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ferences in size and external sculpture, and was accompanied by the evolution of systems to ensure or to facilitate the meeting of the two sexes, and eventual syngamy. This evolution of heterospory cannot be divorced from the evolution of spore traps and the associated chemical and morphological recognition. The seed habit itself, a phylogenetic trend of which heterospory is an integral part,[16] consists of a system of close cooperation between the two sexes, as complex as any in the animal kingdom. The 'existence' of strong co-adaptation between the sexes necessarily creates strong stabilising selection upon the recognition system.

An organism's specific-mate recognition system is of necessity strongly adapted to the natural habitat of the species. It therefore follows that speciation is a process of adaptation. The Palaeozoic arborescent lycopods of the northern hemisphere were large trees, and they flourished in great numbers in the tropical coal forests. The southern hemisphere Gondwana arborescent lycopods, however, were of a more modest size and grew in a strongly seasonal environment in the wake of a long period of glaciation. The group appears to have been adapted to live in close proximity to water—the simple vegetative and rooting structures, lacking any secondary phloem, would restrict the trees to such habitats. Also, the very large megaspores, up to 6 mm in the case of *Cyclodendron leslii*,[6] would almost certainly have been dispersed by water. The large spines of the dispersed megaspores may thus have functioned in binding numbers of these spores together in floating rafts, at a time when showers of microspores were raining down upon them. The presence of the male counterpart attached to the surface, between the spines, is evidence for this. There was some means by which conspecifics adhered, while others of different species did not. Being in close proximity in this manner would facilitate fertilization. The wet surface of the spores would be an ideal medium for the passage of motile antherozoids when archegonia were exposed.

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General problems encountered by the postgraduate first-time electron microscopist

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This article identifies the main problems encountered by the postgraduate first-time electron microscopist. Areas where difficulties arise occurred in the communication and planning of the project, general specimen preparation, safety in the laboratory, and in the use of the electron microscope facility. It is recommended that all students who wish to use the electron microscope as a research tool should attend a short course in EM organised by the EM unit staff in conjunction with the various academic departments whose students make use of the facility, before embarking on their research topic.

Electron microscopy (EM) in South Africa has been hampered by a lack of suitably trained staff familiar with the basic techniques of the discipline. This has long been recognised by the Electron Microscope Society of Southern Africa, and it is mainly through its efforts that the Technikon Pretoria now offers a diploma course in electron microscopy. However, a large proportion of potential electron microscopists are graduates in the biological and physical sciences who undertake postgraduate studies which involve the use of the electron microscope. It is unlikely that these students will avail themselves of the technikon's diploma course to acquire expertise in electron microscopy, and in general are dependent on their project supervisors, electron microscope unit staff and the goodwill of experienced electron microscopists for advice on the techniques of the discipline. Little is known of the problems which such students encounter, if any, in acquiring basic knowledge and skill in EM and applying these to their research investigations.

This article is an attempt to identify the main problems encountered by first-time EM users; to bring these to the attention of project supervisors and novice electron microscopists as well as to offer advice so that these difficulties can be avoided.

In order to gather background information, 17 staff members of nine electron microscope units (at the universities of Cape Town, Durban-Westville, Medunsa, Natal (both Durban and Pietermaritzburg campuses), Pretoria (Onderstepoort), Rhodes, the Witwatersrand, and the South African Institute for Medical Research) were interviewed using open-ended discussion. Three lecturers who supervised postgraduate student projects involving electron microscopy were also questioned as well as eight students who had either just completed a postgraduate course in electron microscopy or were familiar with EM-related projects. All were asked to describe general or specific problems that they had encountered either with first-time EM users or in their own initial dealings with EM, how their expectations had compared to the realities of the technique, what they should or should not have done given the benefit of hindsight, and so on.

These discussions identified both general concerns with principles of research unique to electron microscopy, and specific difficulties dealing with particular preparative techniques or specimen types. This article will deal with the general concerns only; the specific problems will be discussed elsewhere.

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Problems and solutions

Four main areas of conflict were identified during the discussions; those which arose in communication and planning of the project; general specimen preparation; safety in the laboratory; and in the use of the electron microscope facility. The following paragraphs will offer suggestions to overcome these conflicts, thereby dealing with both the problem and the solution simultaneously. No statistical analysis of the results was possible because of the open-ended nature of the information gathered.

1. *Communication and planning*

● Students and supervisors should have discussions with EM unit staff and other experienced electron microscopists before starting a project and should ask questions regarding the following:

– Is an electron microscope really necessary or can light microscopy be used? Remember, electron microscopy is slow, time-consuming and costly—if there is an easier and quicker method, use it.

– Which type of electron microscope, scanning or transmission? Find out what other instruments are available—diffractometers for crystal structure; ion beam etchers to remove layers of material, etc. They might need to be used instead.

– How much time is available for the project and is this enough for the scope of the work?

– What preparation techniques (if any) need to be used? Some specimens do not require any preparation.

● Time should be spent planning the project. A proper literature survey is vital and students should find out what is known about the organism or specimen. Consulting atlases to identify structures is essential.

● Tell experienced electron microscopists what you want to do and bring micrographs and key articles to show what it entails. Ask them if they think your goals are feasible.

● Textbooks on electron microscopy are for recipes and guidelines only, so should not be used as a sole reference source. Remember, books are drawn from published papers and not everything may appear in the textbook. Consult the original paper, but keep in mind that not everything is reported in articles either.

● The student should constantly ask himself what he wants or hopes to see.

● If this groundwork is not done, the student can end up doing three times the amount of work required—or abandoning a worthwhile project in despair.

2. *General specimen preparation*

● Students should learn the technique of electron microscopy using a simple, uncomplicated specimen. They need to master the methodology before they can successfully address the research topic.

● Use the established processing procedure of the laboratory you are working in, in preference to a technique you have picked up from somewhere, unless there is a very good reason to use that method, as in the case of histochemistry or some form of micro-analysis. A routine investigation requires only a routine processing regime.

● Get someone to demonstrate the EM techniques. Listen carefully during the demonstration; little points regarding technique which never appear in the literature make all the difference.

● Always allow sufficient time to carry out a procedure and never skimp, rush or cut down on processing times. If you can see you might not have enough time to finish a procedure, do not start it! In the case of an emergency, quick processing can be done, but be prepared to accept failure. For experimental work always do processing the long way and the same way—do not change the procedure half way through the investigation.

● Always follow the solution recipes and time schedules *exactly*. Common faults are not mixing resins properly and leaving blocks curing in the oven for several days after they have hardened.

● Never use old chemicals which are lying around in the laboratory.

● Think! It is useless to prepare, for example, scanning electron microscope specimens with care, then to touch the surface of interest with the fingers or bring the specimens to the EM unit exposed to dust and dirt in open containers.

● There is no substitute for processing tissue correctly the first time.

● Published pictures are perfect pictures. Students should not be disheartened if they cannot achieve the same result initially.

3. *Safety in the laboratory*

● Students must be made aware of the safety precautions regarding the hazardous solutions used in electron microscopy.

● Gloves should always be worn when working with harmful solutions. These must be resistant to the chemicals worked with. Remember that alcohol can facilitate the penetration of harmful chemicals through the glove into the skin.

● Cleanliness which extends beyond personal care should be exercised. For instance, the EM novice very properly wears gloves but leaves a trail of sticky fingerprints around the laboratory and on the instruments, to contaminate these for the next user.

● Always use a fume cupboard.

● Always dispose of waste chemicals according to a schedule approved by the relevant authorities.

● As a general principle it should be kept in mind that everything used in electron microscopy is bad for you.

4. *Electron microscope units*

● Ask about the rules and regulations of the unit and keep to them. They are there to satisfy the majority of users and to keep the facility running smoothly.

● The staff will be able to teach and advise the student about techniques but are not there to do the experimental work for them, nor to take on the role of supervisor. It is only courtesy for the supervisor to tell the EM unit staff of the prospective student and project.

● Inform the EM unit staff all about your specimens, for example those set in Perspex, which can contaminate the microscope. Tell them of any disasters which occur while using unit equipment, such as when you have lost a specimen in the column.

● Acknowledge EM unit staff in published papers or make them co-authors if their contribution as regards creativity and responsibility warrants it.

Conclusions

From this survey it appeared that it was the EM unit staff in the main who performed the educating role in the training of first-time electron microscopists, with very little acknowledgement for all they do. Generally, this situation arises not through choice nor prior arrangement, but simply because they are the ones who are ultimately presented with all the problems resulting from poor supervision, impossible projects, broken equipment and so on. The success or failure of this educating role of the EM unit staff seems to depend mainly on the interaction between the project supervisor and the EM unit staff. It was striking to note that those students, supervisors and staff of EM units with fewest complaints regarding the training of first-time electron microscopists occurred with students who had undergone a course in EM as part of their postgraduate study, and this of necessity involved both the academic and technical staff.

I believe there is a need to persuade supervisors of student projects to communicate more with EM unit staff regarding the suitability of projects. Furthermore, all students who wish to use the electron microscope as a research tool should attend a short course on the technique organised by the EM unit staff in conjunction with those academic departments whose students make use of the facility, before embarking on their research topic.

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