in a particular growth site is a result of the capability of the species to adapt to the environment according to limitations of the genotype. Atmospheric CO₂ concentration interacts in moss metabolism with the water content of the plant, light flux, and temperature. Each of these factors has a physiological optimum in photosynthesis, but the physiologically optimal combination of these factors for the plant and the realized combination in natural growth sites differ.

23.3.1 Effects of water content in increased CO₂

Bryophytes need water for metabolism, but the supply may be more limited or intermittent compared to that of higher plants. Moss species may be restricted to places with a continuous water supply for active metabolism or else species are somewhat desiccation-tolerant. Bryophytes may also have internal or external water

conducting systems, which effect water movement to the active tissues (Proctor, 1978; Richardson, 1981; Rydin & Clymo, 1989).

Mosses growing submerged or in very moist environments are in a special situation concerning the effect of CO₂ on photosynthesis. The diffusion rates of gases in water are lower than in air by a factor of about 10⁴ depending on water temperature and partial pressure of the gas. The photosynthetic structures of bryophytes are functionally comparable with those of vascular plants and the uptake of CO₂ in water-saturated environments is limited by increasing diffusion resistance (Proctor, Raven & Rice, 1992). In these environments diffusion resistance is an important factor because bryophytes appear to utilize CO₂ taken up mainly from outside the plant and only *Sphagnum cuspidatum* has been shown to use some internally recycled carbon in photosynthesis (Proctor *et al.*, 1992). Rydin & Clymo (1989) showed some transport of CO₂ from lower to upper plant parts, probably in air around the plants.

For maximal photosynthesis in bryophytes, the water content in photosynthetically active tissues has to be at an optimal level in relation to available light. Above the optimal water content, in constant light, temperature and carbon dioxide concentration, the rate of net photosynthesis declines. The decline is due to the increasing obstruction provided by adherent water to the carbon dioxide transfer (Stålfeld, 1937; Grace & Marks, 1978; Proctor, 1978; Lange & Matthes, 1981; Alpert & Oechel, 1985). Below the optimal water content, *i.e.* in drying moss, the decline in net photosynthesis is usually steeper compared to that in plants in very wet environments (Tallis, 1959; Willis, 1964; Lee & Steward, 1971; Busby & Whitfield, 1978; Tobiensen, Slack & Mott, 1979; Alpert & Oechel, 1985; Silvola, 1991). Often there is a minor increase in CO₂ exchange in very dry but still living moss, possibly due to an efflux of CO₂ (Titus, Wagner & Stephens, 1983) or structural changes like the physical effect of inrolling leaf margins in *Polytrichum alpinum* (Oechel & Collins, 1976).

The rate of photosynthesis is determined by the water content of the moss, radiation flux and temperature. An increasing CO₂ concentration forms a new dimension in this response surface. Vascular plants have stomatal control over water use efficiency, but bryophyte gametophytes lack such control (Nobel, 1991) and are therefore more controlled by abiotic factors. In raised CO₂ concentrations, the maximal rate of photosynthesis in *S. fuscum* is shifted towards higher water contents and finally, at high CO₂ concentrations, the typical decrease in the response, found at lower CO₂ concentrations, disappears (Silvola, 1985; Jauhainen & Silvola, 1996; Fig. 1). At the same time, the rate of net photosynthesis is increased in raised CO₂, although the effect decreases with decreasing water content. The loss of saturation of the rate of net photosynthesis in highly raised CO₂ can be explained by the increased partial-pressure of CO₂ in water saturated photosynthetically active tissues, while at lower concentrations it is restricted by slow diffusion rates. In terrestrial bryophytes diffusion resistance has been considered to have but a minor effect on photosynthesis (Proctor, 1978; Sonesson, Gherke & Tjus, 1992).

Increased CO₂ concentrations may also be beneficial under the normally prevailing water contents of mosses. In a characteristic growth site, the capitulum of *S. fuscum* contains 6–10 times its dry mass as water: the mass proportion of water is 600–1000% (Hayward & Clymo, 1982; Luken, 1985; Rydin, 1985; Wallén, Falkengren-Grerup & Malmer, 1988). According to Silvola (1990), within the moisture regime of 600–800%, photosynthesis in *S. fuscum* could benefit from increased CO₂ concen-

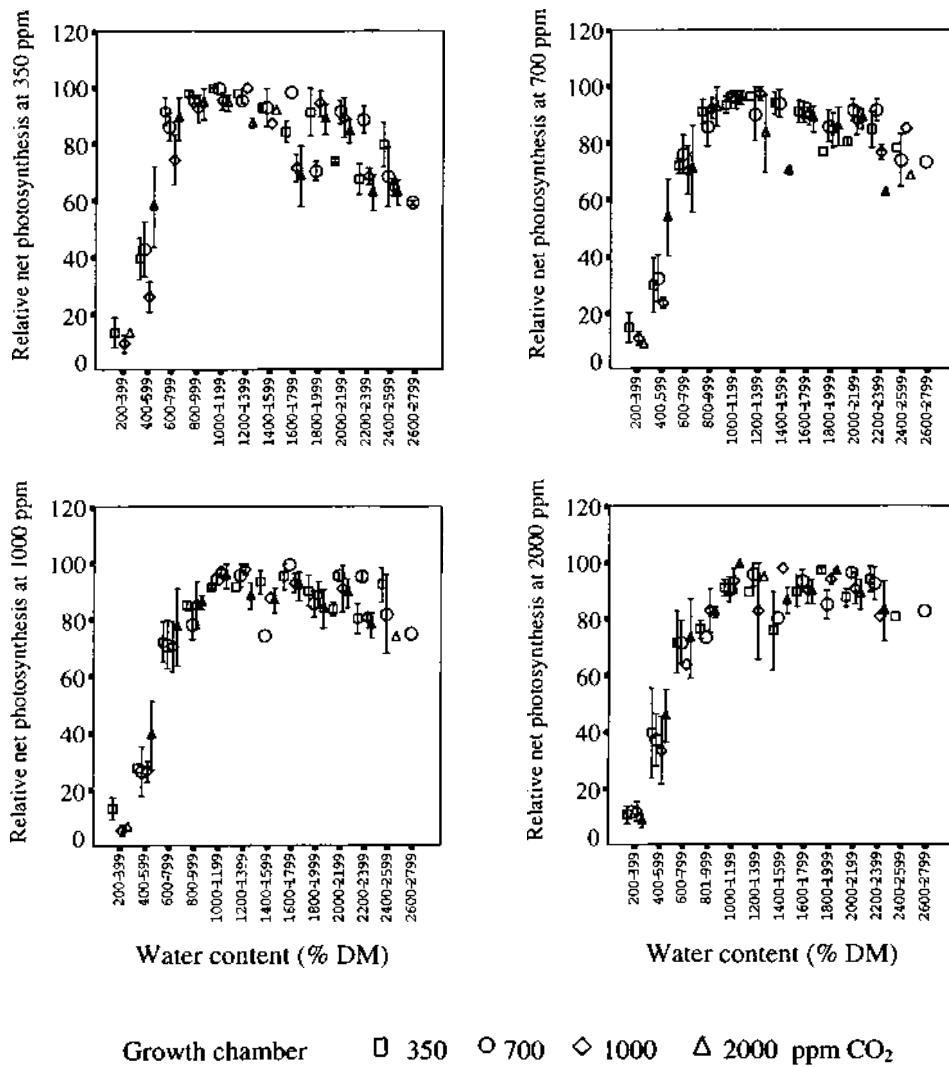


Figure 1. Relative rate of net photosynthesis (% of maximum) of *Sphagnum fuscum* at the short-term CO₂ concentrations indicated and various water contents (% DM). Samples were first grown (long-term) at 350, 700, 1000 or 2000 ppm CO₂ (indicated by different symbols). Temperature 22°C and radiation flux 340 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Mean \pm SE presented (redrawn from Jauhainen & Silvola, 1996).

trations up to 1500 ppm. The water content of mosses growing in moist environments is often too high to permit optimal rate of net CO₂ fixation in naturally available CO₂ concentrations (Silvola, 1990; Sonesson *et al.*, 1992).

23.4.2 Effects of light and temperature in increased CO₂

Photosynthesis is divided into two subprocesses: light reactions and dark reactions. Light reactions form adenosine triphosphate (ATP) and are dependent on the light

flux. Carbon dioxide fixation and the formation of end products in enzyme-controlled reactions take place in dark reactions which are dependent on temperature. Due to the interaction of these processes, an optimum light and temperature combination can be determined. At low temperatures, the dark reactions are slow and restrict the processes and therefore light saturation is reached at a lower radiation flux (Proctor, 1978 and references therein). At higher temperatures, maximal net photosynthesis takes place at a lower water content, partly due to lower CO₂ solubility in water (Lange, 1980).

At increased CO₂ concentration, increasing temperature results in an increase in the rate of net photosynthesis of *Sphagnum fuscum*, *S. angustifolium*, *Dicranum majus* and *Pleurozium schreberi*, causing a change in the temperature dependence curve so that the temperature optimum is shifted to a higher level (Silvola, 1985). This kind of positive interaction between temperature and CO₂ uptake is also detected in field measurements in a mixture of mosses and vascular plants (Grulke *et al.*, 1990). Increase of the CO₂ concentration seems to have only slight or no effect on the dark respiration rate in *S. fuscum*, *S. angustifolium*, *D. majus* and *P. schreberi* (Silvola, 1985).

In low radiant fluxes (PAR) net CO₂ exchange rate remains low, but it is found to increase on increasing CO₂ concentration. In increased CO₂ concentration and low radiant flux conditions the rate of photosynthesis can be higher than in considerably higher radiant fluxes in the ambient CO₂ concentration. Such an effect has been observed, for example, in *Dicranum majus* (Silvola, 1985) and *Hylocomium splendens* (Sonesson *et al.*, 1992). In high radiant fluxes, there is a clear, positive correlation with CO₂ concentration and net CO₂ exchange. For example, at a radiant flux of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, the net CO₂ exchange of *D. majus* was increased twofold by doubling the CO₂ concentration (Silvola, 1985).

Potentially very important is the discovery that prolonged exposure of mosses to raised CO₂ concentration results in homeostatic adjustment to the prevailing concentrations. A short-term increase in the CO₂ concentration in *S. fuscum* clearly increases the rate of net CO₂ exchange, but the effect is smaller after prolonged exposure (Jauhainen & Silvola, 1996). In mosses, a short-term exposure to any CO₂ elevation above the former long-term CO₂ concentration has been found clearly to increase rate of net photosynthesis, and a comparable decrease is found in the response at CO₂ concentrations below the long-term CO₂ concentration (Jauhainen & Silvola, 1996; Fig. 2). Similarly, in a field study of a mixture of mosses and vascular plants, in growth chambers with seasonally increased CO₂ concentration, there was carbon loss in plants after they were re-exposed to lower CO₂ concentration (Grulke *et al.*, 1990).

Raised CO₂ concentrations have also been found to cause a temporary decrease in the light compensation point as well as delayed onset of dormancy at the end of the growing season. In *Hylocomium splendens*, the light compensation point was first lower in raised CO₂, but after a few months' exposure the difference decreased compared to mosses in ambient CO₂ concentration (Sonesson, Callaghan & Carlsson, 1996). Similarly, the light compensation point in a mixture of mosses and vascular plants decreased to a half of the previous value with a twofold increase in CO₂ concentration, but no differences were found after prolonged exposure (Grulke *et al.*, 1990). Dormancy was delayed in a CO₂ mixture of mosses and vascular plants

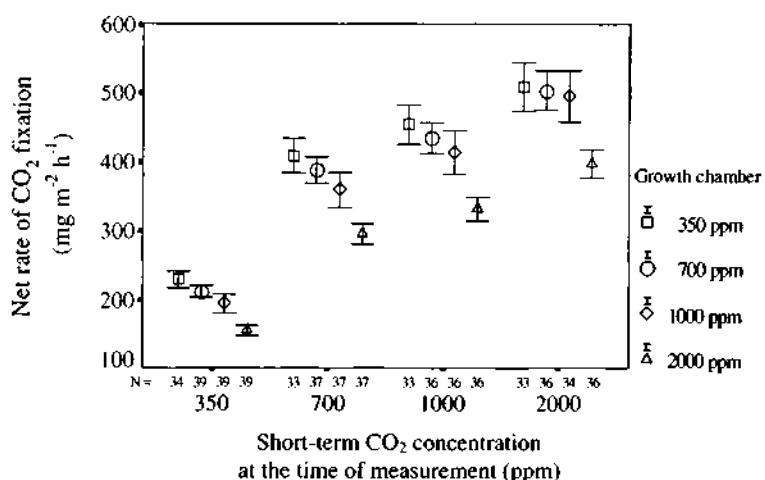


Figure 2. Net rate of CO₂ fixation of *Sphagnum fuscum* in four short-term CO₂ concentrations. Samples were first grown (long-term) at four CO₂ concentrations (indicated by different symbols). Temperature 22°C, radiation flux 340 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR and water content 800–1500% DM. Mean \pm SE presented (redrawn from Jauhainen & Silvola, 1996).

grown in raised CO₂ (Grulke *et al.*, 1990). Tissues of *H. splendens* formed under increased CO₂ in a natural growth site continued high rates of net photosynthesis later into the autumn, while tissues formed under ambient CO₂ (350 ppm), or tissues formed the previous year, did not have any further marked net CO₂ uptake (Sonesson *et al.*, 1992).

23.4 CARBON DIOXIDE AND PRODUCTION

The growth responses of mosses are different from the photosynthetic responses and no clear dependence between the two has been observed. In a four-month laboratory experiment, when four *Sphagnum* species were grown in CO₂ concentrations of 350, 700, 1000 and 2000 ppm, changes in shoot density, dry mass of capitulum and unit dry mass of stem as well as length increment were found to be, for the most part, independent from each other at all CO₂ concentrations (Jauhainen, Vasander & Matero, 1996). Production of *S. angustifolium* was increased in raised CO₂ due to increased length increment and unit dry mass (Jauhainen & Vasander, 1994). Although raised CO₂ concentration caused an increase in the shoot density per unit area and of stem dry mass in *S. fuscum*, the effect was not strong enough to compensate for reduced length increments so, overall, dry mass production was reduced by increasing CO₂ (Jauhainen, Vasander & Silvola, 1994; Table 1). *Sphagnum magellanicum* and *S. warnstorffii* seemed to be least affected by changes in CO₂ concentration as their responses varied most in the four experimental CO₂ concentrations (Jauhainen *et al.*, 1996). Sonesson *et al.* (1996) found an almost 50% lower length increment and shoot mass in *Hylocomium splendens* grown under raised CO₂ (600 ppm) compared to *H. splendens* grown in 350 ppm.

As the growth response of *Sphagna* does not correspond to the response of net

Table 1. The responses of four *Sphagnum* species to increased CO₂ concentrations from 350 to 700, 1000 and 2000 ppm in treatments with a N deposition rate of 10 kg ha⁻¹ a⁻¹. The growth period was 120 d for *S. fuscum*, 71 d for *S. angustifolium*, and 98 d for *S. magellanicum* and *S. warnstorffii*. Factor probabilities are presented by stars; *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, ns = no statistical difference between the treatments.

Species	shoot density	capitulum dry mass	stem dry mass	length increment	dry mass production
<i>S. fuscum</i>	+ **	+ ***	+ *	- **	0*
<i>S. angustifolium</i>	0 ns	+ ***	+ ***	+ **	+ ***
<i>S. magellanicum</i>	- *	0 ns	0 ns	- ns	0 ns
<i>S. warnstorffii</i>	0***	+ ns	+ **	- ***	0 ns

Explanation of symbols:

+ denotes increasing trend in the measure by increased CO₂

- denotes decreasing trend in the measure by increased CO₂

0 denotes variable changes or no real difference in the measure by increased CO₂.

photosynthesis under raised CO₂, one has to ask: what is the fate of increased amounts of photosynthesized carbon? A part of this carbon seems to be allocated to non-structural carbohydrates. According to van der Heijden *et al.* (1996), the concentration of soluble sugars was increased significantly by doubling the available CO₂ concentration for *S. papillosum* and *S. balticum*. The increase in the quantity of soluble sugars explained 10–25% of the increase in the capitulum and stem dry mass in raised CO₂, whereas there was little increase in dry mass production in these species.

23.5 SOURCES OF NITROGEN

The uptake of nitrogen in bryophytes can occur in two basic ways depending on the available nitrogen sources and the structure of the moss. Structurally, mosses can be separated into endohydric and ectohydric species (Richardson, 1981). Endohydric mosses like *Polytrichum* have a well-developed internal water conducting system and are therefore presumably able to take up dissolved nutrients from the soil and transfer them to the living apex (Eschrich & Steiner, 1967; Collins & Oechel, 1974). In ectohydric moss species, the internal conducting system is less developed, but the plant surface in contact with water can adsorb nutrients directly and some ectohydrous mosses like *Sphagnum* have the ability to transport elements externally in water as well (Rydin & Clymo, 1989). Therefore, the uptake of nutrients among the bryophytes is more or less diverse.

Although almost 79% of the atmosphere consists of nitrogen, it is a limiting nutrient in many ecosystems due to the inability of plants to use gaseous nitrogen directly. However, gaseous nitrogen can be converted into more useful nitrogen compounds by cyanobacteria attendant on some bryophytes. The rate of nitrogen fixation by bacteria associated with *Sphagnum* and *Drepanocladus* mosses can be several times higher than the rate of nitrogen input by precipitation in subarctic sites (Granhall & Selander, 1973) and may form as much as 84% of annual N input (Rosswall & Granhall, 1980). Geographically, the highest N-fixation rates are

recorded from mires in lower latitudes and, within mire ecosystems, the highest nitrogen fixation rates are found in fens and rich fens, while the activity is lower in ombrotrophic bogs (Waughman & Bellamy, 1980). Nitrogen fixation associated with mosses colonizing new areas is also important, such as with *Funaria hygrometrica* and *Ceratodon purpureus* in slash-and-burn areas (Brasel, Davies & Mattay, 1986).

Green plants seem to have less ability to use organic nitrogen sources than do fungi (Bolland, 1966). In cultures of *Sphagnum fimbriatum* however, some amino acids, such as glycine, can be almost as effective a nitrogen source as NH_4NO_3 (Simola, 1979). Glycine can be effectively used by *Ceratodon purpureus*, *Funaria hygrometrica* and *Atrichum undulatum* (Pringsheim & Pringsheim, 1935; Burkholder, 1959). The ability to use amino acids seems to be species-dependent and toxic responses may occur even though amino acids are normal metabolites of plant cells (Simola, 1975, 1979). Unfortunately, the ecological importance of organic forms of nitrogen as a nitrogen source for bryophytes is not well understood.

Most of the studies concerning the relocation of nitrogen from plant litter have been made with *Sphagnum* species because they form their own substratum and occupy sites with widely differing nitrogen availability. The rate of decomposition of ombrotrophic moss litter, especially of the remains of *Sphagnum*, is slow due to low pH and it is suggested that the low concentration of nutrients limits microbial activity (Damman, 1988; Verhoeven, Maltby & Schmitz, 1990; Hogg, Malmer & Wallén, 1994). However, nitrogen is effectively recycled within moss stands as the amount of nitrogen in the annual growth of ombrotrophic sites exceeds the input from the atmosphere (Damman, 1978; Rosswall & Granhall, 1980; Malmer, 1988; Urban & Eisenreich, 1988; Malmer, 1992). Minerotrophic fens also receive nutrients from surface water and ground water in addition to airborne deposition. Productivity in fens is high and it has been commonly asserted to be a result of the higher availability of nitrogen and phosphorus (Moore & Bellamy, 1974; Mitsch & Gosselink, 1986).

Deposition of airborne NH_4^+ and NO_3^- is a supplement to available nitrogen for many plants but, for example, in ombrotrophic bogs it is the only external source, if insects captured by carnivorous plants are discounted. Ombrotrophic sites are traditionally considered to be nitrogen-limited, but recent anthropogenic N-sources have increased the rate of N supply in many regions. It has been suggested that in some areas with increased N input, ecosystems may already be limited by low supplies of other nutrients such as phosphorus (Rosswall & Granhall, 1980; Aerts, Wallén & Malmer, 1992). The effects of increased airborne nitrogen deposition on species are therefore also connected with the availability of other nutrients.

23.6 EFFECTS OF AN INCREASED RATE OF N DEPOSITION

23.6.1 Changes in tissue chemical compounds

The effects in experiments of increased nitrogen deposition on bryophytes are inconsistent, mainly because the results from short-term experiments are quite different from the long-term effects. Bryophytes exposed to increased N deposition for a longer period of time have probably been affected by other factors as well, for

example there may have been a change in the relative availability of phosphorus. In short-term experiments where nitrogen supply is increased experimentally, the availability of nutrients and other ions prior to the experiment has an important effect on results. Therefore, responses in mosses from unpolluted and polluted sites differ. Although there have been several studies made to investigate the effects of increased N deposition, the duration of increased N supply, amounts of applied nitrogen and nitrogen supply rates are not usually easily comparable with each other in different experiments.

In *Sphagnum*, the highest nitrogen concentrations on a dry mass basis are always found in the capitula (Malmer, 1962; Clymo, 1978; Damman, 1978; Pakarinen, 1978; Malmer & Nihlgård, 1980; Malmer & Wallén, 1986; Malmer, 1988, 1990). Even a relatively small increase in nitrogen input has increased the nitrogen concentration in tissues of mosses in areas like southern Sweden over recent decades (Malmer, 1988, 1990). Transplantation experiments from low nitrogen deposition to higher N deposition areas show a rapid increase in tissue ammonium-nitrogen concentration in *Sphagnum* and the N-concentration also remains at enhanced concentration (Lee *et al.*, 1987). The relative increase in N-supply compared to other nutrients leads to a change in the ratio of these nutrients in tissues. For example, *Sphagnum* in low nitrogen deposition areas in Scandinavia has a lower N:P ratio compared to specimens from areas with a higher nitrogen deposition (Malmer, 1988, 1990; Aerts *et al.*, 1992). Other environmental factors may also have an effect on the nitrogen accumulation rate. Aquatic mosses (*Fontinalis antipyretica*, *Brachythecium rivulare*), exposed to increased nitrogen concentrations in stream water had an initial high rate of nitrogen accumulation in their tissues but later showed a net loss of N and P, probably because plant metabolism was affected by low oxygen concentration in the water after pollution pulses (Martinez-Abaigar, Nuñez-Olivera & Sanchez-Diaz, 1993).

There are several studies on the isolation of cell organelles in bryophytes in order to localize intracellular enzymes (see Rudolph *et al.*, 1993 for references). At least in *Sphagnum*, the kinetics of NO₃⁻ uptake have been found to differ somewhat from higher plants. *Sphagnum* does not have a long lag phase in the activation of nitrate reductase and therefore it is able to use even episodically available NO₃⁻ pulses immediately (Deising & Rudolph, 1987; Rudolph *et al.*, 1993). It has been proposed by Lee *et al.* (1990) that although endohydric species have thick leaves and internal nutrient uptake mechanisms, they may be able to respond quickly to an increased nitrogen supply while the needed enzyme can be induced by altering the nitrogen supply at least in some endohydric species. Bates (1994) showed in a laboratory experiment that *Pseudoscleropodium purum* could use deposited nutrient (NPK) pulses more efficiently in production compared to *Brachythecium rutabulum*, perhaps due to different nutrient uptake strategies within these species (see also Rincon & Grime, 1989). A short-term effect of increased N deposition has usually been a rapid increase in the nitrate reductase activity in *Sphagnum* growing in areas remote from urban and industrial activity, but in repeated exposures to increased nitrate or nitrite concentrations, the response has lowered proportionally to the rate of nutrient deposition in species like *S. cuspidatum* (Woodin *et al.*, 1985; Press, Woodin & Lee, 1986), *S. fuscum* (Lee *et al.*, 1987) and *S. magellanicum* (Aerts *et al.*, 1992). Due to supra-optimal N availability, *Sphagnum* species growing in high NO₃⁻ deposition areas lose

the capability to react to NO_3^- precipitation events and start to accumulate nitrogen in their tissues (Press *et al.*, 1986; Lee *et al.*, 1987). However, retention of nitrogen seems to be lower in *Sphagnum* grown under supra-optimal N deposition rates for a long periods of time (Press *et al.*, 1986; Lee *et al.*, 1987).

The ammonium form of nitrogen reduces nitrate reductase activity in *Sphagnum* and the reason suggested for this is the ammonium accumulation in the tissues which possibly inhibits nitrate reductase enzyme induction or enhances the degradation of the induced enzyme (Press & Lee, 1982; Woodin *et al.*, 1985; Woodin & Lee, 1987a). This phenomenon must lead partly to a lowered nitrate retention, which is found in *S. capillifolium* after receiving NH_4^+ jointly with NO_3^- (Lee *et al.*, 1987; Woodin & Lee, 1987b). Under moderate ammonium deposition rates, the inhibitory effect of NH_4^+ on the uptake of NO_3^- in mosses in unpolluted areas is lower (Deising, 1987; Pöpperl & Rudolph, 1989; Rudolph *et al.*, 1993).

The response of chlorophyll concentration to nitrogen deposition seems to depend on the species and also on the form of deposited nitrogen. For example, moderately increased supplies of both NH_4^+ and NO_3^- increased the chlorophyll concentration in *S. magellanicum*, whereas an increase in the NH_4^+ supply alone did not (Rudolph & Voigt, 1986). Austin & Wieder (1987) found no effect on chlorophyll concentration in *S. fallax* or *S. pulchrum* exposed to increased NH_4^+ or NO_3^- concentrations, but in *S. henryense*, an increase in NO_3^- supply rate caused an increase in the chlorophyll concentration. The concentration of chlorophyll is also affected by the N-deposition history of the site of origin. Baxter, Emes & Lee (1992) found a higher chlorophyll concentration in *S. cuspidatum* grown under relatively high N availability compared to individuals from low deposition areas. This was perhaps due to the allocation of N from a long term increased supply. Similarly, after exposure of *S. cuspidatum* to an experimentally raised NH_4^+ supply (up to 1 mM), there was a smaller decrease in the chlorophyll content in *Sphagnum* from a high deposition area compared to specimens from lower nitrogen deposition area (Baxter *et al.*, 1992). In a transplantation experiment, after exposure to high nitrogen concentration in stream water, chlorophyll a/b-quotients and net rate of photosynthesis decreased in the submerged mosses *Fontinalis antipyretica* and *Brachythecium rivulare* (Martinez-Abaigar, Nuñez-Olivera & Sanchez-Diaz, 1993).

A clear dependence has been shown between the rate of nitrogen supply and the free amino acid pool in higher plants, especially in concentrations of arginine, glutamine and proline (for example Durzan & Steward, 1967; Kim *et al.*, 1987; Näsholm & Ericsson, 1990; Lähdesmäki *et al.*, 1990). According to Thönes & Rudolph (1983), the capitulum of *S. magellanicum* is normally rich in glutamic acid, asparagine, glutamine and arginine in that order. In N-enriched conditions the most pronounced increase in the free amino acids in *Sphagnum* capitula is in the concentration of arginine (Baxter *et al.*, 1992; Karsisto *et al.*, 1996) (Fig. 3). Arginine also has a significant role in the assimilation of free NH_4^+ in vascular plants (Miflin & Lea, 1977; Givan, 1979; Rabe & Lovatt, 1986). According to Baxter *et al.* (1992), synthesis of asparagine and glutamate serves to minimize carbon utilization for nitrogen storage and their concentrations were increased markedly in enhanced nitrogen input in *S. cuspidatum* and a similar increase was also found by Karsisto *et al.* (1996) in *S. fuscum* and *S. angustifolium* (Fig. 3). The origin of mosses in respect to N deposition rate has a marked effect on the magnitude of response in amino acid concen-

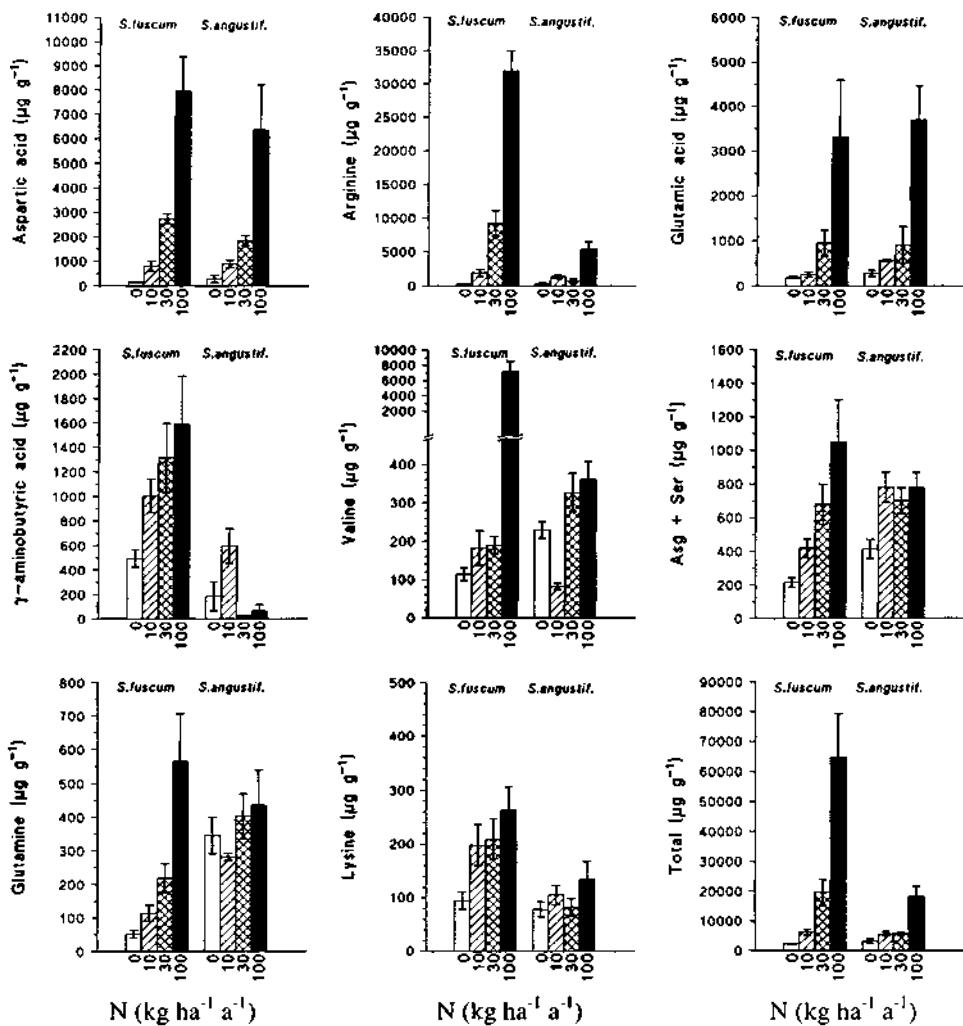


Figure 3. Dominant free amino acids ($\mu\text{g g}^{-1}$ DM) in *Sphagnum fuscum* and *S. angustifolium* capitula at different nitrogen deposition rates. The mosses were grown in growth chambers and treated with NH_4NO_3 solutions, which were applied by spraying the moss surface once a week over a period of 120 d for *S. fuscum* and 71 d for *S. angustifolium* (Karsisto *et al.*, 1996).

tration. Under artificially increased N deposition rates, a smaller change was found in the amino acid concentration in *S. cuspidatum* collected from a N-polluted site compared to that in *S. cuspidatum* from a low N deposition site (Baxter *et al.*, 1992). A suggested reason for the lower response was inhibition of ammonium entry to the cells, or better utilization of amino acids in new tissue formation and growth, in mosses subjected to long-term increased N deposition rates (Baxter *et al.*, 1992). Similarly, there are differences among *Sphagnum* species in respect to both quality and quantity of various amino acids perhaps due to differences in protein synthesis and ecological background. Species that are adapted to a low nitrogen input only,

like *S. fuscum*, accumulated six-fold quantities of some amino acids during one growing season compared to *S. angustifolium* which has a wider ecological amplitude at the same experimental N deposition rates ($1\text{--}10 \text{ g m}^{-2} \text{ a}^{-1}$). This probably indicates severe stress for the former moss species (Karsisto *et al.*, 1996) (Fig. 3).

23.6.2 Growth and production

In many studies, length increment and dry mass production in *Sphagnum* have been measured under increased nitrogen supply. A favourable N:P ratio in tissues for growth in vascular plants is estimated to be between 10–14 (Van den Driessche, 1974; Ingestad, 1979) and due to increased N supply, the ratio may become altered so that growth becomes increasingly phosphorus-limited. According to Aerts *et al.* (1992), experimentally applied extra nitrogen increased *Sphagnum* growth in N-limited areas, *i.e.* in low-N deposition, and there was no response to added phosphorus, but in high N deposition areas, the responses were the opposite. Similarly in The Netherlands, where nitrogen deposition has been high for a long time, added phosphorus increased the growth in *S. subnitens* and *S. fallax*, whereas deposited nitrogen did not have a marked effect (Kooijman & Kanne, 1993).

The response of species to enhanced N supply rates seems to be affected by the origin of mosses in respect to the former N availability and by the prevailing moisture conditions in the micro-environment. For example, within one month, NH_4^+ concentrations up to $100 \mu\text{M}$ in growth media has been found to enhance length increment in *S. cuspidatum* collected from a high N deposition area (Baxter *et al.*, 1992), whereas length increments were decreased under comparable conditions in *S. cuspidatum* collected from a low N deposition area (Press *et al.*, 1986). According to Austin & Wieder (1987), length increment of low hummock forming *S. pulchrum* was induced after receiving NO_3^- solution in concentrations of up to $213 \mu\text{mol}$ over a period of 40–50 d, but the increased N concentration had no effect on the length increment in the hollow form of this species. However, within three months in wet microhabitats *S. fallax* had increased dry mass production in increased N deposition (up to $372 \mu\text{mol NH}_4^+$ or $300 \mu\text{mol NO}_3^-$), but the effect became increasingly negative as the distance of the moss carpet from the water table increased (Twenhöven, 1992).

Ecological amplitude influences the survival of *Sphagnum* species under increased N availability. The more the species is restricted along the gradient of nutrient richness (ombrotrophy-minerotrophy), the more sensitive it seems to be to enhanced N loads. As the reaction of mosses to N increase depends mostly on three factors (species, nitrogen supply, water table depth) it is usually difficult to distinguish the effects of individual factors. In a laboratory experiment, only the wide trophic-status-tolerant *S. angustifolium* maintained a high rate of length increment in deposition rates of 3 and $10 \text{ g N m}^{-2} \text{ a}^{-1}$, though the highest length increments were at the $1 \text{ g N m}^{-2} \text{ a}^{-1}$ input rate in *S. fuscum*, *S. magellanicum* and *S. warnstorffii* (Jauhainen *et al.*, 1996) (Fig. 4). Ombrotrophic *S. fuscum* was particularly sensitive to increased nitrogen supply and its length increment decreased to 1/15 at a deposition rate of $10 \text{ g N m}^{-2} \text{ a}^{-1}$ compared to the control and a significant decrease was found even in meso-eutrophic *S. warnstorffii* (Jauhainen *et al.*, 1996). Also, the dry mass production in the *Sphagna* studied was highest in low and moderate N deposition. The highest N deposition treatment clearly decreased dry mass production in all species

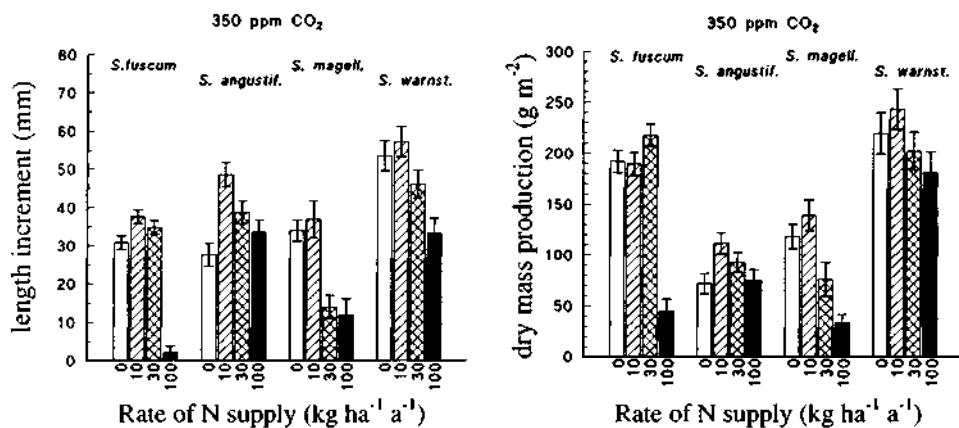


Figure 4. Length increment (mm) and dry mass production (g m^{-2}) of four *Sphagnum* species at four nitrogen deposition rates (0, 10, 30 and 100 $\text{kg ha}^{-1} \text{a}^{-1}$). The growth period (in a growth chamber) was 120 d for *S. fuscum*, 71 d for *S. angustifolium*, and 98 d for *S. magellanicum* and *S. warnstorffii* (Jauhainen *et al.*, 1996).

except in the wide trophic-status-tolerant *S. angustifolium* (Jauhainen *et al.*, 1996) (Fig. 4). Similarly in a four-year field experiment, the growth of *S. fuscum* almost ceased at a deposition rate of 10 $\text{g N m}^{-2} \text{a}^{-1}$ deposition rate whereas *S. angustifolium* continued to grow vigorously (Vasander *et al.*, 1996).

Increased nitrogen deposition has also been found to have an effect on moss densities in some mosses grown at low-N deposition sites. N-fertilization for one season with 4 $\text{g N m}^{-2} \text{a}^{-1}$ increased capitulum density significantly in *S. balticum* (Aerts *et al.*, 1992), as did N deposition rates of 3 and 10 $\text{g m}^{-2} \text{a}^{-1}$ in *S. fuscum* and *S. warnstorffii* (Jauhainen & Vasander, 1994). Contrasting effects were found in *S. angustifolium* and in *S. magellanicum*, which had markedly decreased shoot densities at highly increased (3 or 10 $\text{g N m}^{-2} \text{a}^{-1}$) N deposition rates (Jauhainen *et al.*, 1996).

In a laboratory experiment, increased nitrogen deposition of 3 $\text{g m}^{-2} \text{a}^{-1}$ caused a decrease in capitulum and stem dry masses in *S. fuscum*, *S. angustifolium* and *S. warnstorffii*, but in a highly increased N deposition (10 $\text{g m}^{-2} \text{a}^{-1}$) dry masses were greatly increased due to lowered length increments (except in *S. angustifolium*), which probably indicates lowered vitality in high N input rates (Jauhainen *et al.*, 1996). According to Rudolph & Voigt (1986), there was a significant increase in *S. magellanicum* capitulum mass upon treatment with a solution containing 322 $\mu\text{mol NH}_4^+$ and 153 $\mu\text{mol NO}_3^-$ compared to the control with no mineral supply. Jauhainen *et al.* (1996) found a comparable increase in response of *S. magellanicum* to N deposition rates of 1 and 10 $\text{g m}^{-2} \text{a}^{-1}$ compared to reference treatment with no added nitrogen.

23.7 COMBINED EFFECTS OF CO₂ AND N

There are only a few studies published concerning the effects of simultaneously increased N deposition and CO₂ on bryophytes, but the results indicate a surprisingly

low response. In a laboratory experiment over four months where *S. fuscum*, *S. angustifolium*, *S. magellanicum* and *S. warnstorffii* were grown under CO₂ concentrations of 350, 700, 1000 or 2000 ppm and simultaneous NH₄NO₃ fertilization (0, 1, 3 or 10 g N m⁻² a⁻¹), no general dependence on these factors was found in any of the species studied (Jauhainen *et al.*, 1996). Nitrogen deposition was clearly the controlling factor compared to CO₂ in unit dry mass of shoot, length increment and dry mass production with these particular rates of addition (Jauhainen *et al.*, 1994; Jauhainen *et al.*, 1996). Similarly, Billings *et al.* (1984), in a phytotron experiment with a mixture of arctic vascular plants and mosses, found a significant net ecosystem carbon storage increase after additional nitrogen (25 g m⁻²) was provided, whereas doubling the available CO₂ concentration only had a minor effect. According to Paffen & Roelofs (1991), biomass of submerged *S. cuspidatum* was increased only if the CO₂ concentration in the culture medium was high (1000 ppm), but NH₄⁺ concentration in the culture (from 50 to 1000 µmol) did not have a favourable effect on plant vitality (e.g. colour and senescence) or increase biomass in lower CO₂ concentrations. The only evidence of the combined effects of CO₂ and nutrients on mosses to date is the work of Baker & Boatman (1990) in which increased concentrations of N, P, K and simultaneously raised CO₂ concentration led to increased innovation formation, plant dry mass and inter-fascicle length in *S. cuspidatum* in an axenic culture. However, in this experiment the concentrations of P and K were rather high in the most diluted solution, so the results cannot be regarded as solely due to the effects of N and CO₂.

23.8 CONCLUSIONS AND PROSPECTS

Studies of the effects of increased CO₂ and nitrogen have concentrated on *Sphagnum* which has both ecological and economic importance. Production on a dry mass basis in *Sphagnum* is controlled more by the available nitrogen than by CO₂ concentration.

The rate of net photosynthesis is clearly increased by increasing the concentration of CO₂, but this increase is transient. The effect of CO₂ on mosses may mainly involve increased concentrations of non-structural carbohydrates, such as sugars.

The combined effects of CO₂ and N on mosses have not been studied as much as their individual effects. Also, some enzymatic changes noted by increased N deposition and CO₂ concentrations might be caused by attendant microbes.

In the future, in studies of the effects of both CO₂ and nitrogen deposition on mosses, ecological and physiological aspects should be more closely integrated at the single plant level, as the response to increased CO₂ may also give rise to non-structural production. At the whole mire or ecosystem level, interaction between mosses and vascular plants in growth, competition, decomposition and carbon/nitrogen cycling should be revealed in changing CO₂ and nutrient climates.

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24. *Sphagnum*, the peatland carbon economy, and climate change

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24.1 SUMMARY

Northern peatlands cover about 3% of the Earth's land surface and contain about 600 Gt of carbon — about the same amount as there is in CO₂ in the atmosphere. Species of *Sphagnum* are abundant in most of these peatlands and are amongst the most important fixers of carbon. They decay more slowly than most plants and so come to be over-represented in peat. They are also the main determinants of the porous structure of the surface of most peatlands.

The peat continues to decay anaerobically though very slowly. As the depth of peat increases so the *total* rate of loss increases and the rate of accumulation decreases. Thus the carbon sequestering power declines.

Crudely calculated carbon fluxes into and out from the surface (mol m⁻² a⁻¹) are: influx fixed from CO₂ by photosynthesis 5.2; efflux as CO₂ from aerobic decay 1.1–2.7; efflux as CH₄ from anaerobic decay 0.2–0.5; efflux downward to peat proper 2.0; efflux as dissolved organic matter in runoff 0.5. The net effect on the atmosphere is to remove CO₂ at 3.0 mol m⁻² a⁻¹ and to replace it with CH₄ at 0.2–0.5 mol m⁻² a⁻¹. This CH₄ has, molecule for molecule, perhaps about 20 times the warming potential that CO₂ has so the net effect is the equivalent of *adding* CO₂ to the atmosphere at 1–7 mol m⁻² a⁻¹.

Both mechanisms tend to contribute to the warming potential of the atmosphere. *Sphagnum* is the only bryophyte abundant enough to be capable of having a significant effect of this kind.

KEYWORDS: Carbon dioxide, carbon sequestering, methane.

24.2 INTRODUCTION

The bogmoss, *Sphagnum*, has gone its own evolutionary way and achieved remarkable success in a limited environmental range as a result of its ability to make its environment acid, its ability to grow well in nutrient-poor conditions, and its resistance to decay (Clymo, 1997). At the Centenary Meeting of the British Bryological Society (BBS) I discussed its potential, through the peatlands of which it is the chief engineer, to affect the climate. Here I try to explain some of the ideas involved but make no attempt to review the rapidly growing literature on this subject.

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24.3 THE AMOUNT OF SPHAGNUM

Individual gametophyte plants of *Sphagnum* range from small to robust (as bryophytes go) but all are minute when compared with an average woody tree. At least, they appear to be. We cannot yet tell whether a single genetically distinct patch on a peatland occupies an area a few centimetres across (similar to the typical size of unispecific patches) or interdigitates with other species over many square metres. If the latter were true then one might equate the peat with the mass of structural wood in a tree, and it would become obvious that this single *Sphagnum* plant was similar in mass to a tree. Linked but separate is the question: how old is this individual *Sphagnum* plant? Is it the direct vegetative continuation of a spore that germinated five millennia ago, or did its generating spore begin to grow only a few years or decades ago? By the bicentenary of the BBS the answers to these questions will probably be known. For the time being we can bypass them and try to answer the third of these sorts of question: how much *Sphagnum* is there altogether on the Earth's land surface?

Sphagnum grows in a wide range of habitats and places, avoiding the highly calcareous and the very dry (though a few species essay moderately calcareous and mesohydric conditions). But most species and all the most abundant ones grow best and most commonly in wet acid peatlands, often those dependent on rainwater for their surface water. In such places they may occupy half the surface area or more. The total area of such peatlands is difficult to assess (Clymo, 1996b) because they are difficult to identify by remote sensing and often interdigitate with other types of system or have sufficient trees on them to be categorized as forests. Gorham (1991) estimated that there are about 350 MHa of such peatlands in Boreal and Subarctic North America, Fennoscandinavia and the former USSR. This is 3,500,000 km²: equivalent to a square of side about 1900 km. These northern peatlands thus occupy about 3% of the Earth's land surface.

Most of the live *Sphagnum* is in the top 3 cm of capitulum and topmost branches (Clymo & Hayward, 1982), and has a dry bulk density of about 0.05 g cm⁻³ (Clymo, 1983). If half (0.5) of this northern peatland is covered by *Sphagnum* the total dry mass of these living plants is about $0.5 \times 350 \times 10^6 (\text{Ha}) \times 10^4 (\text{m}^2) \times 0.05 \times 3 \times 10^4 (\text{g}) / (10^6 (\text{t}) \times 10^9 (\text{Gt})) = 2.6 \text{ Gt}$.

The general operation of peatlands is now well-known. New mass is produced at the surface. In the case of *Sphagnum* the capitulum behaves like a factory extruding stem and branches behind it as it grows upwards. Below, the cells die for lack of light. The structure is porous and decay is mainly aerobic producing mostly CO₂. The 5–50 cm thick surface layer is the acrotelm of Ingram (1978). Some plants decay rapidly: *Rubus chamaemorus* is an example. Others, including *Sphagnum*, decay unusually slowly and thus come to be over-represented by the time that collapse and the consequent reduction of hydraulic conductance cause the peat to become permanently waterlogged and thus anoxic, with anaerobically produced CH₄ as a product — the catotelm of Ingram (1978). The proportion of *Sphagnum* in the catotelm is thus likely to be greater than it is at the surface, though we do not know by how much. Here I guess that the peat is 70% *Sphagnum*. Gorham (1991) estimated that the average depth of peat in the Boreal and Subarctic zones is 2.3 m. The mean dry bulk density of the peat is probably about 0.1 g cm⁻³. The total dry mass of

Sphagnum in peat is then about $0.7 \times 350 \times 10^6(\text{Ha}) \times 10^4(\text{m}^2) \times 0.1 \times 230 \times 10^4(\text{g})/(10^6(\text{t}) \times 10^9(\text{Gt})) = 620 \text{ Gt}$. There are several assumptions in this calculation: the true value might be half or double that given.

24.4 CARBON ACCUMULATION IN SPHAGNUM-DOMINATED PEATLANDS

As recently as 30 years ago the general view of peatlands was that they fixed carbon at the surface, lost a small fraction of this in decay and accumulated most of what they had fixed in peat where decay was negligible. *Sphagnum* plants grew onward and upward leaving their dead remains below to accumulate. Peatlands could be viewed as a 'permanent' sink for carbon. Alternating layers of highly humified and less humified peat, if synchronous over a large peatland, represented drier and wetter climates. Carbon-14 dating later showed that in many (but not all) cases these layers corresponded with periods of slower and faster peat accumulation. Apart from these changes, covering a few centuries each, there was no reason to think that carbon sequestration would not continue at much the same average rate until some catastrophic change — another ice age for example — removed the peatlands and all their peat. In the context of current concerns about atmospheric CO₂ concentrations and climate change, peatlands as carbon sinks (and *Sphagnum* as their main agent) were a 'Good Thing' in the sense implied by Sellars & Yeatman (1930).

This simple picture has proved to be incomplete. It is now unclear whether peatlands increase or decrease the temperature-changing potential of the atmosphere. The debate hinges on two matters. First, is the picture of indefinite accumulation of peat at much the same average rate correct, or does the rate decline with time *even if the climate and internal peatland processes remain the same?* Secondly, what is the role of CH₄ produced in the anoxic conditions in waterlogged peat?

24.5 RATE OF ACCUMULATION OF PEAT

The key idea here is that the catotelm is the true peat accumulating layer; the living plants in the acrotelm, among which *Sphagnum* is usually conspicuous, fix carbon and decay predominantly aerobically but at different rates. For any one species one may write, for unit area, something like:

$$\frac{dM}{dt} = p - \alpha M,$$

where M is the accumulated dry mass, p is the rate of addition of dry mass (p for productivity), α is the proportional decay rate, and t is time. The solution to this is:

$$M = (p/\alpha) \times (1 - \exp(-\alpha t))$$

(Fig. 1), which rises from zero at first towards the asymptotic limit p/α (Clymo, 1984). At first there is no material for decay to work on, so the slope of the line at $t=0$ is simply the rate of addition, p: any slope on the graph of M vs t is a rate (productivity) physically similar to p. As time goes on decay can operate on an increasing accumulated mass, so the rate of loss, αM , increases and rises toward the rate of addition. The true rate of accumulation — the net effect of +p and of

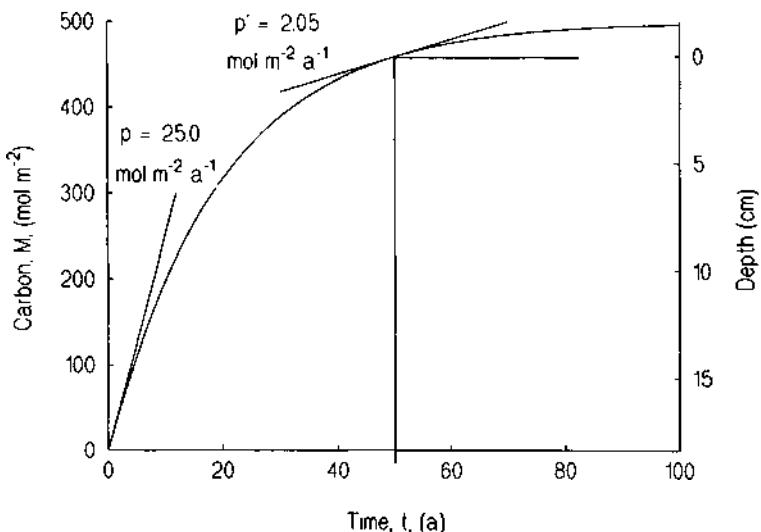


Figure 1. Accumulated dry mass, M , in the acrotelm as a function of time, t , following $M = (p/\alpha)^* (1 - \exp(-\alpha t))$. The rate of addition, p (productivity) is the slope of the line at the origin. The rate of addition to the catotelm, p' , is the slope at the time of collapse when the peat enters the catotelm, after 50 years in this example. The right scale shows the depth (cm) from the top of the steady-state acrotelm. The left scale shows the temporal course of increasing thickness. $p = 25 \text{ mol m}^{-2} \text{ a}^{-1}$, $\alpha = 0.05 \text{ a}^{-1}$.

$-\alpha M$ — is the slope of the curve at any chosen time, and it decreases steadily as time passes. One may argue about the functional form of the decay coefficient, α : is it really constant or does it decrease with time? But it makes little difference to the outcome because within 10 to 100 years structural collapse and the rising water table cut the process short as the catotelm rises to engulf the base of the acrotelm. If p' is the slope at this time then for the acrotelm one has:

$$\frac{dM}{dt} = p - \alpha M - p' = 0.$$

This formalizes two ideas. First, the acrotelm is of fixed thickness and is not a net accumulator of peat once it has reached this thickness after perhaps 50 yr (Fig. 1). Secondly, the acrotelm is a pre-processor: it fixes carbon, loses much of it differentially (50–90% during passage of *Sphagnum* through it; nearly 100% for *Rubus chamaemorus*) and passes on a small part of it at a rate p' to the catotelm where the peat accumulates. Another way to put this is that p'/p is typically about 0.5–0.1 for *Sphagnum* and <0.01 for *R. chamaemorus* leaves. Of course there is variation with climate and microtopography (hummock, hollow). In a hollow the acrotelm may be only 5 cm thick, and p'/p for *Sphagnum* might be 0.5 while in a hummock the acrotelm may be 50 cm thick, with correspondingly longer time for decay to operate, and p'/p for *Sphagnum* might be only 0.05. But the general idea that the acrotelm hands on only a part of what it has fixed to the catotelm, where the peat then accumulates, is the same whatever the exact processes, proportions, and rates.

What happens in the catotelm? If there were no decay *at all* then the catotelm would, as was once thought, be a peat accumulator at rate p' indefinitely. But the

mistake was to confuse *no* decay with a very small rate. That decay does continue is shown, for example, by the CH₄ concentration decreasing from the base to the top of the catotelm (Clymo & Pearce, 1995). We can use the same approach as we did for the acrotelm, but (for variety and in the hope that the main ideas have been established) let us suppose that the decay coefficient, α , is not constant but decreases as the proportion of more refractory matter increases because the less refractory matter has already decayed. Two possibilities are mathematically tractable (Clymo, 1992; Clymo, Turunen & Tolonen, 1998): that the decay coefficient decreases linearly with the amount of dry mass left, and that the decrease is quadratic:

$$\alpha' = \hat{a}'_L (M'_T/M'_0) \text{ linear decay};$$

$$\alpha' = \hat{a}'_Q (M'_T/M'_0)^2 \text{ quadratic decay}$$

where the ' indicates the catotelm, M'_T is the mass of a notional piece of peat which entered the catotelm with mass M'_0. When these (and the constant α' model) are put into $dM'/dT = p'\alpha'M'$ and the equation is solved one gets:

$$M' = (p'/\hat{a}'_C) \times (1 - \exp(-\hat{a}'_C T)) \text{ constant decay};$$

$$M' = (p'/\hat{a}'_L) \times \ln(1 + \hat{a}'_L T) \text{ linear decay};$$

$$M' = (p'/\hat{a}'_Q) \times ((1 + 2\hat{a}'_Q T)^{1/2} - 1) \text{ quadratic decay}.$$

The first is a curve with an asymptote at p'/\hat{a}'_C (similar to Fig. 1). The other two resemble it but have no asymptote: each continues to rise but ever more slowly. To test these one may fit, for several depths at one site, measured cumulative mass (derived from a profile of dry bulk density) to age, derived from C-14 dates converted to dendrochronological age (Clymo, 1992). Or one may use, for the base only of numerous different sites, the measured cumulative dry mass and the corresponding dendrochronological age (Clymo *et al.*, 1998). In the majority of suitable cases there is a fairly good fit, and no difference in the exactness of fit among the three models. Here we are concerned mainly with the fact that all three models curve over, so that the true rate of accumulation, dM'/dT , decreases steadily. This means that the peatland is becoming steadily less effective at sequestering carbon. The sequestering ability of the catotelm, S', may be defined by the rate of accumulation now as a proportion of p' which it was originally: $S' = (dM'/dT)/p'$. For the three models of decay this gives:

$$S' = \exp(-\hat{a}_C T) \text{ constant decay}$$

$$S' = 1/(1 + \hat{a}_L T) \text{ linear decay}$$

$$S' = 1/(1 + 2\hat{a}_Q T)^{1/2} \text{ quadratic decay}$$

For those cases calculated so far (Clymo *et al.*, 1998) one gets values of S' of about 0.7: peatlands now are only 70% as effective at sequestering carbon as they were when they began growth several millennia ago. Of course they may have spread since they began growth and that will counteract the diminishing effectiveness of unit area by increasing the total area. There are too few data yet to be able to make sensible calculations.

24.6 CARBON DIOXIDE AND METHANE

The two main carbon-containing gases that exchange with the peatland surface are CO_2 and CH_4 . Photosynthesis, fixing CO_2 , seems to be the only carbon influx process, but there are several sources and routes of efflux, indicated in Fig. 2. There is no single site (yet) where all these components have been measured so I have taken plausible values for fluxes from several sites. The units are for carbon and are $\text{mol m}^{-2} \text{ a}^{-1}$.

First is the transfer of peat from the acrotelm to the catotelm. The value 2.0 is the best estimate (Clymo *et al.*, 1998) of this rate for peatlands in the concentric bog region of southern Finland. This carbon has been fixed and removed 'permanently' from the atmosphere.

The sideways arrow represents losses as soluble carbon in runoff through the acrotelm. The 0.5 value is the mean for about a dozen peatlands in southern Finland (Kortelainen & Saukkonen, 1996). It is not clear how rapidly this carbon returns to the atmosphere: here I assume that it does so within a few years.

Methane, produced by decay in the catotelm, diffuses up to the acrotelm (Clymo & Pearce, 1995) but the flux is small compared with that of CH_4 produced just below the watertable. Some of this now combined upward flux is oxidized by methanotropic bacteria to CO_2 , but some escapes. And some moves much more rapidly in the low-resistance path of the gas spaces inside the roots of plants. The median value

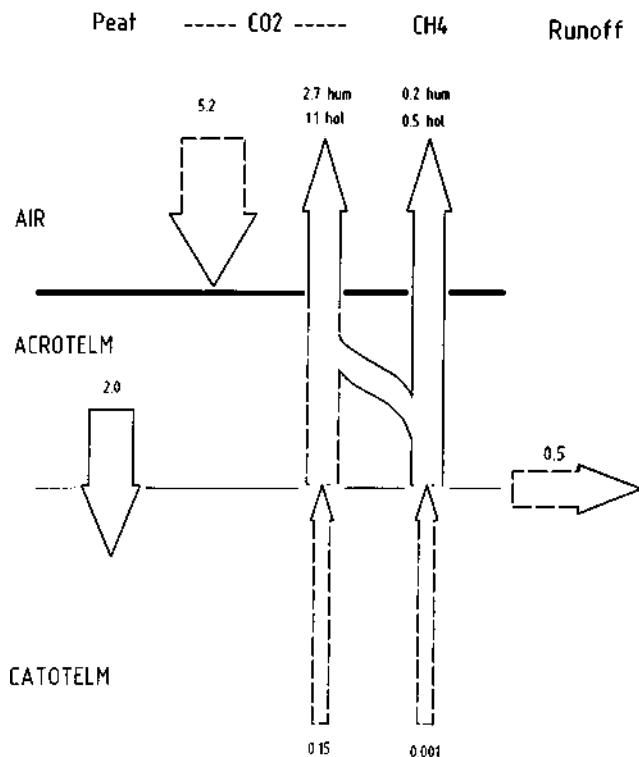


Figure 2. Pathways and approximate fluxes ($\text{mol m}^{-2} \text{ a}^{-1}$) of peatland carbon. Dashed lines show the main sites of production. Efflux of CO_2 is shown separately for hummocks and hollows.

of this combined efflux from ombrogenous peatland in southern Finland recorded by Nykänen *et al.* (1996a) was about 0.35. This agrees well with the values for *Sphagnum*-dominated microhabitats in a peatland in south-west Scotland: 0.5 (hollows) and 0.2 (hummocks) (Clymo & Pearce, 1995).

Some CO₂ is produced in the catotelm but the upward flux is small compared with that of CO₂ produced in the acrotelm. Together their upward flux in the same *Sphagnum*-dominated peatland was about 1.1 (hollows) and 2.7 (hummocks) mol m⁻² a⁻¹.

The most uncertain quantity is the gross input into plant mass. The value 5.2 mol m⁻² a⁻¹ (150 g m⁻² a⁻¹) is similar to that reported for northern Sweden by Svensson & Rosswall (1980) and for *Sphagnum* in northern England (Clymo & Reddaway, 1971). But it gives a value for net CO₂ influx of about $5.2 - (2.7 + 1.1)/2 = 3.3$ mol m⁻² a⁻¹ while Nykänen *et al.* (1996b) measured a value for net CO₂ flux in a southern Finnish bog of about 2.0 for the six growing months. On the other hand the transfer to the catotelm (p') is $2.0/5.2 \approx 40\%$, which may be a bit high.

For the values given above the net influx of carbon in CO₂ is 3.3 from which must be subtracted the 0.3 which is organic matter in solution on its way back to the atmosphere, i.e. a total of 3.0 mol m⁻² a⁻¹. The efflux of CH₄ is about $(0.2 + 0.5)/2 = 0.35$ mol m⁻² a⁻¹. But every molecule of CH₄ has about 20 times the atmospheric warming potential of a CO₂ molecule and $20 * 0.35 = 7.0$, which is greater than the 3.1 removed. If these values are typical then peatlands, particularly those with a large proportion of wet hollows, may already be contributing to climate warming.

24.7 CONCLUSION

It has long been known that peat stratigraphy can be used to infer something about past climate, because the peat accumulating process reflects, to some extent, the climate. Now we can see that there is sufficient peat for it to be possible that peatland processes might affect the climate-changing potential of the atmosphere: the sequestering ability of peatlands diminishes with time though perhaps countered by an increase in area; and it is not clear whether the ordinary processes fixing carbon are more important or less so than the effective conversion of CO₂ to CH₄. In the worst case peatlands are already contributing to the temperature-raising potential of the atmosphere, and almost any alteration, except starting new peatlands, would increase this potential. The truth is probably less dramatic than the worst case, but we lack the data to be sure. Here is a worthwhile problem for the next century.

The bog moss, *Sphagnum*, is the most important constituent of the surface vegetation of northern peatlands and decays unusually slowly so it becomes even more important in peat. *Sphagnum* is thus at the centre of any discussion of peat accumulation. In quantitative terms it is the *only* bryophyte of any importance whatever, but (like the diarist Pepys, who became the first Secretary and main creator of the British Navy) its obscure family, were it able, might well be proud that one of their number had reached such absolute, as well as relative, eminence.

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25. Reproductive biology and life-history strategies

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A classification of life-history strategies in bryophytes, based on trade-offs between gametophyte longevity v reproductive effort and on spore size v spore number, is compared with schemes based on sexuality and gemma production in liverworts, and a statistical analysis of life-history characteristics among species in three large orders of mosses. There is reasonable agreement, although the results suggest that the strategies should be regarded as nodes within a continuous array of reticulate variation rather than as discrete entities.

Evidence is presented that decreasing gametophyte longevity in colonist, fugitive and shuttle species is accompanied by trends towards monoecy and increasing reproductive effort. Support is also provided for the prediction that decreasing longevity will be correlated with lower age of first reproduction, substantial phenological flexibility, and increasing success in establishment from spores. Production of specialized asexual propagules is particularly characteristic of dioecious colonists among the mosses, but occurs more widely among liverworts. These relationships are discussed in terms of factors likely to lead to rarity among bryophyte species.

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