[Reprinted from Protist 149: 293-298, with updated references]

PROTIST NEWS

Meeting Report: XIIth Meeting of the International Society for Evolutionary Protistology, Flagstaff, USA 2-4 August, 1998

It was a good omen. Well, good for me as I boarded the plane at Sydney airport, but bad for the 3 million residents of Sydney. While I headed to Flagstaff, Arizona for the XIIth meeting of the International Society of Protistology (ISEP), the people of Sydney were facing a protist problem of prodigious proportions. Sydney's water was contaminated with *Giardia*. Protistologists know *Giardia* as an amitochondriate diplomonad once thought to represent the most primitive form of the eukaryotic cell. Sydneysiders were introduced to it on the front page of their morning paper as a parasite able to induce gut-wrenching diarrhoea. And if giardiasis weren't bad enough, Sydney water also contained traces of *Cryptosporidium* - "Doubly diabolical diarrhoea for the denizens of Sydney" thought the tabloid sub editor in me. Better to drink beer in preference to water I thought as the plane took off.

ISEP was founded in the 1970's by a group of protistological visionaries who recognised a discipline emerging from previously disparate areas. Today, ISEP remains a small but nonetheless passionate and dynamic society that gathers for a biennial meeting which vacillates between North America and Europe*. In this day and age of plenary lectures, keynote speeches, and papers by invitation only, ISEP seems almost anachronistically egalitarian: everyone gets a talk, 20 minutes, no more no less. The ambience is not unlike a Gordon conference with the notable exception that there are many students and postdocs. Larry Fritz (Northern Arizona University) did a superb job of organisation and Flagstaff proved to be a particularly pleasant location for a meeting.

Euglenozoa

Rich Triemer (Rutgers University) presented the euglenid web site project. This brilliant web site serves up all you need to know about euglenids. There is up-to-the-minute taxonomy, an exhaustive bibliography, superb micrographs, and - best of all - high quality video of euglenids doing their thing. Leeuwenhoek provided the first word on euglenids more than 300 years ago, and he would undoubtedly be delighted to surf down to the website and take in the latest word on euglenids.

Continuing the euglenozoan theme, Mark Farmer (University of Georgia) described some remarkable flagellates from deep-water anoxic environments off the coast of California. In a community dominated by flagellates, some extraordinary euglenozoans were discovered. Covered in exquisitely ordered arrays of epibiotic bacteria, these euglenozoans hint at the existence of metabolic processes not previously recognised in this group. The epibiotic bacteria are likely methanogens, and Farmer demonstrated that numerous double-membrane-bound organelles subtend the plasma membrane of the euglenozoans. These organelles look suspiciously like hydrogenosomes and are reminiscent of the hydrogenosome-like structures recently reported by Alistair Simpson in another euglenozoan (Simpson, et al. 1997). True hydrogenosomes are currently known from parabasalids, chytrids, heterolobosea, and various ciliates, and it seems likely that they are converted mitochondria that have lost their genome (Müller 1997; Palmer 1997). It will be fascinating to learn if euglenozoan mitochondria have also been recruited for hydrogen production in anaerobic environments.

Julius Lukes (University of South Bohemia) described phylogeny of kinetoplastids. *Bodo saltans* is recognised as the closest relative of the trypanosomatids, which are monophyletic. The other bodonids are deduced to be paraphyletic suggesting several independent acquisitions or losses of the parasitic lifestyle. The phylogeny is congruent with kinetoplast DNA (kDNA) architecture and suggests that non-catenated kDNA comprised of minicircles and editing in bodonids was ancestral to the catenated form (Blom, et al. 1998).

Deep eukaryotes?

Are there any extant deep eukaryotes? In recent years our models for the early versions of the eukaryotic cell have come under challenge. Although rRNA and translation factor trees positioned amitochondriate eukaryotes such as diplomonads, microsporidia and parabasalids at the base of the eukaryotes (Sogin 1993), more recent data suggest these protists are secondarily reduced rather than simple and primitive (Germot, et al. 1997; Hashimoto, et al. 1998; Hirt, et al. 1997; Keeling and McFadden 1998; Keeling 1997; Müller 1997; Roger, et al. 1998).

Several ISEP papers addressed this conundrum by sequencing different genes from putatively early protists. John Stiller (University of Washington) has sequenced the RNA polymerase gene from the pelobiont *Mastigamoeba invertens*. This archamoeba lacks mitochondria and could perhaps represent a premitochondrial eukaryote. The RNA polymerase trees position *Mastigamoeba* at the base of the eukaryotes, whereas rRNA trees position it higher up. John Archibald (Dalhousie University) examined subunits of the chaperonin containing TCP-1 complex (CCT) which represent a paralogous family created by duplications at some point during eukaryotic diversification. Similar to later diverging eukaryotes, Archibald showed that *Trichomonas vaginalis* possesses eight subunits suggesting the duplications occurred in eukaryotes prior to divergence of parabasalids. Lorraine Olendzenski (University of Connecticut) focused on the catalytic subunit of V-type ATPases in microsporidia, diplomonads and parabasalids. Phylogenetic analysis positioned *Giardia* at the base of the eukaryotes. The position of parabasalids was not well resolved, emerging polychotomously with numerous other eukaryotes. The microsporidian *Nosema* was found to be sister to fungi, confirming previous data suggesting microsporidia are fungi rather than protists (see Keeling and McFadden 1998 for review).

Naomi Fast (Dalhousie University) described components of the spliceosome from *Nosema locustae* and *Trichomonas vaginalis*. It was initially thought that deep eukaryotes might lack introns (Palmer and Logsdon 1991). This was consistent with the hypothesis that spliceosomal introns originate from Group II introns and that the latter entered eukaryotes via endosymbiosis of an alpha proteobacterium creating the mitochondrion (Palmer and Logsdon 1991). The deep eukaryotes, thought never to have contained mitochondria, were therefore predicted to lack introns. Fast described U6 and U2 snRNAs from *Nosema* (Fast, et al. 1998), and U5 snRNA and PRP8 (a spliceosomal protein) from *Trichomonas*. Identification of these spliceosomal components provides strong evidence for the presence of introns in microsporidian and parabasalid genomes. Fast's hypothesis was confirmed very recently by the discovery of a spliceosomal intron in a microsporidian (Biderre 1998).

Nucleomorphs

Nucleomorphs, the vestigial nuclei of eukaryotic endosymbionts in cryptomonad and chlorarachniophyte algae, were a strong theme at this year's ISEP. Martin Fraunholz, Stefan Zauner and Jurgen Wastl from the laboratory of Uwe Maier (Marburg University) presented progress on the cryptomonad nucleomorph sequencing project being undertaken in collaboration with Tom Cavalier-Smith (University of British Columbia) and Susan Douglas (Inst. for Marine Biosciences). The bulk of chromosome II (175kb) is now sequenced. A preponderance of house-keeping genes appears to exist for the service of just a few genes encoding plastid proteins (*fts*Z, *cpn*60, two ORFs [ORF 292 & ORF 131] with homologues in *Synechocystis,* and perhaps rubredoxin). Most intriguing are the parallels between the nucleomorphs of cryptomonads and chlorarachniophytes. Despite different starting points from separate secondary endosymbioses, both nucleomorphs have converged at similar reduction points. Each nucleomorph has just three chromosomes, all of which carry rRNA genes at the chromosome ends just within the telomeres (Gilson and McFadden 1995). Unlike the chlorarachniophyte nucleomorph, the cryptomonad nucleomorph is depauperate in introns, but both have otherwise very compact organization with miniature intergenic spacers. In extreme cases the spacers are non-existent and genes overlap.

Some of the more intriguing 'finds' in the nucleomorph are tubulins. Patrick Keeling (Indiana University) described alpha, beta and gamma tubulins from the cryptomonad nucleomorph. Although highly divergent, the endosymbiont tubulins still reveal traces of their red algal ancestry (the cryptomonad endosymbiont was a red alga). The function of these tubulins is mysterious since no microtubules are observed in the endosymbiont, not even during nucleomorph division, which occurs without a visible spindle (McKerracher and Gibbs 1981).

It has long been suspected that nucleomorph reduction has involved transfer of genes from the nucleomorph to the host nucleus (McFadden 1993). This transfer is thought to have reached its zenith in organisms like heterokont algae where the endosymbiont nucleus has apparently disappeared completely. James Deane (University of Melbourne) reported two examples of nucleomorph to nucleus gene transfer. Deane cloned the chlorophyll binding proteins of cryptomonads and chlorarachniophytes. Phylogeny shows these genes derive from the endosymbiont nuclei (Durnford, et al. 1998), but they now reside in the secondary host nucleus. Targeting back to the endosymbiont plastid apparently begins at the endomembrane secr etory pathway, as originally suggested by Sally Gibbs (Gibbs 1981).

Ken-Ichiro Ishida (University of British Columbia) provided a rRNA phylogeny of chlorarachniophytes, an unusual group of cercozoans with reduced green algal endosymbionts. Two 18S rRNA phylogenies, one for the host and one for the nucleus of the endosymbiont (nucleomorph), can be made for these chimaeric organisms. Both phylogenies were congruent showing that chlorarachniophytes comprise four distinct lineages exhibiting a range of cell forms: flagellates, reticulopodial amoebae and cysts.

Giardia

In an ironic twist, two talks on *Giardia* were delivered by Australians. Peter Upcroft (Queensland Institute of Medical Research) described karyotyping and rDNA mapping in *G. duodenalis*. Chromosome copy number and size are known to vary dramatically in strains of *Giardia,* and Upcroft showed that the karyotype is highly plastic, a remarkable feature given that *Giardia* is asexual and not known to undergo meiosis. Changes in the chromosomes were largely attributable to rearrangements of the rDNA units and repeated units of cysteine rich proteins, ankyrins and protein kinases, all of which are located subtelomerically. This extraordinary plasticity, which occurs in a clonal line, is a sobering prospect for those undertaking genome projects on *Giardia*.

The superficial similarity between *Giardia* chromosomes and the remnant chromosomes of cryptomonad and chlorarachniophyte nucleomorphs (both have subtelomeric rDNA units on all or most of their chromosomes) is intriguing and might suggest a principal common to reduced genomes. In this respect it is worth noting that certain microsporidia (another group of protists with miniature genomes) also have rDNA units on all chromosomes (Peyretaillade, et al. 1998).

Jacqui Upcroft (Queensland Institute of Medical Research) continued the *Giardia*-down-under theme describing several oxoacid oxidoreductases present in *Trichomonas vaginalis*, (Brown, et al. 1998) *Entamoeba histolytica* and *Giardia duodenalis*. These enzymes perform similar anaerobic energy production functions to the well characterised pyruvate:ferredoxin oxidoreductase (PFOR). However, unlike PFOR the activity of these enzymes does not activate the drug metronidazole, the main line of defense against these parasites. Identification of the enzymes in native gel electrophoresis paves the way for more detailed characterization.

From Basic to Applied

ISEPers are largely involved in basic research, but two talks on thraustochytrids covered the entire spectrum from basic to applied. Leander (University of Georgia) presented a molecular phylogeny of labyrinthulids and thraustochytrids, which appear to be monophyletic and basal within the broader assemblage, referred to either as stramenopiles or heterokonts. On the applied side, Ashcroft (Omega Tech Inc.) outlined the use of a thraustochytrid to produce docosahexaenoic acid as a food additive. Based on exhaustive screening of protists, *Schizochytrium* (which grows readily in airlift fermenters because it doesn't form ectoplasmic nets) was found to be an excellent strain for production of the highly valuable fatty acid used in dietary supplements and infant formula. As much as 70% of fermenter-grown *Schizochytrium* comprised lipids, and almost a third of this was docosahexaenoic acid. Systematists were delighted to hear that good phylogeny had saved Omega Tech Inc. USD $1 million in toxicity tests. Because thraustochytrids are relatives of the heterokont algae and oomycetes (stramenopiles), which are perceived by regulatory agencies as relatively harmless, there were minimal problems in getting approval as producers of food additives. If however, *Schizochytrium* had still been classified as a fungus, approval would have been far more difficult to obtain since fungi produce a panoply of toxins.

rRNA Systematics

A systematic theme was also pursued y Craig Bailey (Bigelow Laboratory for Ocean Sciences) who presented rRNA and *rbcL* phylogenies of xanthophytes (yellow-green algae). Traditionally the siphonous, sexual xanthophytes like *Vaucheria* as are regarded as highly derived, but Bailey's molecular data seem to suggest that *Vaucheria* is an early diverging xanthophyte and that unicellular, asexual forms are more derived. Bob Andersen (Bigelow Laboratory for Ocean Sciences) described attempts to classify eustigmatophytes of the genus *Nannochloropsis*. Small enough (1-4 microns) to defy morphological distinction by microscopical techniques these little nanoplankters also challenge our species concept. Apparently asexual, these tiny algae appear to exist as a number of virtually cosmopolitan lines, each with a unique rRNA sequence. Curiously, some of these lines (species?) have sympatric distribution. Julius Lukes (University of South Bohemia) reported 18S rRNA phylogeny of Sarc ocystidae. Sequences for *Sarcocystis dispersa*, *Sarcocystis sp*., and *Sarcocystis roger* were incorporated into an apicomplexan phylogeny. The trees suggest that *Frenkelia* may belong within *Sarcocystis* (Votypka, et al. 1998). Parasite phylogeny does not mirror the intermediate host phylogeny, but there may be a pattern of Sarcocystidae diversification in relation to the final hosts. Yves Van de Peer (University of Antwerp) tackled the spectre of long branches in rRNA trees. By careful use of appropriate algorithms Van de Peer considers that the burgeoning rRNA database can be extensively utilised to study phylogeny, even though there are high levels of among site rate variation in these sequence s.

Plastids in Apicomplexa

Another theme was the origin and function of the relic plastid of apicomplexan parasites. Chuck Delwiche (University of Maryland) presented the case for secondary origin from a green algal endosymbiont on the basis of TufA phylogeny (Köhler, et al. 1997). Jeff Blanchard (University of Oregon) argued against a green algal endosymbiont on the basis of plastid gene organisation data, which are more congruent with a red algal endosymbiont. Tom Cavalier-Smith (University of British Columbia) argued for a common origin of plastids in Apicomplexa, dinoflagellates and euglenozoa by a single secondary endosymbiosis. In support of his thesis, Cavalier-Smith presented the first dinoflagellate plastid DNA sequen ces. Protistologists have long hungered for any dinoflagellate plastid sequence data, and Cavalier-Smith's presentation of the genes for *psb*A, *psb*B, *psb*C, *rrn*L, *psa*A, and *psa*B was received with much expectation. The genes are extraordinarily divergent and Cavalier-Smith counsels caution in interpreting relationships. Nevertheless, the trees suggest a sister alliance between apicomplexan and dinoflagellate plastids which mirrors the sister relationship between the host components of these two protist lineages.

Apicomplexan plastids and dinoflagellate plastids were in turn the sister group to euglenoid plastids, thereby prompting Cavalier-Smith to adopt a parsimonious scenario in which all three obtained plastids by secondary endosymbiosis via a myzocytotic engulfment of the contents of a photosynthetic eukaryote. However, in his presidential address Øjvind Moestrup (Copenhagen University) described a new apicomplexan-like flagellate with similarities to the sister group of dinoflagellates. Moestrup's new organism seems to lack a plastid making it difficult to invoke a common origin for plastids in apicomplexa and dinoflagellates unless there have been parallel losses of plastids in dinoflagellates, euglenoids, and early but not later emerging apicomplexa.

Ross Waller (University of Melbourne) presented the first data on targeting of nuclear-encoded proteins to the apicomplexan plastid. Immunocytochemistry shows that the products of several nuclear genes are targeted to the plastid in *Toxoplasma*. Targeted proteins carry N-terminal leaders, which were shown to be sufficient to target a reporter protein (green fluorescent protein) into the plastid of *Toxoplasma*. The targeting model, which involves a bipartite leader comprised of a signal peptide and transit peptide, supports a secondary origin for the apicomplexan plastid. Moreover, the targeted proteins suggest that the vestigial plastid could have a function in fatty acid biosynthesis similar to plant and algal plastids, and an inhibitor of plastid fatty acid biosynthesis was shown to have antimalarial activity *in vitro* (Waller, et al. 1998).

Plastid origins were also addressed by Sean Turner (Indiana University) who described ongoing efforts to determine whether or not plastids arose from a single or multiple endosymbioses of cyanobacteria. Turner has steadily increased the catalogue of cyanobacterial rRNA sequences (100 strains now complete) and performed careful phylogenetic analyses in an attempt to determine exactly where plastids emerge from the cyanobacterial divergence. Although the trees indicate a single, early divergence for the plastid lineage, the pattern is not robust, perhaps suggesting that the rRNA sequences do not retain sufficient signal to reconstruct the origins of plastids.

Envoi

An ISEP meeting wouldn't be complete without a new kingdom from Tom Cavalier-Smith. Cavalier-Smith (University of British Columbia) announced kingdom Neomonada, which embraces the choanoflagellates, icthyosporeans, *Corallochytrium*, and subsumes two previous phyla: phylum Apusozoa (small heterotrophic flagellates e.g. *Apusomonas*) and phylum Cercozoa (e.g. sarcomonads, cercozoans, filose amoebae and chlorarachniophytes). K. Neomonada is an unashamedly paraphyletic assemblage, which Cavalier-Smith posits as the wellspring of the four higher kingdoms (animals, fungi, plants and chromists) plu s several protozoan lineages.

And what about Sydney and their protist problems? Not good I'm afraid, a month later and they're still boiling their water. Cysts persist in the water, although they seem to be appearing and then disappearing, which either suggests that they are lodging intermittently in the pipes or that the assay is not entirely reliable. The puzzling part is the lack of any epidemics of giardiasis or cryptosporidiosis. Indeed, levels of diarrhoea do not appear to be any worse than before the crisis, which only serves to highlight the difficulties in monitoring for pathogens. While it is reasonably straightforward to identify parasite cysts in drinking water supplies, it is not yet possible to tell if the cysts are pathogenic or even viable (Morgan and Thompson 1998). In order to avoid unnecessary health scares, protistologists should receive more funding to study simple eukaryotes. At least the collecting will be easy now that the organisms are on tap!

* The next ISEP meeting will be hosted by Julius Lukes in the year 2000 at Ceske Budejovice in southern Bohemia (Czech Republic).

Geoffrey I. McFadden
Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville VIC 3052, Australia.
fax 61-3-9347-1071
e-mail g.mcfadden@botany.unimelb.edu.au

References

Biderre C (1998) A small spliceosomal-type intron occurs in a ribosomal protein gene of the microsporidian *Encephalitozoon cuniculi*. Mol Biochem Parasitol **94:** 283-286

Blom D, de Haan A, van den Berg M, Sloof P, Jirku M, Lukes J, Benne R (1998) RNA editing in the free-living bodonid *Bodo saltans*. Nucleic Acids Res **26:** 1205-1213. Abstract

Brown DM, Upcroft JA, Edwards MR, Upcroft P (1998) Alternative 2-keto acid oxidoreductase activities in *Trichomonas vaginalis*. Mol Biochem Parasitol **94:** 203-214. Abstract

Durnford DG, Deane JA, Tan S, McFadden GI, Gantt E, Green BR (1999) A phylogenetic assessment of plastids and eukaryotic light-harvesting antenna proteins. J. Mol. Evol. **48: 59-68.** Abstract

Fast NM, Roger AJ, Richardson CA, Doolittle WF (1998) U2 and U6 snRNA genes in the microsporidian *Nosema locustae*: evidence for a functional spliceosome. Nucleic Acids Res **26:** 3202-3207. Abstract

Germot A, Philippe H, Le Guyader H (1997) Evidence for loss of mitochondria in Microsporidia from a mitochondrial-type HSP70 in *Nosema locustae*. Mol Biochem Parasitol **87:** 159-168. Abstract

Gibbs SP (1981) The route of entry of cytoplasmically synthesized proteins into chloroplasts of algae possessing chloroplast ER. J Cell Sci **35:** 253-266

Gilson PR, McFadden GI (1995) The chlorarachniophyte: a cell with two different nuclei and two different telomeres. Chromosoma **103:** 635-41

Hashimoto T, Sanchez L, Shirakura T, Müller M, Hasegawa M (1998) Secondary absence of mitochondria in *Giardia lamblia* and *Trichomonas vaginalis* revealed by valyl-tRNA synthetase phylogeny. Proc Natl Acad Sci USA **95:** 6860-6865. Abstract

Hirt RP, Healy B, Vossbrinck CR, Canning EU, Embley TM (1997) A mitochondrial Hsp70 orthologue in *Vairimorpha necatrix*: molecular evidence that microsporidia once contained mitochondria. Curr Biol **7:** 995-998. Abstract

Keeling PJ, McFadden G (1998) Origins of microsporidia. Trends Microbiol **6:** 19-23. Abstract

Keeling PJ (1997) A kingdom's progress: Archezoa and the origin of eukaryotes. Bioessays **20:** 87-95

Köhler S, Delwiche CF, Denny PW, Tilney LG, Webster P, Wilson RJM, Palmer JD, Roos DS (1997) A plastid of probable green algal origin in apicomplexan parasites. Science **275:** 1485-1488. Abstract

McFadden GI (1993) Second-hand chloroplasts: evolution of cryptomonad algae. Adv Bot Res **19:** 189-230

McKerracher L, Gibbs SP (1981) Cell and nucleomorph division in the alga *Cryptomonas*. Can J Bot **60:** 2440-2452

Morgan UM, Thompson RCA (1998) PCR detection of *Cryptosporidium*: the way forward? Parasitol Today **14:** 241-245

Müller M (1997) Evolutionary origins of trichomonad hydrogenosomes. Parasitol Today **13:** 166-167

Palmer J (1997) Organelle genomes: going, going, gone! Science **275:** 790-791

Palmer JD, Logsdon JMJ (1991) The recent origins of introns. Curr Opin Genet Dev **1:** 470-477

Peyretaillade E, Biderre C, Peyret P, Duffieux F, Metenier G, Gouy M, Michot B, Vivares P (1998) Microsporidian *Encephalitozoon cuniculi*, a unicellular eukaryote with an unusual chromosomal dispersion of ribosomal genes and a LSU rRNA reduced to the universal core. Nucleic Acids Res **26:** 3513-3520. Abstract

Roger AJ, Svard SG, Tovar J, Clark CG, Smith MW, Gillin FD, Sogin ML (1998) A mitochondrial-like chaperonin 60 gene in *Giardia lamblia*: evidence that diplomonads once harbored an endosymbiont related to the progenitor of mitochondria. Proc Natl Acad Sci USA **95:** 229-234. Abstract

Simpson AGB, van den Hoff J, Bernard C, Burton HR, Patterson DJ (1997) The ultrastructure and systematic position of the euglenozoon *Postgaardi mariagensis*, Fenchel et al. Arch Protistenk **147:** 213-225

Sogin M (1993) Universal tree of life. Nature **362:** 795

Votypka J, Hypsa V, Jirku M, Flegr J, Vavra J, Lukes J (1998) Molecular phylogenetic relatedness of *Frenkelia* spp. (Protozoa, Apicomplexa) to *Sarcocystis falcatula* Stiles 1893: is the genus *Sarcocystis* paraphyletic? J Euk Microbiol **45:** 137-141. Abstract

Waller RF, Keeling PJ, Donald RGK, Striepen B, E.Handma, Lang-Unnasch N, Cowman AF, Besra GS, Roos DS, McFadden GI (1998) Nuclear-encoded proteins target to the plastid in *Toxoplasma gondii* and *Plasmodium falciparum*. Proc Natl Acad Sci USA **95:** 12352-12357. Abstract

Return to ISEP Home Page