

PROTIST NEWS

Meeting Report: Second European Phycological Congress, Montecatini Terme, Italy, September 20–26, 1999

Is it a good omen that when phycologists meet on a European level for their second time a famous Italian resort town gets almost flooded by exceptionally heavy rainfalls? Definitely yes, because this was a very successful and particularly enjoyable meeting and the weather cleared up almost immediately when the opening ceremony started. Due to the excellent organisation of the Congress and the nice setting of Montecatini Terme it was easy to recapture the excitement of EPC1 in Cologne (Germany) three years ago, as M Dring, Chairman of the Scientific Committee, pointed out at the end of the Congress.

According to a list of delegates circulated at the Congress, there were 373 participants registered from a total of 39 countries (26 in Europe, 13 outside). This meeting included four plenary lectures, 12 morning symposia, 17 mini-symposia with contributed papers and 16 poster sessions with about 200 posters that were on display throughout the Congress. The posters were truly a highlight and 2 hours on each working day of the Congress were set aside to viewing and discussing the posters.

A good balance of contributions concerning photoautotrophic protists (or eukaryotic microalgae), photosynthetic prokaryotes and marine macroalgae was reached. Below we will report on a selection of some highlights of this Congress; other topics not mentioned here may be no less important.

A major focus of the Congress was on the various aspects of biodiversity of algae (including cyanobacteria), i.e. half of all symposia and a quarter of all contributed papers and posters were dedicated to topics such as evolution, speciation, and population genetics. Contributions to these themes may be roughly grouped into those concerning the molecular species concept, population genetics and microalgal diversity, and evolution of plastids and other endocytobioses.

The series of four morning plenary lectures started with a review by M Melkonian (Cologne, Germany) on his 25 years of work on the algal basal apparatus. The basal apparatus is the centrosomal equivalent of flagellate eukaryotes, present in many protists and simply holds flagella and cilia together. After a period dominated by "ultrastructural systematics" it was the centrin Ca^{2+} -binding family of proteins, first discovered in the rhizoplast of the green flagellate *Tetraselmis*, that transformed studies on the basal apparatus "from obscurity to a central position in cell biology". Centrin was shown to be a universal centrosomal protein in eukaryotes including humans. A long series of studies concerning proteins associated with the different elements of the algal basal apparatus as well as on flagellar and basal body development cycle in algae was conducted in the laboratory of M Melkonian. Many of these studies used purified, structurally and functionally intact cytoskeletons and basal apparatuses of the minute green flagellate *Spermatozopsis similis* ("a swimming cytoskeleton") as a model.

Molecular Species Concept

Using a molecular species concept means that only monophyletic groups as they appear in gene phylogenies are accepted, but not paraphyletic taxa. In his review from the viewpoint of a journal editor, WT Stam (Groningen, Netherlands) advocates to describe new species and genera strictly following the rules of a phylogenetic species concept (PSC). Taxonomic changes should be made wherever possible. However, the present nomenclatural rules do not match the PSC and the nomenclatural system should better be changed into a set of rules based on evolution. The pitfalls of the molecular species concept are taxon sampling, stability of the tree and

whether the right sequence (marker molecule) has been chosen to construct the tree. The following three presentations showed intriguing examples on how species of microalgae can be defined based on molecular evidence. A molecular species definition is inevitable in cases where morphological and ultrastructural characters are insufficient. For example, strains of the asexually reproducing coccoid green alga *Coccomyxa* exhibit almost no morphological differences. Species (monophyletic groups of strains) of *Coccomyxa*, however, can be distinguished using differences in the tip regions of the hairpin loops of the secondary structure models from both ITS (Internal Transcribed Spacer of the rRNA operon) regions (T Friedl, Göttingen, Germany). Most of the studies associated with the molecular species concept employed nuclear-encoded ITS-1 and/or ITS-2 rDNA sequences. All three speakers emphasized that the construction of secondary structure models for both ITS regions is essential to achieve an unambiguous alignment of these highly variable spacers. In addition, also plastid-encoded Rubisco-spacer sequences may be valuable tools for resolving species boundaries as was demonstrated by T Ichimura and coworkers (Muroran and Sapporo, Japan). In contrast, nSSU rDNA sequences usually do not show enough divergence to allow phylogenetic analyses of species or mating groups, as shown for *Closterium* (T Ichimura et al.) and the green colonial flagellates of the order Volvocales (AW Coleman, Providence, USA). Group I introns may also be useful for distinguishing monophyletic species (e.g. in *Closterium* and other Zygnematales). Group I intron sequence comparisons, however, can reveal phylogenies incongruent with those based upon ITS-1/ITS-2 sequences because of lateral gene transfer as shown for the lichen green alga *Trebouxia*. Sexual isolation may lead to a great molecular diversification, e.g. in *Closterium*, while few molecular differences may be indicative of asexual reproduction (T Ichimura et al.). In the Volvocales, ITS-based groups correspond precisely with mating groups (AW Coleman). Interestingly, when two strains of Volvocales differed by a compensating base exchange from each other, then mating was not possible.

An overview was given on the rather diverse and very widespread group of picoplanktonic prasinophytes by MJ Chretiennot-Dinet and coworkers (Banyul-sur-Mer and Roscoff, France). Once again, the multiple origins of this basal group of green algae were demonstrated using 18S rDNA sequences. Coccoid forms developed in almost all lineages of prasinophytes independently. The peculiar "budding"-type of reproduction in the coccoid or

pseudofilamentous green algae *Marvania* and *Pirula* were found to be a special type of autospore formation in the detailed ultrastructural study by BJ Foth (Parkville, Australia) and coworkers. 18S rDNA sequence analyses showed *Marvania* and *Pirula* to be members of two independent classes with *Pirula* being most closely related to *Ulva* (Foth et al.).

That 18S rDNA sequence analyses, though now regarded as a classical molecular marker in algal phylogenetics, can still be a real challenge, as shown by M Klingberg and coworkers (Cologne, Germany) for the Euglenophyta. Euglenophyte 18S rDNAs are so diverse that the conventional PCR primers cannot bind and these sequences exhibit an enormous length variation, i.e. some euglenophyte sequences are up to 3 kb long. Molecular phylogenies revealed that the generic concepts of euglenoids need to be reassessed, although they also clearly supported some earlier views based on morphology. The genus *Euglena* is polyphyletic. Non-plastidic colourless forms are basal to the green euglenophytes which are monophyletic. Leucoplast-containing taxa evolved independently in several advanced lineages of the Euglenophyta.

Support for *Coleochaete* as being closest to the common ancestor of embryophytes comes from analyses of the *GapCp* gene which originated from a *GapC* gene duplication early in the evolution of streptophytes (J. Petersen and co-workers, Braunschweig, Germany).

After major breakthroughs using nuclear-encoded SSU rDNA sequences in the first half of the 1990s, the general picture of evolutionary lineages within the diverse phyla of algal protists seems to be generally accepted. Since the current status of our understanding of algal phylogenies may have already been well-reflected on at the 16th International Botanical Congress in St. Louis (USA) just a few weeks before EPC2, there was apparently no need for more general reviews. This may explain why most presentations focused on single groups and taxonomic problems at the genus and species level.

Population Genetics and Microalgal Diversity

The value of maintaining and studying multiple strains of microalgal species in culture collections was highlighted by SI Blackburn and co-workers (Hobart, Australia). Using molecular tools they were able to show a great diversity between strains and populations within a single morphological species. However, there is less within-population diversity for the prokaryotic cyanobacteria compared with eu-

karyotic microalgae. Some dinoflagellate morphospecies may contain several biological species, and interbreeding compatibility closely aligns with molecular genetic patterns of diversity.

There is a significant genetic diversity among clonal isolates of the same species from different phytoplankton populations as LK Medlin (Bremerhaven, Germany) reported. Using different molecular tools an unexpected diversity was discovered in organisms ranging from diatoms, dinoflagellates, to haptophytes and even to viruses or bacteria attacking green and heterokont algae. These studies showed that no single clone of any phytoplankton species can be considered truly representative of that species, but the establishment of clonal cultures has so far been a necessity prior to any analysis. Molecular techniques will allow us to circumvent the need to make clonal isolates for analysing the genetic structure of phytoplankton populations. In that context, ribosomal RNA probes are particularly promising tools because they can be used in a broad range of applications (R Groben and LK Medlin, Bremerhaven, Germany). The high specificity of such probes allows the unambiguous identification of taxa even from environmental samples. In addition, there is the possibility to design hierarchical probes for the identification of microalgae at various taxonomic levels, i.e. from phyla to genera or even species. Ribosomal RNA probes have been developed by M Lange (Bremerhaven, Germany) and co-workers that allow the unambiguous identification of toxic stocks of the dinoflagellate, *Alexandrium tamarense*, which causes paralytic shellfish poisoning. A test system is now available for the detection of even small amounts of toxic dinoflagellates in field samples. Pitfalls of the rRNA probe technique are that it may be difficult to reduce autofluorescence, which may sometimes be very strong, and that cell walls may prevent penetration of the probes. However, Groben and Medlin were optimistic to overcome these problems and already foresee the realization of an automatic identification system for microalgal populations using flow cytometry in the near future.

Another stimulating project that still awaits realisation in the future is a new identification aid for diatoms that may find a wide application, e.g. in environmental research, where diatoms are used as an important tool: ADIAC (Automatic Diatom Identification and Classification) presented by MM Bayer (Edinburgh, Scotland). A motorized microscope equipped with a digital camera automatically scans microscope slides with diatom samples. The system records only those valves that match best stored images of diatom valves. By means of pattern recogni-

tion methods, an intelligent software carries out diatom identification using both valve outline and ornamentation. The basis of ADIAC is a large taxonomic data base with maximum quality digital images of diatoms. As Bayer explained, the problem is to successfully extract morphological characters from a complex background, i.e. striation patterns of the diatom valves. Even if the project should fail, Bayer said, we will have learned much about new techniques to diatom morphology and to describe shape and symmetry axes of diatom valves.

While the morphology of empty dead diatom valves has always fascinated hundreds of phycologists and botanists, the living protoplast and sexuality of diatoms has been the focus of less than a dozen researchers. Therefore, D Mann (Edinburgh, Scotland) in his stimulating plenary talk tried to make the audience look at live diatoms. Sex in diatoms is quite costly as it means an interruption of synthesis. But it is inevitable because of the ongoing size reduction during cell divisions of diatoms, which may continue up to ten years. Therefore, sex is a relative rare event in the life cycle of a diatom in nature, as Mann reported from his long-term direct observations of many diatom species that were mostly from beautifully located lochs and lakes in the Scottish highlands. Size restoration, always associated with sexuality in diatoms, may take only 0.5–2 weeks. However, at least in the Pennales, below a certain threshold size, no sexual reproduction is possible. In diatoms there is an evolutionary trend from oogamy, as in the Centrales, to isogamy associated with the loss of flagella in the Pennales. In SSU rDNA analyses, the centric diatoms form a paraphyletic group that is basal to the radiation of the Pennales which is monophyletic.

Evolution of Plastids and Other Endocytobioses

JD Palmer (Bloomington, USA) in his plenary lecture gave a comprehensive review of present views of plastid evolution. There is increasing evidence that the three lines of primary plastids (those of chlorophytes, rhodophytes, and glaucocystophytes) trace back to the same cyanobacterial endosymbiosis. This evidence comes from genome architecture and gene order as well as from phylogenies of different plastid genes. The same conclusion is drawn from phylogenies based on whole plastid genomes and gene cluster organization (KV Kowallik and B Stöbe, Düsseldorf, Germany with W Martin, Düsseldorf, Germany). However, a different view was expressed by K Valentin and colleagues (Giessen, Germany)

when presenting a phylogeny based on a multigene alignment of plastid-encoded proteins including those from the thermoacidophilic unicellular red alga *Cyanidium*. Valentin's and his coworkers' results showed independent evolutionary origins for green and red plastids.

Secondary endosymbioses by which green or red algal cells were captured and used as plastids occurred many times. This view was once more supported by most recent data from plastid large ribosomal subunit RNA sequences (JD Palmer). Whether the apicomplexan plastids, the most reduced plastid genomes known, are derived from a secondary endosymbiosis event with a red or a green algal-like organism is still unknown. For the chlorarachniophyte plastid which is unequivocally of green algal origin, Palmer suggested that it arose from a charophytic alga. Tertiary endosymbioses is when, for example, a haptophyte that has a plastid derived from a secondary endosymbiosis, was captured by a heterotrophic host. This may have been the origin of the plastid in the toxic dinoflagellate *Gymnodinium breve*. The peridinin-type plastid in dinoflagellates, however, may be of a red algal origin.

Whole organelle genomes have recently been sequenced and these sequences allow detailed insights into their evolution through endosymbiogenesis. UG Maier (Marburg, Germany) presented an update of the almost completed nucleomorph genome of the cryptomonad *Guillardia theta*. In *G. theta*, the genome consists of three very short chromosomes. Analysis of the nucleomorph's gene content demonstrated that it provides genes for maintaining itself and a couple of plastid functions. Secondary endosymbiosis between two eukaryotes may have been only possible when it was preceded by a primary eukaryote/prokaryote endosymbiosis. New intriguing aspects for the endosymbiotic origin of mitochondria were discussed in a paper by C Kurland (Uppsala, Sweden) along with co-authors. The complete genome sequence of the obligate intracellular parasite *Rickettsia prowazekii* (C Kurland and coworkers) revealed striking similarities to mitochondrial genomes, e.g. the lack of many genes involved in the regulation of amino acid biosynthesis as compared to free-living bacteria. Phylogenetic analyses showed *R. prowazekii* to be more closely related to mitochondria than is any other prokaryote studied so far.

Genetic Transformations in Algae

Later than in other botanical fields, genetic transformation became a tool for phycologists. In a symposium dedicated to genetic transformations in algae

the recent progress in that field was summarized and possible applications of transformation systems were discussed. K Apt (Columbia, USA) and colleagues were able to analyse the nuclear-encoded plastid targeted *AtpC* polypeptide. A fusion between the *AtpC* presequence and Green Fluorescent Protein (GFP) was transformed into the diatom *Phaeodactylum*. Sites of targeting and GFP accumulation were visualized by fluorescent microscopy and were readily distinguished between cytoplasm, plastid ER lumen and plastid membranes. The results confirm that the first portion of the *AtpC* presequence targets proteins of the plastid ER, and the latter portion is responsible for targeting the two other plastid membranes. Two other applications of the *Phaeodactylum* transformation system should be mentioned. O Malakhova and C Bowler (Naples, Italy) were able to obtain two lines of transgenic cells of *Phaeodactylum tricornutum* by transformation with bacterial genes that conferred resistance to antibiotics; kanamycin or phleomycin. Both cell lines were then mixed and exposed to various conditions that could potentially stimulate sexual reproduction. As a result, cells resistant to both antibiotics were found and the presence of both genes was confirmed. In this experiment, cells with both resistance genes represent a F1-generation, signalling an otherwise not observed sexual reproduction. The same group was also able to obtain transgenic lines of *P. tricornutum* that express the calcium-sensitive photoprotein, aequorin. This enabled there to screen a range of external stimuli and to identify what diatoms are able to sense in their environment. Other transgenic lines of *P. tricornutum* were obtained which expressed a range of luciferase-based reporter genes. These were used to study the circadian control of growth under different light qualities and quantities, and the presence of a phytochrome-like photoreceptor could be demonstrated.

R Schmitt and coworkers (Regensburg, Germany) studied cell differentiation in the multicellular green alga *Volvox carteri* using different transformation techniques. They were able to isolate and analyse a central controlling gene, *regA*, which controls the maintenance of the somatic state of cells as well as senescence and death in somatic cells. The "somatic regenerator gene" *regA* represses reproductive functions, notably nuclear-encoded chloroplast proteins in somatic cells.

Bacterial antibiotic-resistance genes were successfully integrated and expressed in the nucleus and chloroplast of *Chlamydomonas reinhardtii* by S Purton and colleagues (London, United Kingdom). This fascinating result shows that foreign gene expression may lead to the exploitation of *Chlamy-*

domonas as a cell factory for the synthesis of valuable recombinant proteins.

Cyanobacteria

The prokaryotic cyanobacteria, still regarded as "blue-green algae" by many phycologists, were covered by the plenary lecture of AR Grossman (see below); one symposium, two mini-symposia with contributed papers and two poster sessions which altogether made a total of 36 presentations. Beside these, aspects of cyanobacterial evolution and phylogeny were also treated in the plenary lecture of JD Palmer and as part of many other presentations about microalgal diversity and interactions. These activities documented that aspects of cyanobacteria are still a major field of phycology and should not only be covered by bacteriologists. Both the botanical and bacteriological approaches to cyanobacterial classification are presently widely used, but much work is needed before a convergence between the two systems can be reached. F Garcia-Pichel (Bremen, Germany) suggested a polyphasic analysis which uses ecological aspects as a guiding scheme to attain a unified, phylogenetically coherent system of classification for the cyanobacteria. This approach should integrate morphology, chemotaxonomy, physiology as well as molecular phylogenetic reconstruction. Since cyanobacteria quite often live in extreme habitats, it seems unavoidable to take the specific parameters from such habitats into account because these may represent the sum of a long-term evolution.

R Rippka (Paris, France) gave an overview of recent results from rDNA sequence analyses for tracing evolutionary relationships among cyanobacteria at different taxonomic levels. Morphologically defined orders may not correspond to certain clades in 16S rDNA phylogenies. The order Chroococcales as traditionally defined contains diverse unicellular forms from small picoplankters such as *Prochlorococcus marinus* at around 1–2 µm, to *Chroococcus turgidus* with cells of almost 0.1 mm in diameter, the largest prokaryotic cell known. However, there is no split between the Chroococcales and the filamentous Oscillatoriales in 16S rRNA gene phylogenies as in traditional classification schemes. Unicellular cyanobacteria are of multiple origins (polyphyletic). For example, a close relationship of unicellular cyanobacteria from extreme environments and lichen symbioses (e.g. *Chroococcidiopsis* and *Gloeocapsa*) with the filamentous *Nostoc* is seen in 16S rDNA phylogenies (DP Fewer, Göttingen, Germany). Rippka found that determining the genetic di-

versity among isolates of *Nostoc*, a widespread prokaryote in many symbioses with fungi and plants, is hampered because of extensive intragenomic variation of ITS rDNA. The different types of ITS in *Nostoc* are due to the presence/absence of one or several tRNA genes that interrupt the ITS sequences. As an alternative, spacer regions between coding regions for phycocyanin subunits (e.g. the *cpcBA* spacer) may be suitable for resolving relationships among closely related taxa with the advantage that these sequences are specific for cyanobacteria. An analysis of *cpcBA* sequences by CJS Bolch (Hobart, Australia) showed that the species complex of *Microcystis aeruginosa/flos-aquae*, toxic bloom-forming cyanobacteria, consists of several closely related genotypes forming a monophyletic clade. It is distinct from the morphologically similar *M. wessenbergii*. This is a quite stimulating finding since the discrimination of a cyanobacterial species is often very difficult due to a morphological continuum and numerous transitional forms, combined with conflicting taxonomic views.

That thermophily is polyphyletic within the cyanobacteria was reported in a co-authored paper presented by RW Castenholz (Oregon, USA). Thermotypes exist in several distantly related genera of cyanobacteria, such as the filamentous *Oscillatoria* and *Calothrix*, and the unicellular *Pleurocapsa* and different groups of *Synechococcus*. A considerable genetic diversity was observed within a continuous green *Synechococcus* film in a thermal gradient over the range of 45 °C to 70 °C, though all cells were morphologically indistinguishable. In contrast to previous assumptions, a clade of ecotypes adapted to very high temperatures, was not evolutionarily derived from predominant types at lower temperatures.

In his plenary lecture on the "Acclimation of photosynthetic organisms to adverse environmental conditions" AR Grossman (Stanford, USA, co-authored by colleagues) reviewed the fascinating processes in response to nutrient deprivation and excess light. He focused on systems for sensing environmental changes and the role of Nbl proteins that are involved in the degradation of phycobilisomes using the cyanobacterium *Synechococcus* PCC7942 as a model.

A nitrogen stress specific gene, *ntcA*, has been discovered in many cyanobacteria (AF Post and co-workers, Eilat, Israel). It encodes a DNA-binding protein required for the transcription of operons for N-acquisition systems. Response to low ammonium availability involves the expression of *ntcA* and only then can N-sources, other than ammonium be used. Therefore, *ntcA* can be used to monitor the N-status

of cyanobacteria. Comparisons of *ntcA* coding regions allow the assessment of the genetic diversity of cyanobacteria and to reconstruct their evolutionary relationships.

Besides the above-mentioned topics, there were symposia on algal viruses, morphogenesis and gene regulation in algae, the extracellular matrix of microalgae, and altogether six symposia and minisymposia that dealt with the ecology and ecophysiology of microalgae or cyanobacteria. Six other symposia covered topics related to macroalgae only, and two were devoted to applied phycology. The latter was the topic of an additional meeting that was held right after EPC2 at the same site.

As the Steering Committee of the EPS (European Phycological Society) has asked for offers to host

the next Congress to be submitted by the end of 1999, no decision was made at EPC2 as to where the Third European Phycological Congress is to be held. However, it is certain that it will be in summer 2003, and will attract at least as many participants as the very successful Congress in Montecatini.

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