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THE APPLICATION OF CELL CULTURES IN BRYOLOGY *

By Siegfried Huneck

CELL CULTURES of higher plants have been used extensively for the elucidation of biosynthetic pathways, the synthesis of pharmaceutically-interesting drugs and for physiological experiments. How is the state of art concerning bryophytes? To date relatively few papers have been published on the biochemistry and chemistry of cell cultures of bryophytes. It is the aim of this review to summarize these publications and to encourage further research in this new and interesting branch of biology and bio-organic chemistry.

Ohta et al. (1977) introduced a special medium (Murashige and Skoog's medium 1, MSK-1) for cell suspension cultures of *Marchantia polymorpha*. They found that the cultures did not grow appreciably in the dark. Subsequently Katoh et al. (1980) developed a modified medium, MSK-2, for cell cultures which contains 111 mM glucose, 3.13 mM phosphate, 0.3 mM malic acid, 13.7 µ M rhamnose, 4.5 µ M 2,4 - dichlorophenoxyacetic acid, 5.5 x 20^3 µ M inositol, 8.2 µ M nicotinamide, 2.96 µ M thiamine, HC l, 2.66 µ M pyridoxine. HC l, 4.12 x 10^{-2} µ M biotin and 1.48 x 10^{-2} µ M cyanocobalamin. A cell suspension culture was initiated from green haploid callus of *Marchantia polymorpha* L. and protoplasts were isolated from the cultures cells by enzymatic degradation of cell walls (Ono et al,1979).

A suspension culture of *Marchantia polymorpha* used CO_2 in air as the sole carbon source. The growth rate in terms of cell dry-weight during the exponential phase was 0.171, and the doubling time was 1.76 days; the highest content of chlorophyll was 24 mg/g dry weight (Katoh et al., 1979). According to Ohta et al., (1981) the organic acids of the TCA cycle support the growth of the cell suspension culture of *Jungermannia subulata*. Ohta and Hirose (1982) analysed the induction and characteristics of cultured cells from *Jungermannia sublata* and *Calypogeia granulata*; they investigated especially the utilization of different nitrogen sources.

Schieder and Wenzel (1972) and Taylor (1979) used tissue cultures of *Sphaerocarpos donnellii, S. texanus, Marchantia polymorpha* and *Lophocolea heterophylla* for the enzymatic isolation of protoplasts. The yields ranged from $10^2 - 10^3$ protoplasts per ml. Ohyama et al. (1982) went a step further and described a method for the isolation of chloroplast DNA from cell suspension cultures of *Marchantia polymorpha*. The DNA showed a covalently closed circular form, and restriction endonuclease digestions proved that the plastid DNA was uncontaminated by DNA from other organelles. Ohyama et al (1983) used cell suspension cultures of *Marchantia polymorpha* for the physical mapping of chloroplast DNA by restriction endonucleases BamHI, SmaI, KpnI and XhoI. Yamano et al. (1985), determined the complete nucleotide sequences of 4.5 S rRNAs in chloroplasts from the liverworts *Jungermannia subulata* and *Marchantia polymorpha*.

Handa and Johri (1979) examined the role of purine and pyrimidine ribosides, nucleotides and substituted xanthines in the differentiation of chloronema filaments in suspension

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Footnote.* A contribution to the Research and Development column edited by R. Mues and J.G. Duckett. For addresses, see Bryol. Times, 31:9.

OUT AND ABOUT

In the People's Republic of China

By Pan-Cheng Wu

CHINESE BRYOLOGY made progress in several fields in 1985.

In memory of the late Prof. Pan-chieh Chen's great contribution to Chinese bryology, the leader of the Academia Sinica decided to give him second place in the awards of the Academia Sinica for the Genera Muscorum Sinicorum. So far it is the highest award made for any Chinese bryological work.

After great efforts in recent years, the Bryoflora of Xizang (Tibet) and A glossary of terms and names of bryophytes were both published in 1985.

Last May, Prof. Paul L. Redfearn and his wife Alice once again visited the Institute of Botany, Academia Sinica, under the co-operative programme, to produce a catalogue of the mosses of China, which will include about 2264 taxa, and which is to be published in the Annals of the Missouri Botanical Garden. Before Prof. Redfearn left for Hiroshima University he gave a very interesting lecture entitled " The vegetation of the interior Highlands of the United States".

Dr. T. Koponen made his second trip to China in July 1985. He brought his wife, Dr. A. Koponen, and his young son Hekki, and they stayed until the beginning of September 1985. Three Chinese bryologists accompanied them on a visit to one of the forest regions on Hainan Island, the mangrove forest and the Academia of Tropical Agriculture. Another two Chinese bryologists organized an expedition to south Yunnan and south-west Yunnan with the Koponens. They both gave lectures in Guangzhou, Kumning and Peking, Dr. T. Koponen dealing with an expedition to New Guinea and Dr. A. Koponen with her study of the family Splachnaceae.

[contd. on page3]

Application of cell cultures in Bryology (contd. from p.1).

cultures of the moss *Funaria hygrometrica*. Cyclic adenosine -3', 5'-monophosphate (cAMP) and mono- and dibutyryl cAMP gave the maximum response in wild-type protonema. Wang, Horgan and Cove (1981) found that gametophore-over-producing mutants of the moss *Physcomitrella patens* grown in liquid culture synthesized large amounts of cytokinins: 4.6 - 7.2 µg/g fresh weight N^6 - (Δ^2-isopentenyl) adenine and 0.5 - 5.0 µg/g fresh weight zeatin. Cell cultures of three cytokin-in-over-producing mutants of *Physcomitrella patens* have been shown to convert [8-^{14}C] adenine to N^6 - [^{14}C] - (Δ^2-isopentenyl) adenine (Wang, Beutelmann and Cove, 1981).

Liverworts synthesize numerous and unique sesquiterpenoids (Asakawa, 1982; Huneck, 1983) and it would be a great advantage to produce these compounds on a large scale by means of cell cultures. Takeda and Katoh (1981) successfully cultivated cells of the gametophyte of *Calypogeia granulata* and compared the pattern of sesquiterpenoids from these cultured cells with the composition of the sesquiterpenoids from the original plant. The cells grew photoheterotrophically, but not in the dark and yielded after 20 days 1,4-dimethylazulene (1*; 43% of dry weight of the cells), ledene (2; 1.5%), bicyclogermacrene (3; 17%), 1, 4-dimethyltetrahydroazulene (4; 1.5%), 1,4-dimethyl-dihydroazulene (5; 0.6%), an indene type aldehyde (6; 2.8%) and two compounds (I; 4.5%, II; 5.1%) of unknown structure. In comparison the intact plant gave the following values: 1: 51%, 2:1.0%, 3: 3.8%, 4: 0.1%, 5: trace, 6:2.8%, comp. I: 2.6%, and comp. II. 2.4%. Subsequently Takeda and Katoh (1983 a) elucidated the structure of 5 as 1,4-dimethyl-3, 10(S)-dihydroazulene and the structure of 6 as 3,7-dimethylindene-5-carboxaldehyde. A further analysis of the volatile oil from cultured cells of *Calypogeia granulata* led to the additional isolation of β-elemene, bicycloelemene, eremophilene, allo-aromadendrene, anastreptene, 3-acetoxybicyclogermacrene, 3-hydroxybicyclogermacrene, trinoranastreptene (7), 2-acetoxy-3-hydroxybicyclogermacrene (8) and 3-acetoxy - 2 - hydroxybicyclogermacrene (9) (Takeda and Katoh, 1983 b).

Lunularic acid (10) (LNA) is a dormancy factor from *lunularia cruciata* and has been detected in numerous other liverworts, but not in mosses. To study the LNA accumulation in the cells under various environmental conditions, and its metabolism, Abe and Ohta (1983) analysed the cell cultures of 4 liverwort species and found concentrations between 0.04 and 10.8 µg LNA /mg dry weight. Careful chromatography of the extract from cells of *Marchantia polymorpha* led Ohta et al. (1983, 1984) to the isolation of a probable precursor of LNA, prelunularic acid (11) which is the first example of an intermediate with "prearomatic" structure in the phenylpropanoidpolymalonate pathway. Finally, Abe and Ohta (1984) determined the contents of lunularic and prelunularic acids in *Marchantia polymorpha* cell cultures and found 2 - 4 µ g/g dry weight 10 while the concentration of 11 exceeds this value by about a hundredfold.

If further investigations confirm that cell cultures of liverworts contain the same secondary products as the intact plants, this method will be extremely useful for the bryochemist for (a) the cultivation of liverworts which are difficult to collect in nature in large quantities; and (b) for following the biosynthetic pathways of terpenoids and phenolics.

* See formula in Figure 1.

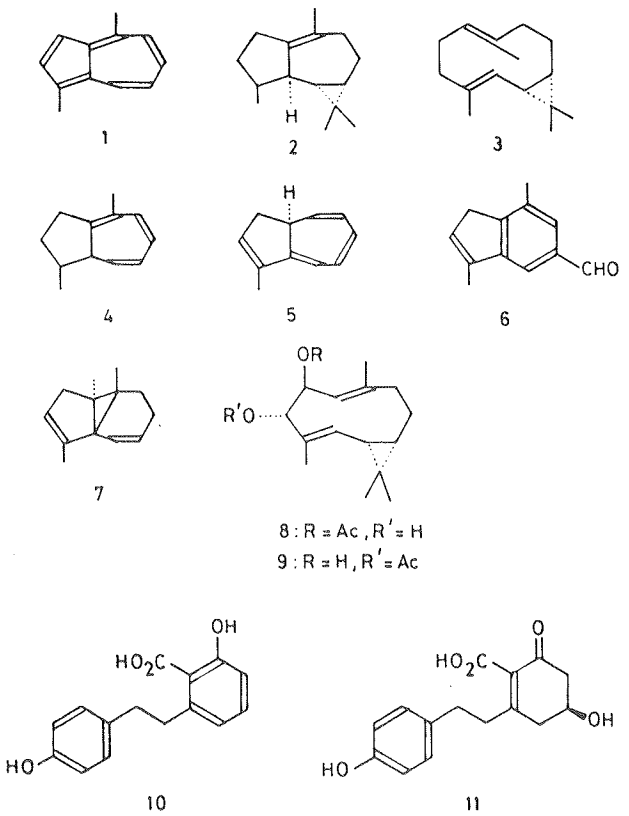

Figure 1. Formulae

1: 1,4-dimethylazulene; 2: ledene; 3: bicyclogermacrene; 4: 1,4-dimethyltetrahydroazulene; 5: 1,4-dimethyl-3, 10(S)- dihydroazulene, 6: 3,7-dimethylindene-5-carboxaldehyde; 7: trinoranastreptene; 8: 2-acetoxy-3-hydroxybicyclogermacrene; 9: 3-acetoxy-2-hydroxybicyclogermacrene; 10: lunularic acid; 11: prelunularic acid.

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Out and About in the People's Republic of China

(continued from page 1.)

Several new winter hosts for the aphids of gallnuts, including several species of *Brachythecium*, *Pleuropus* and *Thuidium* have been found in southern-central China. It seems that the bryophytes will be increased in quantity in the near future, and used in the production of gallnuts in southern China.

Of very great interest was the news that local scientists had achieved success in the use of ecological methods in their research on epiphyte lichens, bryophytes and city trees, as indicators of air pollution. They have produced an air-pollution map of Guangyang, the capital city of the province of Gunagzhou. In another piece of work they used lichens and mosses as monitors to compare pollution levels in two factories, and this also proved successful.

Five Chinese bryologists are undergoing training abroad, i.e. in the Hattori Botanical Laboratory, the Missouri Botanical Garden and Alberta University. Some are studying for degrees.

The sad news was that Prof. W.X. Xu of Yunnan University, one of Professor Chen's oldest students, died at the age of 66, on August 29th, 1985. His main work was on the bryoflora of Yunnan. He introduced a number of students to bryology.

In 1985, another new herbarium belonging to the Department of Botany, Shanghai Museum of Natural History, was opened in the west of Shanghai. It occupies 1,500 sq. metres, and the seven-floor building houses vascular plants, algae, fungi, lichens, bryophytes and ferns. More than 25,000 specimens of mosses and liverworts were collected, mostly in SE China, S. China and SW China in 1985.

Institute of Botany, Academia Sinica, Beijing, Republic of China.

Bryological Herbaria: Additions to the Compendium of Bryology, 1985.

SOSNOWIEC: HERBARIUM OF DEPARTMENT OF PHARMACEUTICAL BOTANY. Silesian Medical Academy in KATOWICE, Jagiellońska 4, 41-200, SOSNOWIEC, POLAND.

STATUS: Institutional
FOUNDATION: 1978
DIRECTOR: Krzysztof Jędrzejko/ synecology, phytosociology, influence of anthropopressure on bryophytes, floristics of bryophytes and vascular plants /medicinal plants / and nature protection.
CURATOR: J. Żarnowiec/ Musci. H. Klama/ Hepaticae.
CORRESPOND TO: K. Jedrzejko
SPECIMENS: MUSCI: 14,000
HEPATICAE: 6,000
GEOGRAPHICAL AREAS: Western Carpathians/Beskid Żywiecki, B. Ślaski, B. Mały/ and adjacent territories/Racibórz-Oświęcim Basin/, Silesian Upland and Upper Silesian Industrial District, KRAKOW-WIELUN Upland particularly.
LOAN: available
EXCHANGE: available
PUBLICATIONS DESCRIBING HERBARIUM: "Musci macroregioni meridionali Polaniae exsiccati" and "Hepaticae macroregioni meridionali Poloniae exsiccati".
IMPORTANT COLLECTIONS: K. Jedrzejko/original/; J. Żarnowiec/ original/; H. Klama/original/.

Chlorophyll Extraction in Mosses through Dimethyl Sulfoxide (DMSO)*

By Geert Raeymaekers

Introduction

DETERMINATION OF CHLOROPHYLL from plants has been used to estimate primary production or to assay physiological stresses. The extraction through 80% acetone has been used mostly, although the extraction with dimethyl sulfoxide (DSMO) is more reliable and time saving (Raeymaekers & Longwith, 1986). Although DSMO was known as a solvent for proteins and starch before it was used to extract photopigments from algae, there have been no reports, until recently (Alpert, 1984), on its use to extract chlorophyll from bryophytes.

The advantages of using DMSO to extract chlorophyll from mosses are numerous:

1. The chlorophyll can be extracted without maceration of the moss sample. This is important because many mosses have thick cell walls, hard stems and, in general, low chlorophyll concentrations.

2. The turbidity of the extract is very low.

3. More chlorophyll can be extracted with DMSO than with 80% acetone.

4. Chlorophyll is more stable in DMSO than in 80% acetone.

5. The DMSO method is faster than the acetone method and requires less time on the part of the researcher.

6. Dry weight can be obtained after extraction, if necessary, so that chlorophyll can be extracted immediately upon sampling. The DMSO on the sample can, in this case, be removed from the sample after the extraction by washing it in methanol, followed by drying to take the dry weight.

I have been using DMSO to extract chlorophyll from mosses without major problems. A drawback is that DMSO acts by increasing membrane permeability, and it is therefore advisable to prevent spilling the DMSO. DMSO by itself is not toxic, but will carry toxic substances through the skin. (Wash hands before working with DMSO). Rubber gloves are good but very "bulky" when handling small cuvettes, especially when cuvettes stick to the gloves. The extraction time differs among the mosses; this means that one should determine the optimal extraction for each moss (see note 1). The smell and taste of DMSO, or at least from its degradation products, is rather obnoxious. A spill on your hand will carry the solvent in a few minutes through your body up to the taste buds in your mouth!

Materials and methods

Material: Balance, dimethyl sulfoxide (DMSO, supplied by Fischer, Inc.), methanol, test tubes, test tube rack, aluminium foil, tissue papers (Kleenex, Kim wipes, etc.), drying oven, cuvettes and spectrophotometer (visible light).

Method: It is advisable to work in subdued light conditions to prevent any possible chlorophyll degradation.

Air-dry the moss samples for 24 hours in the dark room at room temperature. Do not dry at 80° C (often used in the drying of plant materials) since such a high temperature can degrade the chlorophyll. Store mosses in desiccator and take the air-dried weight (DW) of the moss sample. An approximate sample of 10-15 mg DW is sufficient. A larger sample may cause uneven extraction, or may need more DMSO. Rewet the moss sample, blot it dry between tissue paper and put it in the test tube. Fill test tube with 5 ml of concentrated DMSO. Cover test tube with aluminium foil cap. Do not use cork or rubber stoppers. DMSO has a corrosive effect on rubber and will extract pigments from the cork, yielding a brown extract. Put test tubes in test tube rack and cover test tube with aluminium foil. Place rack in pre-heated oven at 65° C. A water bath instead of the oven can cause condensation of water inside the test tubes. This will change the amount of solvent used, and the extractability of chlorophyll.

After the extraction time remove the test tube rack from oven. The extract should have a translucent green color. It is possible to store the extracts in the refrigerator for up to 64 hours. The DMSO freezes in the refrigerator, but this will not affect the chlorophyll. It will take a few hours to defrost the extract. (Although no statistical differences showed up after a storage of 64 hours in the dark at 4°C, there is a consistent exponential degradation during this time. It is thus better to go to the next step as soon as possible.) Pipet about 3 ml chlorophyll extract in a cuvette and read the absorbance of 750 nm (turbidity), 663 nm (chl a), and 645 nm (chl b) against a DMSO blank which has been put through the same steps as the samples. Rinse cuvette with methanol between readings.

The chlorophyll concentration can be calculated as follows, based on Arnon's equation (Arnon, 1949):

Concentrations in mg. Chlorophyll / g Dry Weight (see note 2).

$$C_a = (12.7\ A_{663} - 2.7\ A_{645}) * S/W$$

$$C_b = (22.9\ A_{645} - 4.7\ A_{663}) * S/W$$

$$C_{a+b} = (20.2A_{645} + 8.0A_{663}) * S/W$$

where S = amount of solvent (DMSO) in test tube (ml) and W = dry weight of sample in test tube (mg).

The 750 nm absorbance values should be low, (about 0.038 - 0.042). Skewed optical densities can be caused by detached leaves in the solvent. This happens occasionally, but normally the leaves will settle down below the beam path of the spectrophotometer.

Note 1.

Determine the optimal extraction time for each moss species you are dealing with. This can be done as follows: Proceed the same way as described above, but pipet at regular time intervals (e.g. every 2 hours) 3 ml into a cuvette and read absorbance at 750 nm, 663 nm and 645 nm. Return the pipeted solvent back to the sampled test tube and continue extraction. The optimal extraction time lies between 12 and 20 hours, depending on the species, being longer in xerophytic and less in mesophytic species. You should expect a degradation of some of the chlorophyll which shows up after the optimal extraction.

Note 2.

I have found that the peak positions of pure chlorophyll shift somewhat to the higher wavelengths in DMSO as compared to 80% acetone. (665 nm for chl a, and 649 nm for chl b), i.e. reading at 665 nm and 649 nm in DMSO solvent will result in higher absorbance values than at 663 nm and 645 nm. Nevertheless, I think that Arnon's equations are still usable to express relative changes in the chlorophyll content. A formula for the exact chlorophyll content using DMSO needs to be developed. The correct peak positions are known, but the exact extinction coefficients, based on the absorbance values of a known amount of pure chlorophyll a and b in DMSO, need to be calculated.

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[continued over]

* A contribution to the Techniques Notebook column, edited by Janice M. Glime. For her address, see Bryol. Times, 31:9.

AN APPARATUS FOR DRYING BRYOPHYTES IN THE FIELD *

By

J.-P. Frahm & S.R. Gradstein

EVERYBODY KNOWS how much of a problem it can be to dry bryophyte specimens during long field trips, especially in the wet climates of, for instance, the tropics or the arctic. Commonly-used methods, such as keeping the specimens in cotton sacks (flour sacks as available in food shops in tropical countries can be recommended), in plant presses or in nets, do not work well during rainy periods.

Drying bryophytes in plant presses, like phanerogams, is particularly time-consuming and tiring. It requires frequent changes of paper and leaves you with lots of wet paper and specimens still wet. Another method used less often is to spread the specimens on the floor of a tent, allowing access of wind through closed mosquito-nets. The advantage of this method is that the specimens can be left there unguarded for long periods and during short periods of rainfall. This is a better method than spreading all the specimens on soil where a sudden gust of wind can blow them away. Some long periods of low air humidity are also needed, however, to get rid of the moisture from the specimens.

The most effective and fastest method seems to be to dry the specimens over heat. Previous generations of botanists made camp fires of wood for this purpose. During the 1982 BRYOTROP expedition to Peru we used a kerosene stove surrounded by aluminium boxes and above it the wire-netting from a plant-press, on which the specimens were "toasted". However, this method needs constant attention as it requires turning and changing the specimens, hence taking lots of time to dry specimens from one-day "plundering" expeditions. More comfortable is the method described by T.B. Croat in Taxon, 28: 573-580 (1979), who does his fieldwork with a pick-up truck equipped with a professional herbarium dryer heated by butane.

For those people who do not have the opportunity to use a fully-equipped "herbarium - van" during their collecting trips, we recommend a small, but effective, light-weight structure (fig. 1). This type of specimen dryer is often used in wet, tropical surroundings by botanists from Utrecht and other places. The workshop of the Department of Engineering of the University of Duisburg has now constructed one for the 1986 BRYOTROP expedition to Borneo.

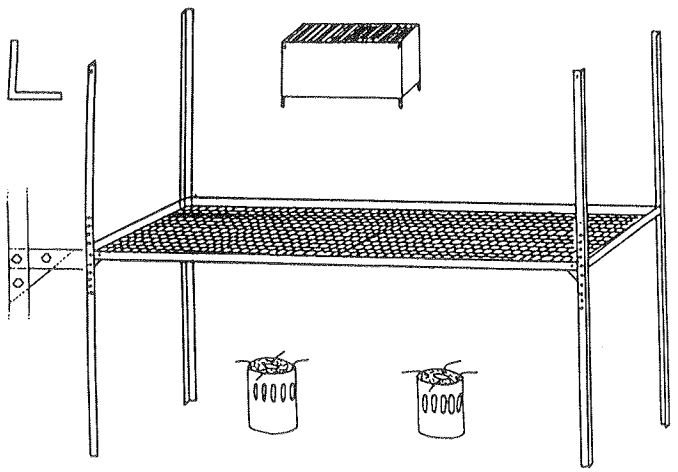

Figure 1. Details of dryer with accompanying kerosene stoves.

The construction of this dryer, as shown in fig. 1, is so simple that anybody can tinker with it himself. It is made from L-shaped aluminium pieces, and consists of 4 aluminium legs of ca. 1 m long, each connected by an aluminium frame to hold wire nets of plant presses. It is surrounded by a cotton curtain (polyester or nylon is more inflammable and should not be used). The structure is held together by a few screws and can be taken to pieces and packed into a bundle of less than 1 m in length and 2.5 kg. in weight. The total price of all the items required is less than U.S. $ 20.00. The dimensions can be altered easily and can be adapted to the dimensions of plant presses, or the amount of specimens to be dried. Also the height of the frame is variable and can be adjusted to suit the temperatures of the stoves. In our experience, one or two stoves are sufficient and their flames may be kept very low as the surrounding curtain keeps the heat inside very well.

The specimens can be placed on the wire nets, either in paper bags placed loosely on the frame to allow air to pass through, or in plant presses in an upright position (mixed with cardboard sheets) or as large folders made from newspaper (also in an upright position). Users of this method should regularly check the flames to prevent the specimens from becoming overheated (or even burnt!). The adjustment of the height of the flames is merely a matter of experience. Eventually, specimens can be left unguarded on the dryer, for instance, overnight, until dry. Dry specimens should be placed carefully in plastic bags to prevent them from picking up moisture from the air again. Preferably large, sturdy, plastic bags should be used for this purpose to protect specimens from damage during transport. Be sure, however, that there is absolutely no moisture left among the specimens packed in plastic, otherwise your collection may become more interesting to mycologists ...

The above construction can also be used in the lab with an electric heater, or can be modified for permanent use in the lab. In the latter case aluminium frames are not required, and wooden plates instead of the cotton curtain can be applied.

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* A contribution to the Techniques Notebook Column edited by Janice M. Glime. For her address see Bryol.Times, 31:9.

Chlorophyll extraction in mosses through dimethyl sulfoxide (DMSO) [continued from page 4.]

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Keeping them Dry*

By S.W. GREENE

READING THE PRECEDING article by Frahm & Gradstein (1986) reminded me of cold. windy, rain-filled days in the Southern Chilean rain forests where, in early 1976, Gabriela Hässel and I had to battle hard to dry specimens collected during field work for the Transecta Botanica de Patagonia Austral (Hässel de Menéndez, Greene & Matteri, 1984). Our dryer was made of chicken wire, and was affectionately called the "mouse-trap" - but that is another story!

To keep the dried specimens dry, we used activated crystals of silica gel ($SiO_2 .n H_2 0$) sealed into the tops of heavy-duty polythene bags with our specimens. The crystals were brought into the field in cotton bags and then, as required, were transferred into some of the empty paper bags we were using for our specimens. These crystal-filled bags were dried on the dryer along with our specimens to drive off adsorbed moisture. Once activated in this way one or two bags of crystals were sealed into each polythene bag as we finished filling it with dried specimens. Thus we tried to ensure that any moisture that might get in due to incomplete sealing of the neck of the bag would be taken up by the crystals and not the bryophytes.

The method worked excellently and we came home to no mycological nightmares! Since silica gel is very cheap and easy to obtain, it is well worth taking on collecting trips to humid parts of the world.

References

Frahm, J.-P. & S.R. Gradstein, 1986. An apparatus for drying bryophytes in the field. Bryol. Times, 38: 5.

Hässel de Menéndez, G.G.,Greene, S.W. & C.M. Matteri, 1984. The occurrence and distribution of bryophytes in southern Patagonia between latitude 51° and 52° S. J. Hattori bot.Lab., No. 55: 45-64.

Dept. of Botany, Reading University, Berkshire RG1 5AQ, U.K.

*Contribution to Techniques Notebook Column.

BRYOLOGICAL WORKS

Published by the late J. Cramer of Braunsweig.

BOOKS and other publications of the IAB published before summer 1985 by J. Cramer can still be ordered from J. Cramer publishers at the new address:

J. Cramer, Am Hasengarten 23A, 3300 Braunschweig.

i.e. the Compendium of Bryology, the Advances in Bryology I and II, and the Beiheft of Nova Hedwigia 71 "Bryophyte Taxonomy". Also all other Cramer publications from before summer 1985 can be ordered at this address.

Periodicals and other series previously published by J. Cramer are now continued by

Gebrüder Borntraeger Verlagsbuchhandlung, Johannesstrasse 3A, D-7000 Stuttgart 1, West Germany.

including the bryologically relevant series Advances in Bryology, Nova Hedwigia, Herzogia, (see Bryol. Times, 35:7), and Bryophytorum Bibliotheca. A full catalogue of the available Cramer books and serials can be obtained from the Borntraeger Publishing House at the above address.

BRYOPHYTORUM BIBLIOTHECA continues under new editors

BRYOPHYTORUM BIBLIOTHECA is a serial of works in the field of bryology consisting of volumes of at least one hundred printed pages in length. Manuscripts are printed in offset from camera-ready typescripts, and are in English, German or French. All manuscripts intended for Bryophytorum Bibliotheca should be sent to either of its new editors, Prof. Dr.J.-P. Frahm, Universität Duisburg, Biologie, Gesamthochschule Duisburg, 4100 Duisburg 1, Postfach 919, Lotherstrasse 65, West Germany: or Dr. S. R. Gradstein, Institute of Systematic Botany, University of Utrecht, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands. Manuscripts will be reviewed by at least 2 specialists before acceptance. Authors will receive 25 copies free, and further copies may be purchased at a discount from the publisher. Guidelines for the preparation of manuscripts are available on request from either of the editors.

S.R. Gradstein, Institute of Systematic Botany, State University of Utrecht, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands.

Postal Problems in Finland

WE ARE INFORMED that parcels and letters addressed to Finland have been returned simply marked "undeliverable" because a long strike of public employees paralyzed our postal services.

We are especially concerned because some of you may have sent us loans which we have requested. The Botanical Museum of the University of Helsinki still exists, and we are the same as ever, and are anxious to receive requested loans, to continue exchange programmes, etc.

So, if you had problems in reaching us, please try again!

Pekka Isoviita, Botanical Museum, University of Helsinki, Unioninkatu 44, SF-00170, Helsinki, Finland.

Language Assistance

A Progress Report

THERE WAS A VERY ENCOURAGING response to Janice Glime's notice (Bryol. Times, 35 : 6), announcing Language Assistance, an IAB scheme to assist colleagues with the presentation of papers in English. With offers of help coming from as far afield as New Zealand and Hawaii, the list of volunteers willing to correct manuscripts or make tape recordings of papers presently numbers 27. Several requests for assistance have been dealt with, and I hope that some working partnerships are now established.

Anyone wishing assistance or wanting to volunteer to correct manuscripts, or make tape recordings, is requested to contact the undersigned.

A.J. Harrington,
British Museum (Natural History),
Cromwell Road, London SW7 5BD,
England.

Recent Publications

Australas. Bryol. News, No. 14: 1-11, 1986.

Bryol.News Letter, Indian Bryol. Soc., No. 1: 1-29, 1984; No. 2: 1-28, 1985.

Bryologist, 88(4): 285-434, 1985.

Crypt. Bryol. Lichén., 7(2): 95-188, 1986.

Gradstein, S.R. (Ed.), 1985. Contributions to a monograph of the Lejeuneaceae subfamily Ptychanthoideae. Beihefte Nova Hedwigia, 80. 7-253. [Mededelingen van het Botanish Museum en Herbarium van de Rijksuniversiteit te Utrecht, No. 542.]

This volume comprises a collection of 10 papers by colleagues (Y. Asakawa, R. Matsuda, B. Thiers, C. Vanden Berghen) and students (M. van Slageren, R.Kruijt, T. Buskes, J. van Beek) of the editor, as well as contributions of his own. The main emphasis is on the taxonomy and distribution of members of the sub-family Ptychanthoideae, known in the older literature as the Holostipae: but data pertinent to the family as a whole are also included. The work is arranged in two sections, Section I (with 3 papers) being called Keys and Characters, while Section II (with 7 papers) is entitled Taxonomic revisions. The work concludes with an Index to taxa in which the use of different type faces shows at a glance those which are accepted names, new names and synonyms. Available from Utrecht, see Column 3 on this page.

Kumar, S.S., Sharma, M.L. & Sunita Anand, 1986. All India Conference on Bryology, February 25-27, 1986. Abstracts of contributed papers, invited lectures, list of participants. Chandigarh, Panjab University, 47 pp.

This slim paper-back volume gives an interesting insight into the range of topics discussed during the three-day meeting sponsored by the Panjab University, Chandigarh, and the University Grants Commission, New Delhi, and held at the Department of Botany, Panjab University, Chandigarh, India. The meeting was convened by Professor S.S. Kumar, and from the list of participants, appears to have been attended only by bryologists from India.

An inaugural talk was given by R.S. Chopra (Bryophytes - A view), and there were five invited lectures, viz: P.N. Mehra (Experimental observations in vitro in some hepatics); R.N. Chopra (Regeneration in bryophytes); G.C. Mitra (moss protonema - its morphological nature); and D. D. Pant (Palynology of bryophytes and a second lecture entitled Fossil history of bryophytes). There were about 40 contributed papers on a variety of topics, including morphogenesis, ultrastructural studies, chemical constituents, taxonomy, floristics and ecology.

Li, Xing-jiang (Ed.in Chief),1985. Bryoflora of Xizang. The Comprehensive Scientific Expedition to the Qinghai-Xizang Plateau, Academia Sinica. (The series of the Scientific Expedition to Qinghai-Xizang Plateau.). Beijing, China Science Press. 581 pp. with 238 line drawings. Hard covers.

This illustrated flora of the mosses and hepatics of Tibet has a list of 17 editors, the first of whom is cited as editor-in-chief. Dr. Li is a staff member of the Kunming Institute of Botany, Academia Sinica, Helongtan in Yunnan. The author(s) of each family is given in footnotes throughout the text. The text is almost entirely in Chinese, but Latin nomenclature is used as is Latin for the diagnoses of taxa described as new. Keys to genera and species are provided within families, but there is no general key to the mosses or the liverworts. Basionyms and some synonyms are cited with their places of publication, but where a taxon has been described in a Chinese publication, the reference to the latter is in that language. The work lacks a bibliography, but there is a 25-page consolidated index of accepted taxa and synonyms.

The precise date of publication is not known, but the dedication on the reviewer's copy is dated June 1985. Otto Koeltz of Koenigstein, West Germany, first advertised the flora as a new work on 25 April 1985 (at a price of DM 62). Some information in English on the background to this work will be found in Mu, Z. & X-j Li, 1982, (Beihefte Nova Hedwigia, 71: 337-339) and in Wu, P-c, 1984 (J.Hatt. Bot. Lab., No. 56: 29-38).

The editor-in-chief and all involved in the great amount of work that must have gone into the writing of this first bryoflora of Tibet are to be congratulated on its completion in what has surely been a remarkably short period of time. While respecting their reasons for publishing the work in their own language, a summary in English or one of the other major languages familiar to bryologists the world over would have greatly increased the usefulness of this important work. It might also have given some understanding of the extent of field work that has taken place in Tibet, the amount of material studied and its location, the basis of the taxonomic concepts used, how much it was possible to check the identity of Chinese material against specimens from other parts of a taxon's range; how much use was made of type material, etc., etc. But all in all, this flora is a very welcome contribution to bryological literature.

VAN SLAGEREN, M.W., 1985. A taxonomic monograph of the genera *Brachiolejeunea* and *Frullanoides* (Hepaticae) with a SEM analysis of the sporophyte in the Ptychanthoideae. Published as Mededelingen van het Botanisch Museum en Herbarium van de Rujksuniversiteit te Utrecht No.544, 309 p., 54 plates, December, 1985. (Also published privately as a thesis, 15 November, 1985.). Price D. fl. 30 or US $ 10.-. Send orders to Mr. T. van Geffen, Sales Department, Institute of Systematic Botany, P.O. Box 80.102, 3508 TC Utrecht, The Netherlands.

This is a richly illustrated monographic treatment of two New World genera of the liverwort family Lejeuneaceae. All species are fully described and illustrated. Keys and distribution maps are provided, as well as detailed analysis of the sporophyte of the subfamily Ptychanthoideae based on the results of scanning electron microscopy. Traditionally treated as subgenera of one single genus, *Brachiolejeunea* and *Frullanoides* are shown to belong to different, newly-recognized tribes of the Ptychanthoideae, characterized by their fundamentally different sporophyte structure.

S.R. Gradstein, Institute of Systematic Botany, University of Utrecht, The Netherlands.

De Zuttere, P., J. Werner & R. Schumacker, 1985. La bryoflore du Grand Duché de Luxembourg: taxons nouveaux, rares ou méconnus. Travaux scientifiques du Musée d'Histoire Naturelle de Luxembourg, Vol. 5: 1-153. [Issued March, 1985. Available from Musée d'Histoire Naturelle, Marché-aux-Poissons, L-2345, Luxembourg.].

Although this work is basically an alphabetical checklist of the 111 liverworts and the 342 mosses now known from the Grand-Duché, 381 of which are new, rare or poorly-known taxa since the publication of Koltz's Prodrome over 100 years ago, it contains much additional information of value. Thus ecological data are given for each taxon, as is a statement of the European phytogeographical element to which each belongs. The distribution within the country is itemized for each of two major districts, Oesling to the north and Gutland to the south. All records are documented by reference to specimens. For 41 of the taxa distribution maps are provided based on a 1 kilometre grid system.

DIARY

For explanation of acronyms, see Bryol. Times, 31: 7-8, 1985.

July 20-30. DBLWG. Summer field camp nr. Champagnole, French Jura. Further information from: Han van Dobben, Mariaplaats 16, 3511 LJ Utrecht, The Netherlands.

July 23-30. BBS. Summer field meeting (1st week), Fort William. Local Sec.: Mr. G.P. Rothero, Benmore Centre, By Dunoon, Argyll, Scotland.

July 28- 2 Aug. NBS. Summer excursion - annual meeting, Fröskog, Dalsland, SW Sweden. Organizer, T. Hallingbäck. Further information from Sven Fransen, Botaniska Institutionen, Carl Skottsbergsgata 22, 413 19, Göteborg, Sweden.

July 30 - 6 Aug. BBS. Summer field meeting (2nd week), Gairloch, Wester Ross. Local Sec.: Mr. D.G. Long, Royal Botanic Garden, Edinburgh EH3 5LR. For details of both weeks, see Bull. BBS, 47.

Aug. 2-4. BSJ. 15th Annual Meeting at Beppu, Oita-ken, with paper-reading sessions. Excursion to Shinyabakei Gorge, and photo contest. Further information from Mr. M. Ohtsuku, Minamit suruta Shinden 790-17, Usa-shi, Oita-ken, 870 Japan.

Aug. 10-14. ABLS. Annual Meeting, University of Massachusetts, Amherst. Further details from the Program Chairman, Dr. William L. Culberson, Dept. of Botany, Duke University, Durham, North Carolina 27706 (Tel. 919-684-3715). For details of pre-meeting field excursion to places in southern Vermont, contact Dr. Cyrus B. McQueen, Dept. of Botany, University of Vermont, Burlington, Vermont 05405-0086 (Tel. 802-656-2930).

Aug. 26-29/30. CEBWG. Leipzig, DDR. For preliminary announcement, see Bryol. Times, 34:8.

Aug. 28-1 Sept. SBLS. Field Meeting. Fideriser Heuberge (Prätigau, Ct. Grison). Further information from Klaus Ammann, Syst.-Geo-Bot. Institut, Altenbergrain 21, CH-3013, Bern, Switzerland.

Sept. 7. VWGB. Field trip to Lummen and Herk-de-Stad, Prov. of Limburg. Meet 09.30 hrs. at the church in Lummen.

Sept. 12-14. BLAM. Field Meeting in Nordschwarzwald, BRD, based on Karlsruhe. Organizer and leader, Dr. G. Philippi, Erbprinzenstr. 13, 7500 Karlsruhe, B.R.D.

Sept. 19-21. Eleventh annual LeRoy Andrews Foray. Peconic Dunes Camp of Suffolk County, Long Island, New York. Further information from Dr. W.R. Buck, New York Botanical Garden, Bronx, NY 10458-5126, U.S.A.

Sept. 20-21. BBS. A.G.M. and paper-reading meeting. University of Leeds. Local Sec.: Prof. D.J. Cove, Dept. of Genetics, University of Leeds, Leeds LS2 9JT. For preliminary details, see Bull. BBS, 47.

Oct. 3-5. 2nd Blomquist Bryological Foray, Tennessee. Staying at Pickett State, Rustic Park, Jamestown, Tennessee. Further details from Dave Smith, Dept. of Botany, University of Tennessee, Knoxville 37996, U.S.A.

Oct. 4-5 or 11-12. SBLS. Taxonomic Workshop with Dr. J. Váňa on *Jungermannia* and *Marsupella*. Zürich, Botanical Institute of the University. Further information from Klaus Ammann, Syst.-Geo-Bot. Institut, Altenbergrain 21, CH-3013, Bern, Switzerland.

Oct. 5. VWGB. Field trip to Oosterzele-Balegem, Prov. of Oost-Vlaanderen. Meet 09.30 hrs. at the church in Oosterzele.

Nov. 1-2. BBS. Taxonomic Workshop, University of Reading. Local Sec.: Dr. R.E. Longton, Dept. of Botany, The University, Reading RG6 2AS, Berkshire, England. For details, see Bull. BBS., 47.

Nov. 9. VWGB. Field trip to Hoogaarden-Meldert, Prov. of Brabant. Meet 09.30 hrs. at the church in Hoogarden.

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April 1-8. BBS. Springfield meeting, Penzance, Cornwall. Local Sec.: Mrs. J.A. Paton, Fair Rising, Wagg Lane, Probus, Truro, Cornwall TR2 4JU. For details see Bull. BBS, 47.

July 17-23. IAB. Mainz. Bryological Methods Workshop. For further information see Bryol. Times, 34:7.

July 24-1 Aug. XIVth IBC Berlin (West). Preceded by IAPT Nomenclature Session, July 20-24. For Second Circular, see this issue, page 8. Congress address: XIV IBC. Bot. Garden and Museum, Königin-Luise-Str. 6-8, D-1000, Berlin (West) 33, Germany.

July or Aug. BBS. Summer field meeting, Achill Island, Co. Mayo and Westport. Local Sec.: Dr. D.M. Synnott, National Botanic Gardens, Glasnevin, Dublin 9, Ireland. For preliminary notice see Bull. BBS, 46.

Sept. BBS. A.G.M. and paper-reading meeting, Wye College, Kent. Local Sec.: Dr. M.A.S. Burton, Paris House, East Malling, Maidstone, Kent ME19 6AU. For preliminary notice, see Bull. BBS, 47.

Nov. BBS. Taxonomic Workshop. University of Manchester. Local Sec.: Dr. S.R. Edwards, The Herbarium, Manchester Museum, The University, Manchester M13 9PL. For preliminary details see Bull. BBS, 47.

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June 6th. CEBWG Meeting, Liblice (Village near Mělník), Czechoslovakia. Further details will be announced later.

THE INTERNATIONAL ASSOCIATION OF BRYOLOGISTS published The Bryological Times every two months, the Bulletin of Bryology twice a year and the Advances in Bryology every two years. Material for the Bryological Times can be sent at any time, but submission dates for the Bulletin and the Advances should be discussed with the Editors, Dr. Diana G. Horton (University of Iowa) U.S.A. and Dr. Norton G. Miller (Albany) U.S.A. respectively. The Editors do not accept responsibility for the views of authors.

For details regarding members of the International Association of Bryologists (currently U.S. $8.00 p.a.), write to the Honorary Secretary, Dr. S.R. Gradstein, Instituut voor Systematische Plantkunde, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands.

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ITEMS FOR THE NEXT ISSUE to be with the Editor, Dr. S. W. Greene, Department of Botany, The University of Reading, London Road, Reading RG1 5AQ, Berkshire, England (Telex 847813 RULIB) by the 1st of August at the latest. Items for the regular columns should be sent direct to the column editors, whose names and addresses will be found in Bryol. Times, 31:9, 1985.