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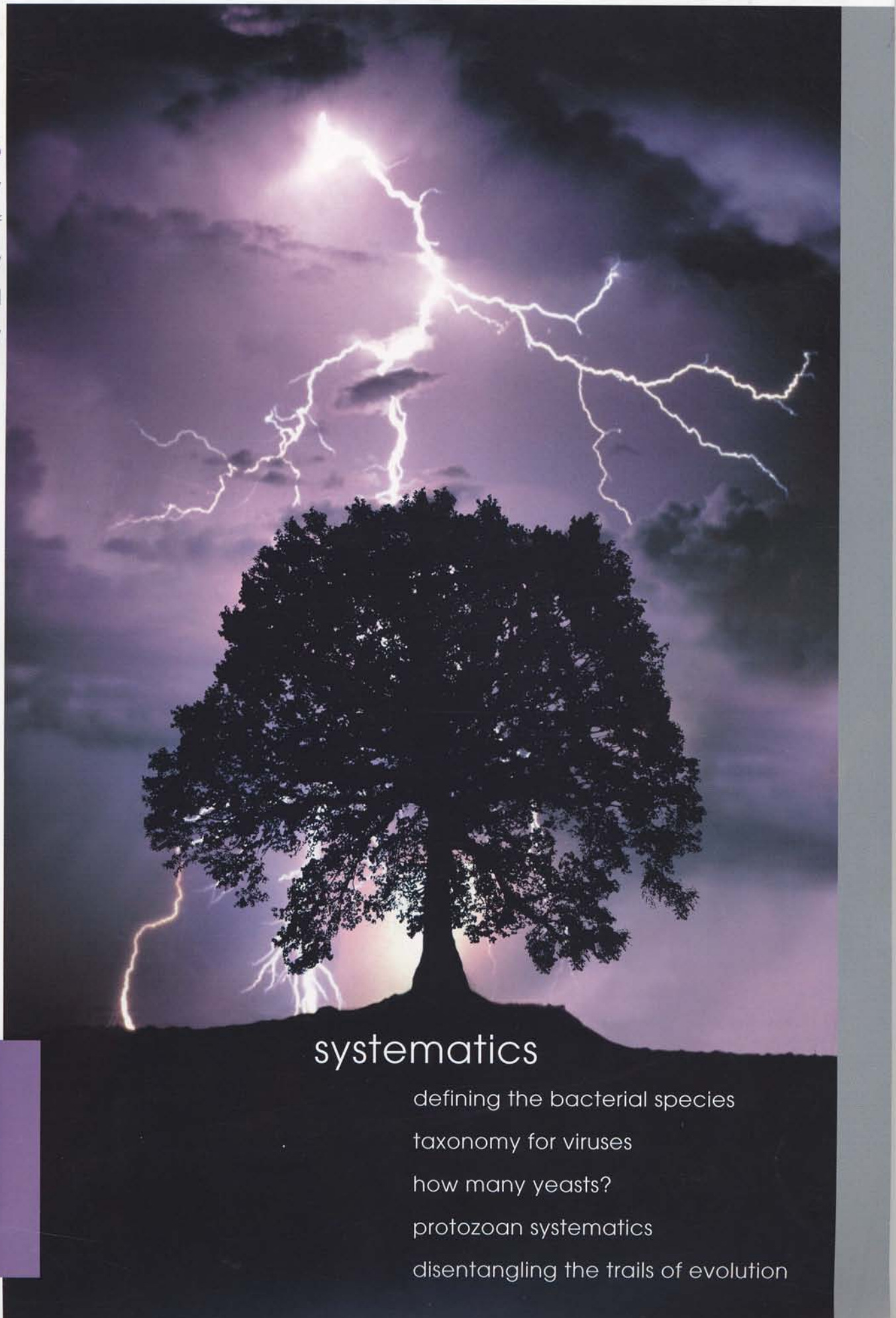

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Cover image Lightning striking a tree. *Mike Agliolo / Science Photo Library*

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news

New Editor for *Microbiology Today* – Matt Hutchings

I am delighted to be taking over from Gavin Thomas as Editor of *Microbiology Today*. I have a long-standing interest in microbiology which began as a PhD student in the Biochemistry Department at Southampton University.

In 1998 I moved to Stephen Spiro's laboratory at the University of East Anglia to investigate nitric oxide-dependent gene regulation in *Escherichia coli* and *Paracoccus denitrificans* and, after 3 years at UEA, I was offered another postdoctoral position in Mark Buttner's laboratory at the John Innes Centre. It was here that I started working with *Streptomyces coelicolor*, the model organism for the genus *Streptomyces*. The streptomycetes have a complex life cycle which is unusual among bacteria and which includes hyphal growth and sporulation. More importantly, they produce most of the antibiotics in clinical use today. Earlier this year I made the short trip back across the Norwich Research Park to UEA to set up my own laboratory as an RCUK fellow in Molecular Microbiology.

My research is focused on learning more about the ways in which *Streptomyces* species sense and respond to their environment. The streptomycetes belong to a much larger group of bacteria called the actinomycetes and one of my aims as Editor of *Microbiology Today* is to introduce this diverse group of bacteria to a wider audience. I am also interested in the effects that microbes have on our lives. This includes not just bacteria but all micro-organisms. Those that live on or inside us, either as pathogens or commensal organisms, those that produce primary or secondary metabolites that are used by the pharmaceutical, food and agricultural industries and the free-living microbes which have both positive and negative effects on the ecosystem. Traditionally, we have studied only those microbes which are culturable in the laboratory, a tiny fraction of the total microbial life on this planet. However, recent projects to sequence DNA from the soil, the oceans and the atmosphere have revealed just how little we understand about this diverse microbial world. I hope to cover some of these topics during my time as Editor. I also hope we can use *Microbiology Today* to communicate these ideas to a wider audience and make people appreciate why microbiology is so important.

News of members

Professor Hilary Lappin-Scott, SGM Scientific Meetings Officer, has become President of the International Society for Microbial Ecology, with effect from August 2006.

Congratulations to Education Officer **Dr Sue Assinder** on her appointment as Director of the Academic Development Unit in the School of Education at the University of Wales, Bangor.

The Food Standards Agency has appointed **Professor Harry Flint**, Head of the Gut Microbiology and Immunology Division at the Rowett Research Institute, to its Advisory Committee on Novel Foods and Processes.

The Society notes with regret the death of **Dr Andrew Robinson**, Health Protection Agency, Porton Down (member since 1973).

New Corporate Member

Welcome to **VH Bio**, a manufacturer of synthetic olignucleotides, PCR reagent supplier, UK distributor for sepsis PCR diagnostics, electrophoresis equipment, sequencing and PCR purification kits, DNA kits and instruments. For further information contact Andrew Hewitt, VH Bio Ltd, Unit 11b, Station Approach, Team Valley Trading Estate, Gateshead NE11 0ZF (e ahewitt@vhbio.com; w www.vhbio.com).

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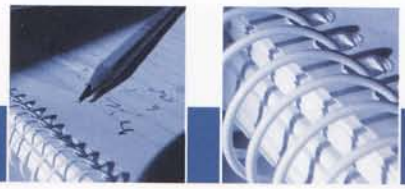
BioSciences Federation

As a result of recent elections, the following have been welcomed as new members of the Council of the BioSciences Federation:

Professor John Coggins, Institute of Biomedical and Life Sciences, University of Glasgow

Dr Pat Goodwin (*SGM member*), Head of Pathogens, Immunology and Population Studies, The Wellcome Trust

Professor Frank Odds (*SGM member*), Professor of Medical Mycology, University of Aberdeen.



SGM Council

June Meeting Highlights

SGM journals open access policy

It is now a condition of Wellcome Trust grants that publications arising from work funded by the Trust must be made freely available within 6 months of publication, or immediately if an open access charge has been paid. The Medical Research Council has recently agreed to follow a similar policy, while other UK Research Councils have set rather more relaxed requirements. The publishing policies of all SGM journals have been modified to include an open acess option in exchange for an author-side payment (for details see Ron Fraser and Robin Dunford's article in the August issue of *Microbiology Today*, p. 140). Further developments on this front will be closely monitored by SGM as open access publishing is the subject of intense debate and rapid change.

Budget 2007

The main business of the summer meeting of the SGM Council was the approval of the budget 2007. This was set with either no changes or inflationary increases to membership subscriptions and journal prices. Institutional subscriptions to journals were increased in line with inflation, the increase in the size of the journals and the small annual decline in the market. In the case of the *Journal of General Virology*, an allowance was made for the income stream due to the new arrangements with open access conditions. SGM journals represent excellent value for money, generally being one-third to one-quarter of the price per page of journals published by commercial publishers.

The funds available for SGM grants have been increased from approximately £332k to £385k as a result of significant increases in the joint meetings and travel funds (see below).

Council members

This was the last Council meeting for **Hugh Pennington** as SGM President and chair. Council members thanked him for all his efforts, in particular for his interaction with the media which has helped to raise the profile of microbiology in general and that of the SGM in the public eye. Council also wished him well in his role as Chairman of a Welsh Assembly Inquiry into a recent *E. coli* O157 outbreak. The incoming President, **Robin Weiss**, attended the Council meeting as an observer and will take over from Hugh in September 2006. **Jeff Cole**, **Jeff Errington**, **Peter Andrew** and **Gavin Thomas** have also completed their term on Council, and the President thanked them for their expert input and engagement.

SGM Grants and Funds

The SGM Grants and Funds schemes have been revised, and the overall funding has been increased (see p. 146 for further information). Details of the schemes and extended eligibility criteria will be available on the SGM website (www.sgm.ac.uk) in due course. Until the end of 2006, the present conditions apply.

***Ulrich Desselberger**, General Secretary*

Staff News

Congratulations to Staff Editor **Ashreena Osman** and her husband Jason on the birth of a son Ajay in June.

Although the baby appeared on the scene rather earlier than expected and had to stay in hospital for while, he is now at home with his mum and doing well.

Young Microbiologist of the Year

The York meeting saw the finals of the SGM's science communication contest. This year a bumper field of 11 keen postgrads or postdocs, who had been selected as finalists by the Special Interest Groups and Irish Branch on the basis of their offered presentations (either oral or poster) at recent SGM meetings, gave 10 minute talks on their research. The standard was amazingly high and the judges, provided from the Group Committees and chaired by Jo Verran, Education & Training Group Convener, had a very difficult job. The winners were announced at the Society Dinner later that evening. The first prize of £500 went to **J.D. Neufeld** (Warwick) for his presentation *Methylotrophs in the spotlight*. The second prize of £200 was won by **P.A. Rowley** (Aberdeen) and the third prize of £100 by **P.C. Fineran** (Cambridge). All finalists will get free SGM membership in 2007.

Further details of the competition and an entry form are available on the meetings page of the SGM website. Why not enter by submitting an offered poster or oral presentation for the spring meeting at Manchester next year? The closing date for abstracts is **27 November 2006**, so get cracking!

New Council Officers

With effect from 12 September 2006, **Professor Robin Weiss** (University College London) commences his 3-year term as President. A profile of Professor Weiss appeared in the August issue of *Microbiology Today*. **Dr Matt Hutchings** (University of East Anglia) also commences his 3-year term as Editor of *Microbiology Today* (see p. 142).

New elected members of Council

The following will serve on Council for 4 years from 12 September 2006: **Professor Michael R. Barer** (University of Leicester), **Dr Richard M. Hall** (GlaxoSmithKline) and **Dr Catherine O'Reilly** (Waterford Institute of Technology).

Mike Barer

I am Professor of Clinical Microbiology at Leicester University. I trained in Medicine and Immunology at University College London and in Clinical Microbiology at St George's, London and in Newcastle. My principal research interests lie on the interface between bacterial physiology and infection and most of my current research is concerned with tuberculosis and the study of bacterial cells that are difficult to culture. I firmly believe clinical microbiologists should base their research and practice on understanding micro-organisms and seek to promote better interchange between basic science and clinical practice in this area.

Catherine O'Reilly

I graduated in Genetics at Trinity College Dublin in 1978 and continued in the same department to do my PhD on proline biosynthesis and insertion sequences in *Salmonella typhimurium*. I then moved to the Max Planck Institute in Cologne for 2 years as an EMBO postdoctoral fellow where I worked on transposable elements in maize. Following that I worked as an EU postdoctoral fellow in the Department of Botany, Durham, working on opine biosynthesis in *Agrobacterium tumefaciens*. The next 10 years were spent at the University of Sunderland. There I developed an interest in microbial cyanide and nitrile metabolism. I moved to Waterford in 1996 and have continued to work on nitrile and cyanide metabolism. Current research is mainly focussed on the genetics of nitrile metabolism in *Rhodococcus* and the use of nitrile-hydrolysing enzymes in biotransformation.

Richard Hall

I have worked in the pharmaceutical industry for over 20 years and presently head the Microbial Culture Sciences group located within Gene Expression and Protein Biochemistry at GlaxoSmithKline in Harlow. My main interests are in the expression and scale-up of recombinant proteins. Following graduation (BSc Biological Sciences) I remained in Manchester and was appointed as a Research Assistant in the University's Department of Child Health, studying the effects of malnutrition on brain development. I had the good fortune to then join Colin Ratledge's group (University of Hull) working on aspects of iron metabolism in mycobacteria and was awarded my PhD in 1983. I remained at Hull for a postdoc, focussing on *Mycobacterium leprae* (causal organism of leprosy).

In 1985, I joined Glaxo, working in a natural products group based at Greenford. Following the merger with Wellcome in 1995, I moved to Stevenage, where I was particularly involved in aspects of biotransformation, including the use of microbial systems to mimic mammalian metabolism. GlaxoSmithKline was formed in 2000 and I moved to my present location in 2001.

I am married with two daughters both at university. Hobbies include fishing, football (I'm a long-time supporter of Manchester City) and walking (I come from the Lake District so it is in the genes!). I joined the SGM in 1984 (I think Colin talked me into it) and have previously worked with the Fermentation & Bioprocessing Group. I have recently offered my services to the Education & Training group and am delighted to be joining the Council.

Microbiology Research Matters

SGM has just published a colourful booklet to help communicate the importance of microbiological research.

Millions are spent each year on research involving micro-organisms. However, the public can perceive research as being esoteric and unlikely to have a significant impact on their lives. Attention tends to be confined to controversial issues such as avian 'flu and MMR vaccination, representing the microbiological interests of only a fraction of UK scientists. The idea of the booklet is to alert the public to some of the amazing discoveries being made by microbiologists in the UK.

The booklet is the output from a workshop on *Communicating Microbiology* organized by the SGM. Ten young microbiologists joined Myc Riggulsford, a professional facilitator with extensive experience of writing science for the general public, to learn about the principles of effective science communication. The challenge for the participants was to then apply the lessons learnt to produce a 1-page article that communicated the key points of their research.

The diversity of the work described in the booklet is striking. Projects include the identification of novel drug targets by the molecular unravelling of pathogen genomes, the effect of surface chemistry on microbial 'stickiness' and the use of chocoholic bacteria as a source of alternative energy. All of the projects illustrate the relevance of microbiological research to society and demonstrate the need to provide continued support for the work of the next generation of UK microbiologists.

The booklet will be distributed free of charge to schools and the public. It will also be sent to attendees at SGM Microbiology Awareness Campaign events at the House of Lords, the Welsh Assembly Government and the Scottish Parliament, and will be distributed at all forthcoming MAC events. Members wishing to obtain a copy can email **pa@sgm.ac.uk**

Groups

New committee members, elected by postal ballot for the Physiology, Biochemistry and Molecular Genetics Group or elected unopposed (all other Groups) to serve for 3 years from 12 September 2006 are as follows:

Cells and Cell Surfaces

A. Cunningham *University of Birmingham*
J.R. Fitzgerald *University of Edinburgh*
R. Massey *University of Oxford*

Clinical Microbiology

S. Lang *Glasgow Caledonian University*
D. Mack *University of Wales Swansea*
S. Patrick *Queen's University Belfast*
D. Ready *Eastman Dental Hospital, London*

Clinical Virology

P. Cane *Health Protection Agency, Porton Down*
E. MacMahon *St Thomas' Hospital, London*
D. Pillay *University College London*
J. Breuer *Barts & The London* has taken over as Group Convener

Education and Training

W. Ashraf *University of Bradford*
S. Burton *University of Exeter*
R. Dixon *University of Lincoln*

Environmental Microbiology

D.W. Hopkins *University of Stirling*
R. Pickup *Centre for Ecology and Hydrology, Lancaster*
C. Whitby *University of Essex*

Eukaryotic Microbiology

S.K. Whitehill *University of Newcastle*
A. Goldman *University of Sheffield* has taken over as Group Convener

Fermentation and Bioprocessing

D. Charalampopoulos *University of Reading*

Food and Beverages

K. Jones *University of Lancaster*
W. Morrisey *Green Isle Foods*

Irish Branch

J. Morrissey *University College Cork*
C. O'Byrne *National University of Ireland Galway*
E.M. Doyle *University College Dublin* has taken over as Group Convener

Microbial Infection

B. Kenny *University of Newcastle*

Physiology, Biochemistry and Molecular Genetics

G.W. Blakely *University of Edinburgh*
F. Sargent *University of East Anglia*

Systematics and Evolution

R. Goodacre *University of Manchester*
L. Hall *Barts and the London School of Medicine*

Virus

M. Cranage *St George's, University of London*
L. Roberts *University of Surrey*
A.J. Sinclair *University of Sussex*
J.A. Walsh *Warwick HRI*

Grant schemes reviewed

The Treasurer has recently carried out an extensive review of SGM grant schemes, to reflect both demand and changing circumstances. A range of schemes currently provide funding for members to attend non-SGM scientific meetings in the UK and overseas. Most of these have been directed at early-career scientists, such as the President's Fund, but others have offered funding to established scientists provided they meet certain criteria e.g. IUMS Congress grants. These grants have proliferated over the years, become increasingly difficult to administer and resulted in uneven distribution of money.

Some schemes have been merged and others amended. The new rules will take effect from January 2007 and details and application forms will be published shortly on the website.

Please note:

Postgraduate Conference Grants for SGM meetings remain unchanged. For meetings in 2006, you should apply to the current schemes.

SGM has a wide range of grant schemes to support microbiology. See **www.sgm.ac.uk** for details.

Any enquiries should be made be made to the Grants Office, SGM, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG (t 0118 988 1821; f 0118 988 5656; e grants@sgm.ac.uk).

Scientific Meetings Travel Grants

This scheme will be open to an extended range of early-career microbiologists resident within the EU, ranging from postgraduate students to first postdocs and newly appointed lecturers. Funding will be tiered according to the location of the meeting. The maximum grants will be as follows: **UK (or country of residence)** – £200; **within Europe** – £350; **Rest of World** – £500. These grants may also be used to support attendance on short courses.

President's Fund for Research Visits

Grants are available to support short research visits (1–3 months) by early-career microbiologists resident within the EU, ranging from postgraduate students through to first postdocs and newly appointed lecturers. Funding is limited to a maximum of £3,000. Retrospective applications will not be accepted.

Closing dates: **30 March** and **12 October 2007**.

This scheme results from a merger of the President's Fund – Research Visit and International Research Grant scheme with funding now directed at early-career scientists.

Microbiologists in developing countries seeking funding to support a short research visit should apply for an UNESCO–IUMS–SGM Fellowship (www.iums.org/Travelgrants/fellowships.htm).

Student Schemes

GRADSchool Grants

Postgraduate student members registered for a PhD in a UK university can apply for funding to support the full cost of course fees for a national GRADschool. Students funded by Wellcome Trust, BBSRC, NERC, MRC or EPSRC are entitled to a free place on a GRADSchool course and should not apply to this scheme. Applications, on the appropriate form, are considered throughout the year but must be made before booking a place on a course.

Postgraduate Student Meetings Grants

Grants cover travel and accommodation expenses for attendance at one SGM meeting each year. Applicants must be Student Members resident and registered for PhD in an EU country. Closing date for the Manchester Meeting: **23 March 2007**.

Elective Grants

Funding for medical/dental/veterinary students to work on microbiological projects in their elective periods. The closing dates for applications in 2007 are **27 April** and **26 October**.

Vacation Studentships

The 2007 scheme is now open for applications. As described on p. 182 the scheme offers a great opportunity for undergraduates to work on microbiological research projects during the summer vacation before their final year. The awards, which are made by competition, aim to give students experience of research and to encourage them to consider a career in this area. The studentships provide support at a rate of £180 per week for a period of up to 8 weeks. An additional sum of up to £400 for specific research costs may also be awarded. Applications must be from SGM members on behalf of named students. The closing date for applications is **16 February 2007**.

Student Society Sponsored Lectures

These cover the travel and other expenses of up to two speakers on microbiological topics per Society each year at student society meetings.

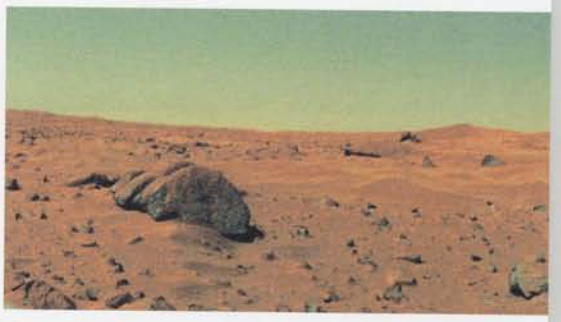
Other schemes

Public Understanding of Science Awards

Are you planning any projects to promote the public understanding of microbiology? Have you got a National Science Week event in mind? Grants of up to £1,000 are available to fund appropriate activities. Applications are considered on a first come, first served basis throughout the calendar year.

Life on Mars?

Distinguished US microbiologist Howard Gest, who wrote a Comment in the August 2005 issue of *Microbiology Today* on his views on astrobiology, has published a short article expanding on the content of the Comment, which addresses the usefulness of this new 'field' of research and reviews new claims of organic matter reported to have been discovered in Martian meteorites. The article entitled *The 2006 Astrobiology Follies: Return of the Phantom Martian Microbes* can be accessed through Professor Gest's website at **www.bio.indiana.edu/~gest/**



▶ The surface of Mars as photographed by the Viking 1 lander. *NASA / Science Photo Library*

SGM membership subscriptions 2007

The following rates were agreed at the AGM of the Society on 12 September 2006.

Membership category		Annual subscription		Additional subscriptions for publications (print only)							
				Microbiology		*JGV*		*IJSEM*		*JMM*	
		£	US$	£	US$	£	US$	£	US$	£	US$
Ordinary		49	90	98	180	98	180	98	180	52	96
Postgraduate Student		21	38	44	81	44	81	44	81	44	81
Retired		21	38	44	81	44	81	44	81	44	81
Technician		20	NA	44	81	44	81	44	81	44	81
Undergraduate		10	NA	NA	NA	NA	NA	NA	NA	NA	NA
School		10	NA	NA	NA	NA	NA	NA	NA	NA	NA
Corporate	Tier 1	350	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Tier 2	500	NA	NA	NA	NA	NA	NA	NA	NA	NA

For airmail despatch of *Microbiology Today*, add £17/US$30 to subscription.

Members are reminded that their 2007 subscriptions are due for payment by **1 December 2006**.

As in previous years, no journal or meetings information will be despatched to members who are in arrears, and there will be no guarantee of provision of back numbers of journals for members who pay their subscription late.

Payment Against Invoice

Invoices were despatched recently to all members who pay by this method. If you did not receive one, please inform the Membership Office.

New secure online credit card renewal payment

If you pay against invoice, from now on you will be able to renew your subscription online via the SGM website (www.sgm.ac.uk/members) with either a credit or debit card. Please see your invoice for details.

Payment by Direct Debit

Subscription notices were despatched recently to all members paying by direct debit. To continue your present status and journal requirements, no further action is necessary. To change your membership status or journal requirements for 2007, you should have amended your subscription notice and returned it to the membership office by **17 November 2006**. However, if you have missed this deadline, your amended notice will be accepted if it is submitted immediately.

Please note

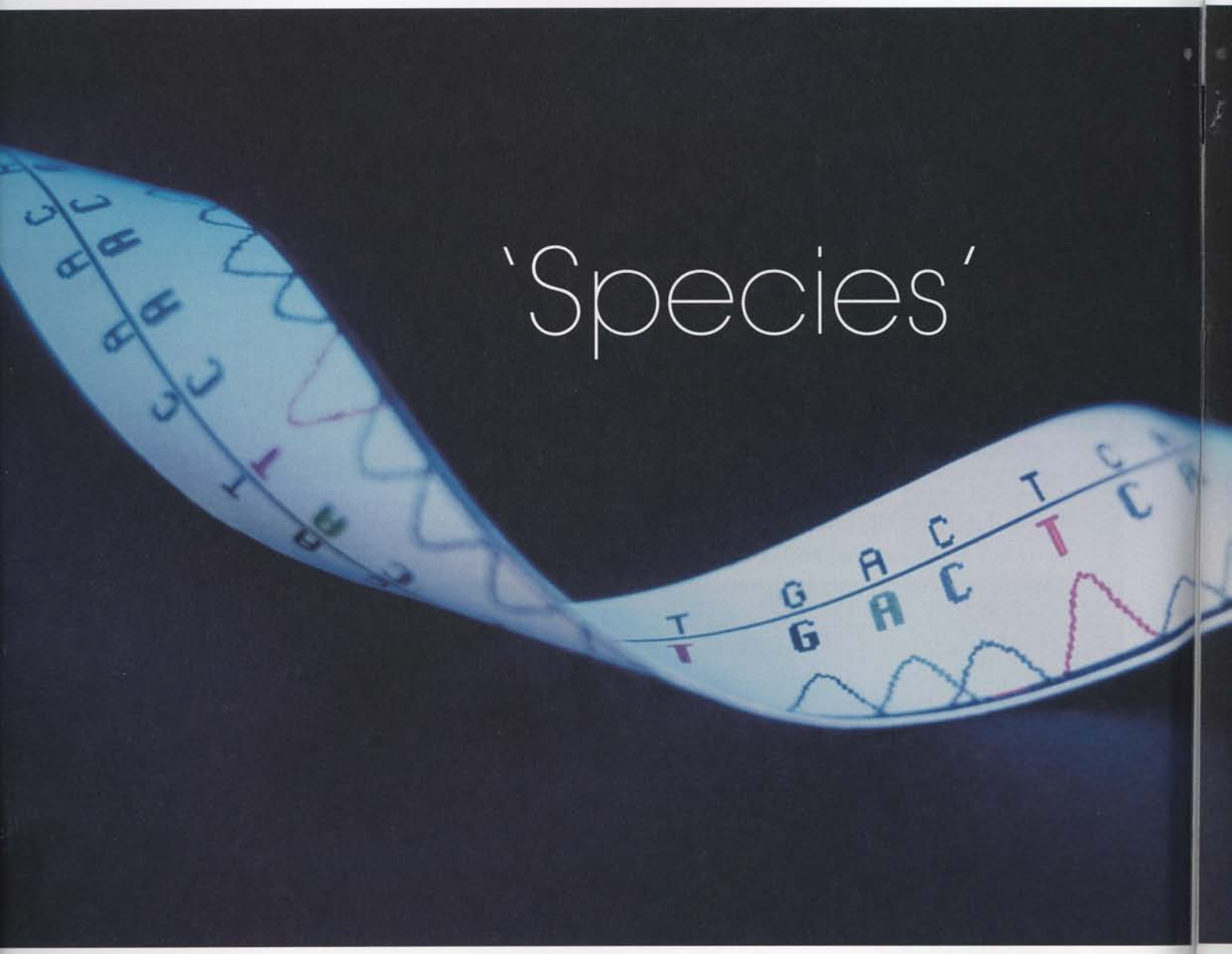
Continuous credit card payments are no longer available. Alternative methods are by direct debit (for UK bank account holders) or one-off credit/debit payment online.

Subscriptions Waived for Unemployed Members

As in previous years, subscriptions may be waived at the discretion of the Society for unemployed members under the age of 35 who are resident in the UK. If you are eligible and wish to benefit in this way in 2007 you should send a signed statement that you are currently unemployed to the Membership Office before **30 November 2006** (Please note that no increase in journal requirements will be permitted).

Income Tax Relief on Membership Subscriptions

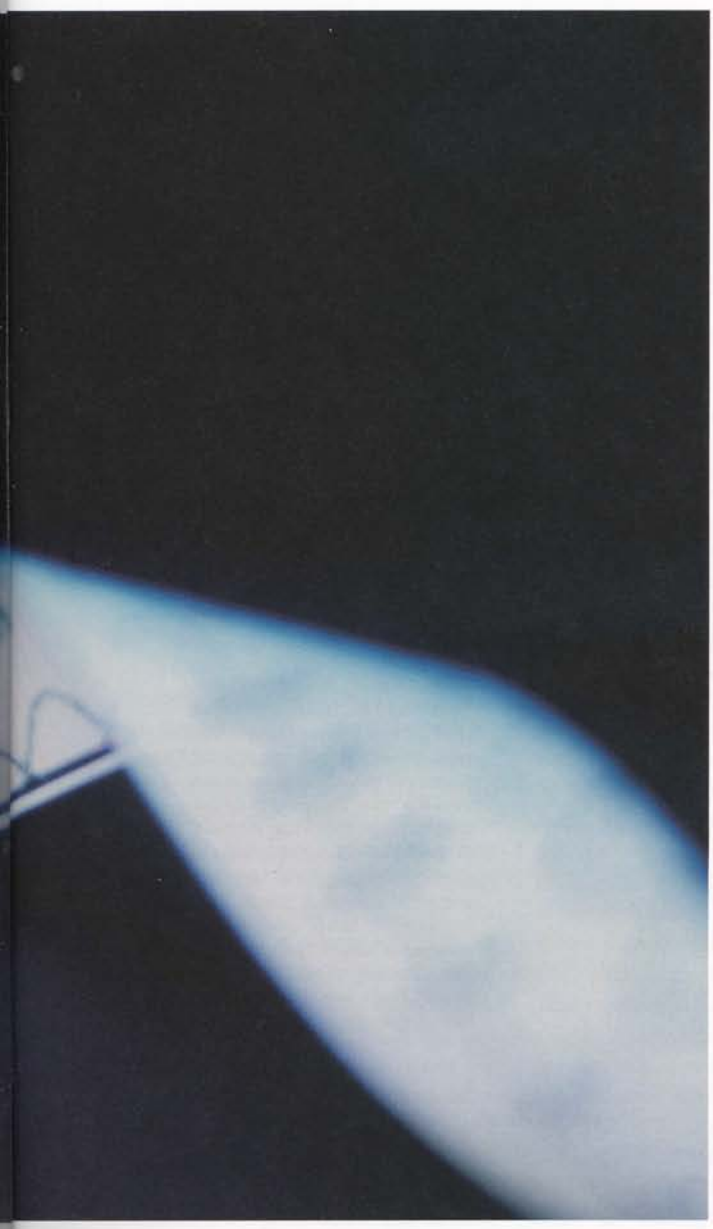
Members who are liable for UK income tax are reminded that their annual subscriptions to the Society have been approved by the Inland Revenue as qualifying for income tax relief. Any member who would like further information or has difficulty in obtaining this relief should contact the Executive Secretary.



'Species'

Biologists have long struggled with the problem of defining and recognizing species. Even animal and plant species are sometimes difficult to delimit, and philosophers disagree about the ontological referents of the word. But microbes – especially prokaryotes – seem to pose special problems. Their small size and general uncultivability (only a very small percentage can grow in the lab) confound efforts to describe and archive type specimens. Worse, prokaryotes reproduce asexually and are thus in principle unable to conform to Ernst Mayr's popular Biological Species Concept – '*groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups*'.

Thus microbiologists have in general been willing to admit that practical needs for identification and naming might best be met by some widely agreed-upon but provisional (and at least in some sense arbitrary) species definition, while the more theoretically driven search for a unifying species concept that would explain patterns of microbial diversity in ecological and population genetic terms could wait for the accumulation of more data, especially gene and genome sequence data. Operationally, molecular definitions have won the day, and species are usually expected to share at least 70 % binding in standardized DNA–DNA hybridization and/or over 97 % gene-sequence identity for 16S ribosomal RNA (rRNA) (*see the article by Stackebrandt & Ebers on p. 152*).



◀ *Lawrence Lawry / Science Photo Library*

What is a bacterial species? **W. Ford Doolittle** discusses how genome data are giving microbiologists cause to think carefully about how to define the 'species'.

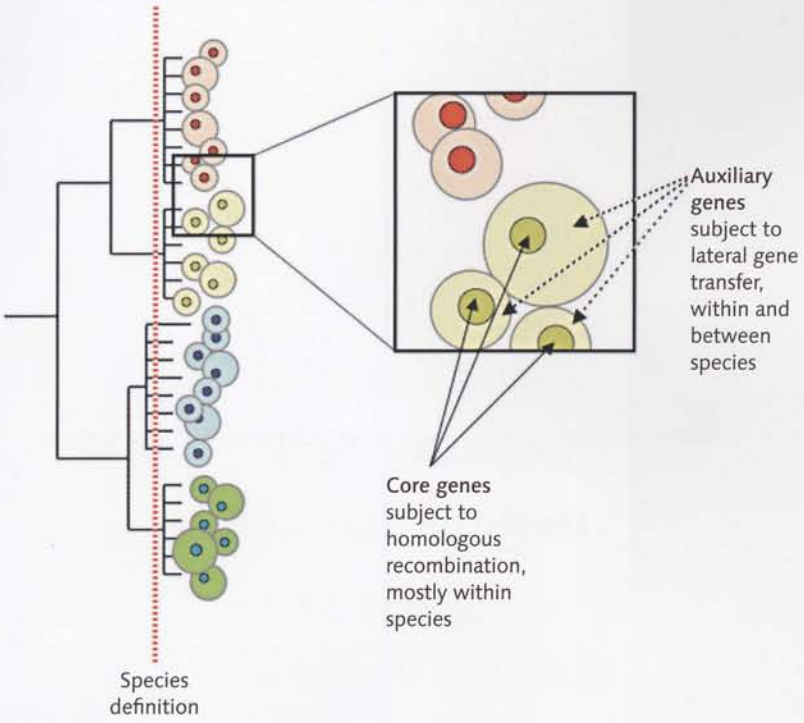
Diversity within diversity

As a bonus, molecular methods allow us to identify and enumerate species in the environment without isolating and cultivating any organisms – for instance, using specific PCR primers to amplify and sequence 16S genes from unfractionated environmental DNA preparations. What we often find is an astonishing number and diversity of apparent species, with few 16S sequences assignable at the 97 % level to any cultivated isolates – including isolates from the same sampling site. Moreover, many species, as defined by the 97 % 16S identity cut-off, will themselves be represented by multiple different but similar individual sequences in any sample (Fig. 1).

Such gene sequence 'microdiversity' is the rule rather than the exception with environmental sampling of many genes (in addition to 16S), and may be matched by another kind of variation at the level of genome composition (gene content). Martin Polz and collaborators have used pulsed-field gel electrophoresis to show that *Vibrio splendidus* isolates (with >99 % 16S sequence identity) from a sample site on the Massachusetts coast can differ by as much as a megabase in size, comprising '*at least a thousand distinct genotypes, each occurring at extremely low environmental concentrations (on average less than one per millilitre)*'.

Gene content diversity has also emerged as a principal message of more 'traditional' complete genome sequence studies, based on cultivated isolates. When such activities began, a decade ago, the thought was that one sequence (that of strain K12) would surely be enough to define *Escherichia coli*, one would do for *Bacillus subtilis*, and so forth. Completion of the second *E. coli* (O157:H7) gave us a shock. This sometimes lethal food contaminant proved to have 1,387 genes not present in K12, scattered in hundreds of small or large clusters around its genome. (Reciprocally, K12 had 528 genes not in O157:H7.) The two genomes were otherwise (aside from one inversion) colinear, exhibiting 98 % average nucleotide identity (ANI) between shared genes.

Now that there are more than three dozen species with more than one strain sequenced, results like this seem to be almost the norm. Strains which are very close on the basis of the sequences of the genes they do share



◀ Fig. 1. Diversity and microdiversity. Trees based on 16S rRNA or other phylogenetic marker genes sequences often show 'microdiversity', exhibiting clusters of sequences more closely related than the value accepted as defining species (vertical hatched red line). Even in species so defined, genomes can show substantial (up to 30 %) variation in size and gene content. A 'species genome' or 'pangenome' can be imagined to comprise core genes shared by all its strains, and a set of auxiliary genes found only in some strains. These two classes of genes may have different evolutionary modes and tempi. *W.F. Doolittle*

may nevertheless differ by up to 30 % in gene content, the differences being attributable to scores or hundreds of events of gene gain (mostly by lateral gene transfer) and gene loss after divergence from a common species ancestor. Although many variable sequences are phages or transposable elements, others are genes vitally important in defining a strain's specific niche. Sometimes such genes are transferred together: 'pathogenicity islands' exemplify this, but the phenomenon is not limited to pathogens. Endosymbiotic nitrogen-fixing strains of *Mesorhizobium*, for instance, possess an approximately 500 kb 'symbiosis island' which encodes not only the dozen and more genes needed to form root nodules, but most or all genes needed for nitrogen fixation and the island's own strain-to-strain transfer.

It has recently become popular to think in terms of 'species genomes' or 'pangenomes' (Fig. 1), consisting of a core or backbone of genes shared by all strains and an auxiliary or flexible gene pool found only in one or some strains. For some groups, the number of such auxiliary genes already exceeds the number of genes in the core, and just keeps on climbing with each new genome sequenced. Core genes in contrast get fewer with each new genome. But still there will be hundreds to thousands of core genes for any group we might want to call a species, and they will usually comprise a colinear backbone, within which laterally transferred genes and islands can be seen to be embedded. We might use concatenated (strung-together) core gene sequences to define species with greater precision and nuance than either DNA–DNA hybridization or 16S allow. Konstantinidis & Tiedje noted that an ANI value of greater than 94 % for core genes characterizes most species defined by other means.

We might also use phylogenetic trees of concatenated core gene sequences to establish lineage relationships of strains within a species, although for this, homologous recombination poses a complication. Increasingly, many bacteria (and some archaea) turn out to avidly indulge in between-strain homologous recombination. Recombination means that different genes will have different evolutionary histories, and there will be no unique phylogeny relating the genomes of different strains of a species. The rate of recombination in bacteria will of course never approach that of animals, who must recombine every time they reproduce, but still it can exceed mutation as a generator of evolutionary novelty. This raises the possibility that the Biological Species Concept might appropriately be applied to some bacteria after all, at least in so far as it entails sharing of core genes by recombination in a common gene pool.

Getting the concept

Homologous recombination is one process that confers genomic coherence (within-species similarity and between-species divergence) in prokaryotes, just as it does in animals. Members of species A will more closely resemble each other than they do members of a sister species B, because at most core gene loci they share alleles that have arisen within the A gene pool. Periodic selection is another coherence-generating

We will be more likely to realize a full understanding of microbial diversification if we accept that the word 'species', for all its utility, may have no precise referent.

process, which will also homogenize gene content. In this mode, favourable mutations sweep to fixation within a physically or ecologically bounded finite asexual population, carrying the rest of the genome in which they first occurred (and whatever auxiliary genes it might bear) along for the ride. Mutations at other loci than that under selection may of course occur along the way, and if homologous recombination is frequent, in the end only the favoured mutation – not the genome in which it first appeared – will achieve fixation. In different groups and at different times these two forces will vary in strength to promote coherence in gene sequence and/or gene content, but there is no guarantee that either will maintain or create sharp species boundaries. Divergence in sequence suppresses recombination, but recombination with divergent sequences might often offer more significant selective advantage. Agents of genetic exchange (phages and conjugation systems) have recognition specificity, but as selfish elements are under pressure to broaden, not restrict, host range. Sometimes genetic processes and ecological selective forces may create groups as genomically coherent as animal species, but there is no compelling theoretical argument that they must always or usually do so, and so far there are insufficient data to say that they generally have.

Lateral gene transfer is no respecter of species boundaries and disrupts genomic coherence, however defined or achieved. More to the point, transfer is often the source of those genes whose expression in phenotype most strongly differentiates closely related taxa, and concerns us most as practising microbiologists. So molecular definitions of species based on whole genomes may be only poorly coupled to the ecological drivers of the processes of diversification and adaptation we would like to think of as speciation. For this reason Konstantinidis & Tiedje suggest that microbiologists might better bring their ideas about species in line with those of zoologists by '*including only strains that show a >99% ANI* ***or*** *are less identical at the nucleotide level, but share an overlapping ecological niche...*' (emphasis mine). They are willing to be flexible about using overall genomic coherence in recognizing species, when phenotypic differences (which will often be due to laterally transferred genes) seem to warrant it. Our current species definitions and the more genomically based criteria likely to be adopted soon serve their practical purposes as well as we can expect, but there can be no very precise mapping to any unitary model of diversification and adaptation. That is, it seems unlikely that we will soon, if ever, have a uniform species concept that will allow us to give unqualified (definition-independent) answers to such questions as how many prokaryotic species are there at some particular site, or in the whole world. It's not that there is no hope for a full understanding of microbial diversification, adaptation and dispersal – it's just that we will be more likely to realize that hope if we accept that the word 'species', for all its utility, may have no precise referent.

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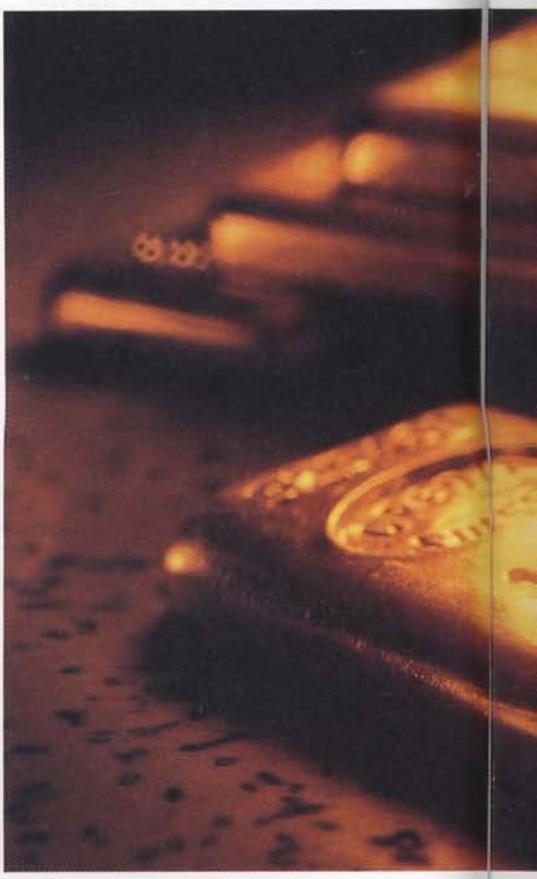
Taxonomic parameters revisited: tarnished gold standards

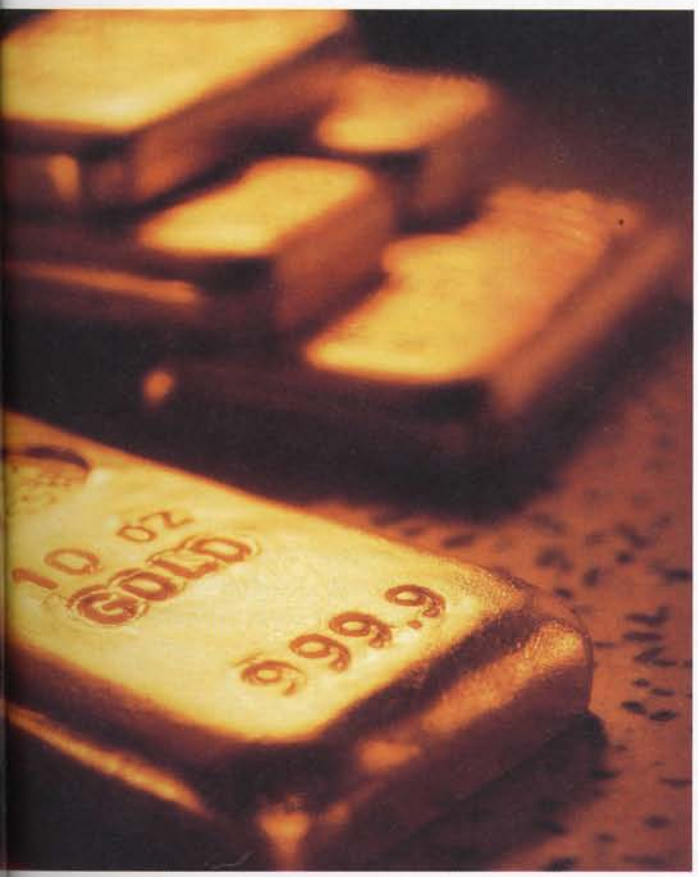
Molecular properties are included in the definition of a 'species'. Exciting new findings announced here by **Erko Stackebrandt** and **Jonas Ebers** show that a 16S rRNA gene sequence similarity range above 98.7–99 % should be mandatory for testing the genomic uniqueness of a novel isolate. This overturns the old value of 97 % and will greatly facilitate the work of taxonomists.

With the inclusion of defined genomic properties in 'minimal standards' of taxon descriptions, molecular data are now fully acknowledged in systematic studies of prokaryotes. Depending on the rank of a taxon, these approaches are either mandatory or optional. At the taxonomic level of 'species', molecular properties serve two requirements: first, to verify the morphological, biochemical and chemotaxonomic coherence of strains of a 'species' by their similarities (preferably identity) at the genomic level and, second, to delineate this taxon from phylogenetically neighbouring species of the genus (Wayne *et al.*, 1987; Rosselló-Mora & Amann, 2001). As the taxon 'species' represents populations that themselves are the



result of different mechanisms and tempi of evolution (Stackebrandt *et al.*, 2002; Gevers *et al.*, 2005), the degree of deviation from nearly absolute phenotypic and genomic identity (as expected to occur in clones) requires from taxonomists a balanced judgement of evolutionary processes that they may possibly not be aware of. In order to facilitate and harmonize taxonomic decisions in a field in which the Biological Species Concept does not apply, an arbitrary and artificial definition has evolved over a century of bacterial taxonomy (Staley & Krieg, 1984; Stackebrandt, 2000); today, the description of the construct 'species' is more stringently controlled by recommendations than that of any other taxon. While in the pre-Approved Lists era, taxonomists were allowed to follow their own subjective judgements, the past 25 years have witnessed a more objective and internationally controlled verification process of 'species' descriptions.

The predictability of the uniqueness of a '*species novum*' has been largely strengthened by the universal applicability of molecular data. Methods applied, to name a few, embrace approximate characterization of the chromosome by determination of the base composition (mol% G+C content) and degree of reassociation of single-stranded DNA (DNA–DNA hybridization) as well as comparison of one-dimensional restriction and PCR patterns (Pukall, 2005); other methods focus on genes and operons, encoding rRNA and proteins, including typing and sequencing. Each of the methods applied has its strength in elucidating a defined range of the 4-billion-year evolution of prokaryotes. Though several molecular methods have their merits in taxonomy, two approaches, the 'gold standards', play a dominant role: DNA–DNA hybridization for 'species' delimitation, and 16S rRNA gene sequence similarities for unravelling more distant relationships among strains. DNA–DNA hybridization can be expressed as percentage reassociation similarity or ΔT_m of reassociated DNA strands (Wayne *et al.*, 1987), but only the first parameter is in general use. This judgement appears objective when browsing through the past 15 or so volumes of the *International Journal of Systematic and Evolutionary Microbiology* (*IJSEM*) (formerly *International Journal of Systematic Bacteriology*), the official publication of the International Union of Microbiological Societies (IUMS). Almost every species description contains a phylogenetic analysis of the type strain based on 16S rRNA gene sequence similarity comparison and many novel species are delineated from their phylogenetic neighbours by DNA–DNA reassociation values below 70 %.



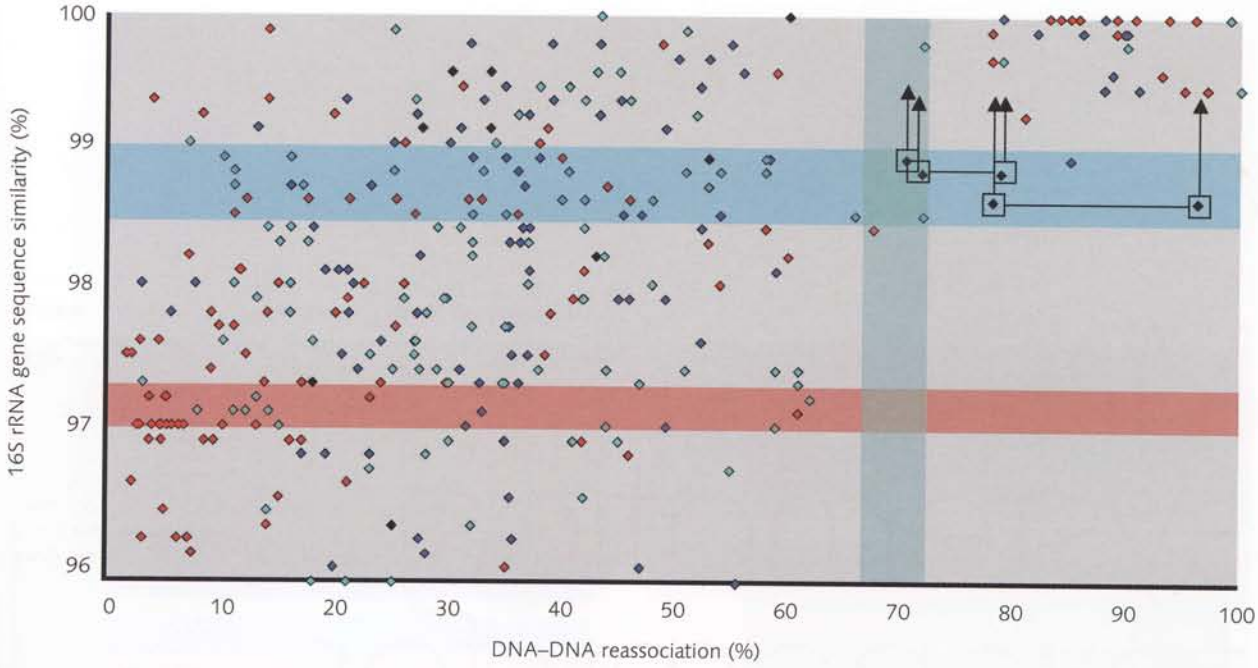
◀ Gold standards. *Digital Vision*

▶ Fig. 1. Comparison of 16S rRNA gene sequence similarities and DNA–DNA reassociation values. Data have been compiled from publications containing species descriptions from *IJSEM* **55** (2005). The different colours refer to broad categories of reassociation methods: red, microtitre plate technique, e.g. Ezaki *et al.* (1989); dark blue, spectrophotometric technique, e.g. De Ley *et al.* (1970); light-blue, membrane filter method, e.g. Tourova & Antonov (1987); black, other methods, e.g. dot hybridization (Amakata *et al.* 2005), or not defined. Horizontal rules between squares indicate data obtained by two different reassociation methods. Arrows point to the position of *in silico*-recalculated binary 16S rRNA gene sequence similarity values of sequences deposited by Amakata *et al.* (2005). The horizontal blue bar indicates the threshold range above which it is now recommended to perform DNA–DNA reassociation experiments; the horizontal red bar indicates the threshold values published previously (Stackebrandt & Goebel, 1994). *E. Stackebrandt & J. Ebers*

Despite the importance of the DNA–DNA reassociation approach, most microbial taxonomists are not in a position to perform these studies and need collaboration with specialized laboratories. Experience is needed in isolation and purification of DNA and, although one can choose from a variety of different hybridization methods (Rosselló-Mora, 2005), none of these is straightforward to apply without thorough training. But these are not the only reasons for the aversion to this technique: the method of reassociation of denatured DNA strands of two different strains unfolds the homologous genome stretches that are involved in the reassociation process. In these times of complete genome sequences and the teaching of sequence techniques to undergraduates, this failure to examine the mechanisms behind the process makes DNA–DNA reassociation seem like a method salvaged from the past. Also, a significant number of physico-chemical parameters, genome size, the presence of large plasmids, DNA purity and other factors, influence the hybridization results; reciprocal values may differ by up to 15 %. Unlike sequences, which must be deposited in public databases for inspection of quality, no reviewer of a new species description is in a position to judge DNA reassociation values. Last, but not least, as the data are not cumulative, studies on a large number of closely related species (i.e. in the genera *Streptomyces*, *Pseudomonas*, *Aeromonas* or certain groups of *Bacillus*) may become a search for the end of the rainbow. However, it must be conceded that the requirement to provide evidence for overall genomic and phenetic similarity among members of a species on the one hand, while proving dissimilarity of character traits between members of different species on the other, works well, and has set the stage for stability in prokaryotic taxonomy, keeping in mind the artificial definition with which 'species' are described.

At the beginning of the 1990s, with the release of the avalanche of 16S rRNA gene sequences, it became obvious that sequence similarities and DNA reassociation values obtained for the same strain pairs do not show a linear relationship (Rosselló-Mora & Amann, 2001; Fox *et al.*, 1992; Stackebrandt & Goebel, 1994). It could be demonstrated on the basis of a limited dataset that, below a threshold value of 98.5 % gene sequence similarity, the corresponding DNA reassociation values were always lower than 70 %. In order to reduce the workload involved in DNA–DNA reassociation experiments, it was suggested that reassociation experiments need only be performed for strains that shared 16S rRNA gene sequence similarities higher than about 97.0 % (Stackebrandt & Goebel, 1994). This value was lower than that determined from the literature, but was suggested from a taxonomically conservative point of view. Having been cited more than 1,350 times since 1994, this demarcation value indeed turned out to be a guide for researchers and reviewers. In order to update the correlation between these two taxonomic parameters, we screened all articles published in volume 55 of *IJSEM* and are now able to revise the results published in 1994 (Fig. 1). Rather than 97.0 %, we now recommend a 16S rRNA gene sequence similarity threshold range of 98.7–99 % as the point at which DNA–DNA reassociation experiments should be mandatory for testing the genomic uniqueness of a novel isolate(s). The graph compiles 380 data points obtained by all major hybridization techniques performed on representatives of most phyla of prokaryotes (only values above 96 % similarity are shown in Fig. 1). Only two studies on three strain pairs revealed that, at 16S rRNA gene sequence similarity lower than 99 %, the corresponding DNA reassociation values were higher than 70 %.

Our criticism is also directed towards a somewhat careless handling of 16S rRNA gene sequences. Many sequences deposited in public databases appear to be direct downloads from computer printouts, lacking rigorous inspection of quality and secondary-structure feasibility. As sequence errors decrease rather than increase similarity values, the relatedness between organisms with erroneous sequences is lowered. Indeed, there are some sequences of highly related strains which show deviations from highly conserved secondary structure features. We have critically analysed the quality and similarity values of 16S rRNA gene sequences for one data set that show higher than 70 % DNA reassociation values at 16S rRNA gene sequence similarities below 99 % (marked in black in Fig. 1). Though we had access to neither the strains nor the original data, *in silico* corrections of nucleotide idiosyncrasies meant that the similarity values increased by



up to 0.8 %, pushing them over the 99 % line: these values are indicated by arrows in Fig. 1. It appears to be critically important to check the quality of sequences according to secondary features prior to deposition in public databases.

This recommended increase of about 2.0 % in 16S rRNA gene sequence similarity will significantly facilitate the work of taxonomists without sacrificing the quality and precision of a 'species' description. As indicated above, DNA–DNA hybridization constitutes the bottleneck of taxonomic studies among closely related 'species', and taxonomists should acknowledge the updated correlation curve and welcome the expected reduction in workload. As the artificial cut-off value of around 70 % reassociation may not have phylogenetic significance, rare examples may exist and will arise in the future in which reassociation values around and above 70 % emerge at corresponding 16S rRNA gene sequence similarities around 99.0 %. In these cases, taxonomists are reminded of the article of Wayne *et al.* (1987), summarizing an overall concern of these authors '*that any phylogenetically based taxonomic schemes that result must also show phenotypic consistency*'.

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Virus systematics: taxonomy for the tiny

The classification of viruses poses different problems from those of classifying more elaborate life forms as **Anne-Lise Haenni** and **Mike Mayo** describe.

A question frequently asked of virologists by non-virologists is – are viruses living? Perhaps one answer to this chestnut is in the nature of virus systematics. Although molecular in scale (circoviruses have ~20 nm diameter virions and ~2 kb ssDNA genomes; viroid genomes consist of ssRNA of only ~0.3 kb), viruses and viroids are replicated by error-prone mechanisms that result in variations from which natural selection yields distinctive forms fitted to particular niches. Thus one can study and describe virus variation, investigate the causes and consequences of this variation and manipulate the data to produce a system of classification. This constitutes a classic definition of systematics and can be said to describe much of current virological research activity.

Background

Intrinsic to any biological research are **classification** – the production of a logical system of categories, each containing any number of organisms, that allows easier reference to its components, **nomenclature** – the construction of a system of names and a formal guide to their use in taxonomy, and **taxonomy** – the process of naming organisms and classifying them hierarchically to express their mutual relationship in a simplified way and according to internationally agreed codes of practice. In virology, these activities are undertaken by the International Committee on the Taxonomy of Viruses (ICTV). ICTV consists of a network of some 500 virologists organized into Study Groups for particular groups of viruses (e.g. a family) that report to a subcommittee for each of the major groups of hosts (vertebrates, invertebrates, plants, prokaryotes, or fungi, protozoa and algae). The Chairs of these subcommittees form the backbone of the Executive Committee of ICTV.

It is feasible to administer virus taxonomy (including nomenclature) through such an organization because of the relatively small number of virus species currently recognized. In contrast to the approximately 17,000 new species

Genome	Family	Genera	Hosts	Genome size (kb)
dsDNA	*Myoviridae*	6	Pro	169
	Siphoviridae	8	Pro	43
	Podoviridae	4	Pro	40
	Tectiviridae	1	Pro	15
	Corticoviridae	1	Pro	10
	Plasmaviridae	1	Pro	12
	Lipothrixviridae	3	Pro	16
	Rudiviridae	1	Pro	33
	Fuselloviridae	1	Pro	15
	Guttaviridae	1	Pro	20
	Poxviridae	11	V	170
	Asfarviridae	1	V,I	180
	Iridoviridae	5	I	150
	Phycodnaviridae	6	Algae	335
	Baculoviridae	2	I	134
	Nimaviridae	1	I	293
	Herpesviridae	10	V	130
	Adenoviridae	4	V	31
	Polyomaviridae	1	V	5
	Papillomaviridae	16	V	7
	Polydnaviridae	2	I	245
	Ascoviridae	1	I	120
	Unassigned genera	3		
ssDNA	*Inoviridae*	2	Pro	6
	Microviridae	4	Pro	6
	Geminiviridae	4	Pl	6
	Circoviridae	2	V	2
	Nanoviridae	1	Pl	6
	Parvoviridae	9	V	5
	Unassigned genera	1		
RT	*Hepadnaviridae*	2	V	3
	Caulimoviridae	6	Pl	8
	Pseudoviridae	3	I,Pl,F	6
	Metaviridae	3	I,Pl,F	5
	Retroviridae	7	V	7
dsRNA	*Cystoviridae*	1	Pro	13
	Reoviridae	12	V,I,Pl,F	24
	Birnaviridae	3	V	6
	Totiviridae	3	F	5
	Partitiviridae	3	F,Pl	4
	Chrysoviridae	1	F	13
	Hypoviridae	1	F	10
	Unassigned genera	1		
–ssRNA	*Bornaviridae*	1	V	9
	Rhabdoviridae	6	V,Pl,I	12
	Filoviridae	2	V	19
	Paramyxoviridae	7	V	15
	Orthomyxoviridae	5	V,I	10
	Bunyaviridae	5	V,Pl,I	12
	Arenaviridae	1	V	11
	Unassigned genera	4		
+ssRNA	*Leviviridae*	2	Pro	4
	Narnaviridae	1	F	3
	Picornaviridae	9	V	7
	Dicistroviridae	1	I	9
	Marnaviridae	1	F	9
	Sequiviridae	2	Pl	10
	Comoviridae	3	Pl	10
	Potyviridae	6	Pl	10
	Caliciviridae	4	V	8
	Astroviridae	2	V	7
	Nodaviridae	2	I	4
	Tetraviridae	2	I	8
	Luteoviridae	3	Pl	6
	Tombusviridae	8	Pl	4
	Coronaviridae	2	V	28
	Arteriviridae	1	V	13
	Roniviridae	1	I	26
	Flaviviridae	3	V,I	11
	Togaviridae	2	V,I	10
	Bromoviridae	5	Pl	8
	Tymoviridae	3	Pl,I	6
	Closteroviridae	3	Pl	16
	Flexiviridae	8	Pl	6
	Barnaviridae	1	F	4
	Unassigned genera	14		
Viroids	*Pospiviroidae*	5	Pl	0.4
	Avsunviroidae	2	Pl	0.3

Table 1. Virus families and some representative properties

Abbreviations: Pro, prokaryotes; V, vertebrates; I, invertebrates; Pl, plants; F, fungi. Colours indicate main hosts, gradients signify more than one type of host. Genome sizes are for a representative species.

of animals that are described every year, the virus world consists of a mere 1,950 species. As parasite diversity surely parallels host diversity, this number is clearly a massive under-representation, but is probably held in check because to establish a new virus species is a laborious research exercise and only 'important' hosts such as man are studied intensively.

Viruses are diverse in genome type and size and infect hosts in all major types of organisms. The table shows the currently recognized virus families clustered according to genome type. Taxonomy above the level of families is at present limited to the recognition of three orders; most families are not clustered into orders and some genera are as yet unassigned to taxa above the level of genus. This reflects some of the uncertainties in current virus taxonomy.

The nomenclature of virus taxa, in particular species, differs fundamentally from that of the rest of biology. It is regulated by the International Code of Virus Classification and Nomenclature, which is published on the ICTV website (www.danforthcenter.org/iltab/ICTVnet/asp/_MainPage.asp) and in reports such as the current *8th ICTV Report*. This article is concerned only with the classification aspects of ICTV's work, in particular the factors that complicate virus classification: the intrinsic properties of viruses and the probable evolutionary origins of viruses.

Complicating factors – properties of viruses

As for many microbes, morphological characters are of limited use (although the advent of electron microscopy did lead to significant taxonomic advances) and small genetic changes can result in substantial changes in pathological impact. Virus genomes are small (see Table 1) and can often vary rapidly. However, it is now possible to obtain nucleotide sequences very quickly, sometimes without isolating the virus from its host. By using appropriate software, evolutionary distances between sequences can be deduced and phylogenetic trees obtained. This assumes that the sequences are derived from a single ancestral sequence and

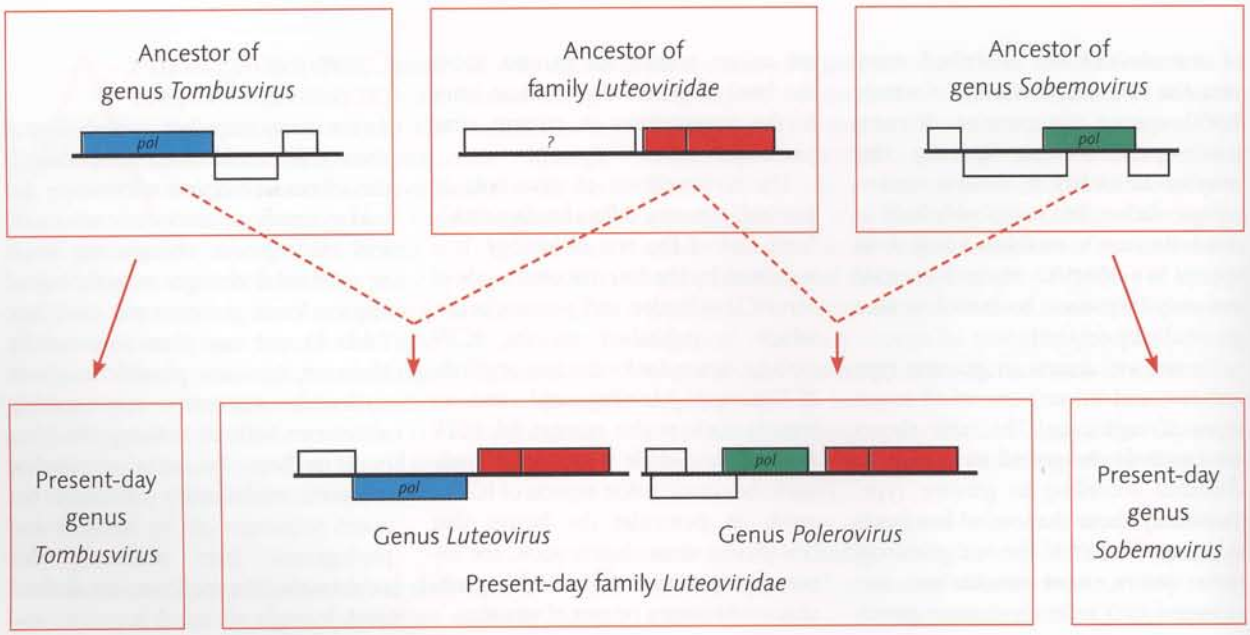
that differences have arisen by mutation of single bases. Such a model seems most reasonable when sequences are clearly similar, such as when viruses in a genus are compared. When other sorts of sequence variation have happened, such as recombination, these simple comparison techniques are likely to lead to erroneous conclusions. This is vividly illustrated by the genomes of viruses that are classified in the family *Luteoviridae*. There is no dispute that viruses in this family are related both in sequence details and in their biology (all are transmitted by aphids in a circulative fashion that involves virions crossing several barriers inside aphid bodies). The RNA genomes of luteovirids consist largely of two gene blocks, one of structural genes and the other of replication-related (*pol*) genes (Fig. 1). The phylogenetic trees obtained by comparing luteovirid structural genes confirm the family cluster and show a separation of species into the constituent genera. But when *pol* genes are compared with those of other viruses, the genera *Luteovirus* and *Polerovirus* differ greatly and each seems more closely related to one or the other non-luteovirid genus. It seems that recombination between gene blocks has happened during the evolution of these viruses (Fig. 1). Expecting sequence comparisons and the resulting trees to produce a durable classification is thus, at least in some instances, a naïve approach to virus classification. Whereas it works for some virus taxa, for others a more arbitrary and broadly based approach that uses multiple criteria is clearly necessary.

Complicating factors – evolutionary history

Biological taxonomy is well suited to classifying organisms that are related to one another via simple branched and diverging descent, with relatively long evolutionary distances between successive branch points. However, virus evolution differs from this simple paradigm in several ways.

1. Genomes of some viruses, such as luteovirids (see above; Fig. 1), have been formed by recombination (joining of parts of separate genomes to produce a new genome) and/or reassortment (making new combinations among separate parts of the genomes of viruses with multipartite genomes, such as influenza viruses), resulting in chimeric viruses with polyphyletic (i.e. hybrid) genomes whose origins cannot be represented in a monophyletic scheme.
2. Genomes of some viruses seem to contain genes acquired from their hosts.
3. Viruses are unlikely to be descended from one original 'protovirus'; they probably arose more than once, but when and how many times is unclear.
4. Genomes of some viruses (e.g. retroviruses) integrate into the genomes of the hosts, where they are subject to selection pressures

▼ Fig. 1. Diagram of possible origins of the genomes of viruses in different genera in the family *Luteoviridae*. The upper boxes contain hypothetical genomes and assume that the ancestors of tombusviruses and sobemoviruses had genomes like those of their current-day counterparts. Red boxes represent blocks of structural protein genes. *pol* signifies replication-related genes. ? signifies a gene block of unknown type. Further details can be found in the *8th ICTV Report*.



different from those applying to independently replicating genomes.

5. Some viruses infect more than one type of host (one often serving as vector for transmission between other hosts) and therefore will have evolved in response to complex selection pressures.

Any or all of these factors greatly complicate, if not vitiate, attempts to deduce phylogeny from studies of current genome sequences and thus, whatever the taxonomic arrangement, some viruses and/or taxa will always seem to be misfits.

Conclusion

Notwithstanding the complications, some sort of classification is fundamental to most virological research, whether it be deducing the nature of a new disease agent by working out which of the known taxa it most resembles or developing a better understanding of the nature of a virus by predicting properties based on its assumed classification.

To develop and maintain such a classification, ICTV communicates with virologists in several ways. Via its website, ICTV provides many of the details of its organization as well as current issues in virus taxonomy. ICTV also publishes regular formal reports (such as the recent *8th ICTV Report*) and occasional notes in the Virology Division News section of *Archives of Virology*. Also, workshops, such as the recent meeting on virus evolution and taxonomy which has been summarized by U. Desselberger, are held and, as work progresses, ICTV expects to organize further such workshops.

New viruses are being discovered all the time. Some can be fitted into existing taxonomic structures (e.g. *Severe acute respiratory syndrome coronavirus*; SARS-CoV), making it possible to predict their properties, and others are unlike anything seen previously (e.g. *Acanthamoeba polyphaga mimivirus*). Thus, virus taxonomy must have both an internal logical justification and a capability of expansion to accommodate novelty. Whatever the virological endeavour, virus taxonomy should provide a provocative framework to which all virologists can contribute. Current ICTV practice aims to achieve this.

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Virus taxonomy must have both an internal logical justification and a capability of expansion to accommodate novelty. Whatever the virological endeavour, virus taxonomy should provide a provocative framework to which all virologists can contribute.

How many yeasts?

Faced with classifying more than 300 novel yeasts isolated from insect guts, **Meredith Blackwell** has had to consider many systematics issues.

One, two, three ... 300? At a time when systematists are interested in biodiversity, it is important to be able to enumerate numbers of organisms. For example mycologists believe that many fungi remain to be discovered to reach the total estimated 1.5 million species. Numbers of species are dependent to some extent on a choice of a species concept, a topic that has been the subject of heated discussions among systematists of all organisms. My colleagues and I study yeasts that inhabit the gut of insects feeding on fungi. After 6 years of periodic collecting in Panama and the southern USA, a limited geographical range, we believe we have approximately 300 novel ascomycete yeast species in a group (Saccharomycetales) currently estimated to contain about 800 species. Yeasts are essential to decomposition in many environments and some are important economically. We hope to discover that our yeasts are both ecologically and economically significant, but for the moment we are preoccupied with determining and describing unique species from the poorly investigated beetle gut habitat.

A dramatic shift in my research occurred when I put aside the study of life cycles and phylogenies of insect-dispersed fungi with complex life histories, some involving

two unrelated hosts and as many as three distinct morphological states in the same life cycle. I was diverted from such remarkable fungi by Dr Sung-Oui Suh, a postdoctoral researcher in the laboratory, to the study of yeasts from the gut of mushroom-feeding beetles. Our research emphasis changed, therefore, not only to fungi with an extremely simple morphology, but also to resolving different questions set at lower taxonomic levels. Because of the presence of relatively few morphological characters in yeasts, physiological traits have been used as phenotypic characters, but such traits often are too plastic to serve as stable markers for species.

What is a species?

Our need for a species concept became urgent when, in our first year of studying insect gut yeasts, we discovered almost 100 potentially undescribed taxa. Then there were 200, and today, almost 300 undescribed yeasts. We spoke glibly about numbers of 'species' without considering exactly how we defined them. There is no shortage of species concepts from which to choose, and, perhaps much of the confusion surrounding the topic rests with separating theory from practice. An excellent discussion of species concepts applied specifically to fungi by John W. Taylor and colleagues, distinguishes theoretical and operational aspects of the question. In their evaluation of species concepts, outlined by R.L. Mayden, they accepted the Evolutionary Species Concept (ESC) of G.G. Simpson as a primary theoretical species concept. Briefly, the ESC defines a species as a group of organisms with a history of common descent that '*maintains its identity from other such lineages...*'. Mayden considered other concepts: Morphological Species Concept (MSC), Biological Species Concept (BSC) and Phylogenetic Species Concept (PSC) to be secondary to, but compatible with, the ESC.

Taylor and colleagues discussed species concepts in terms of recognition criteria and pointed out that these are not specified by the ESC, although they are inherent in secondary, operational concepts such as the MSC, BSC and PSC. Moreover, theoretical and operational concepts can be separated

◀ Far left. Wood-boring passalid beetles attempt an escape into their tunnels. The beetles inhabit wood previously infected by white rot basidiomycetes that have removed cellulose and lignin, making the wood suitable for beetle invasion. These beetles harbour very different yeasts when compared to those of fungus-feeding beetles, and their hind gut microbiota includes xylose-fermenting yeasts. *Meredith Blackwell*

◀ Far right. Where are all the yeasts? Novel gut yeasts are known from the beetle *Ellipticus* sp. (Erotylidae) shown feeding on sporulating patches of *Tinctoporellus epimiltinus* (Basidiomycota). The basidiomycete is one of the species that is widespread in Louisiana as well as other parts of the Caribbean, but thus far associated insects and yeasts have much more restricted distributions. *Joseph V. McHugh*

▼ Left. Yeast-harbouring endomychid beetles are found feeding on a variety of fungi, including some ascomycetes, the dark patches seen here. *Joseph V. McHugh*

▼ Right. The larger erotylid beetle (*Megischysus* sp.) and three scaphidiine (Staphylinidae) beetles feeding on a resupinate polypore. The presence of different beetles on the same fruiting body has been postulated as providing opportunity for host switching by their gut yeasts. *Joseph V. McHugh*

on the basis of 'species concept' and 'species recognition'. The criteria for recognition of species are inherent in the name of the concepts: basically (1) morphology, (2) reproductive competence and (3) position in a phylogenetic tree. For most fungi morphology is very simple – in fact that is why yeasts (and bacteria) were distinguished by physiological traits early in the history of their systematics. Physiological traits, while no longer considered to be the best characters for recognizing species, do provide clues to ecological function and help in the recognition of yeast guilds that share similar nutritional resources. Tests of the BSC relying on reproductive compatibility do not work well for most fungi, because many are asexual and many more are self-compatible and do not outcross. 20% of all fungi are estimated to be asexual; for ascomycete yeasts the proportion is even greater with 215 asexual to 265 ascospore-producers recorded in 1998. That many of the ascospore-formers are non-outcrossing yeasts increases the count even more.

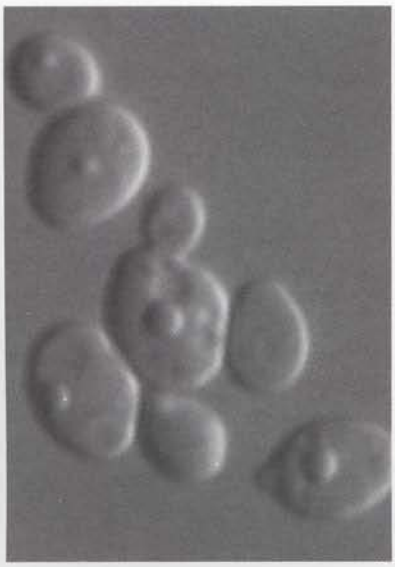
The use of two of the criteria, morphology and sexual compatibility, to distinguish species has been played down on the grounds that these recognition criteria cannot be applied with an expectation of precise success. The criteria, however, should not be completely disregarded, because they have been the basis of so many currently recognized fungi. Recent comparisons of fungal species based on morphological or reproductive compatibility criteria, however, indicate that species numbers usually, although not always, are underestimated by comparison with species recognized on the basis of DNA analysis, because changes in morphology and reproductive capacity often lag behind genetic change. Phylogenetic species recognition is also favoured because it can be applied readily to all fungi. Fungi share characters from DNA sequences that, regardless of morphology or reproductive capability, can be collected and analysed to produce gene genealogies of potential species.

Lines of demarcation

At this point an operational problem arises: where to draw the species line? Is it appropriate simply to draw a line based on a clustering of terminal taxa in a tree? Taylor and colleagues applied a non-arbitrary method, genealogical concordance, that relies on agreement of several different gene genealogies to indicate placement of the line of species limits. Varying degrees of discordance among genealogies indicates that lineage sorting and fixation of genes is not complete and isolation of the population has not happened. Phylogenetic Species Recognition by genealogical concordance has been applied successfully to a wide variety of basidiomycetes and ascomycetes as well as non-fungal organisms, including prokaryotes.

Delimiting 300 new species?

How do we delimit and describe 300 new yeasts within the 6-year period of grant funding? This is a huge problem because of the constraints of community expectations and nomenclature code restrictions. We characterize the yeasts using DNA sequences, a feat rapidly accomplished by a team of hard-working undergraduates. By 1998 Cletus Kurtzman and colleagues had sequenced the D1/D2 region of the large subunit nuclear ribosomal RNA gene (LSU rDNA) of all available yeast



▲ Top. Many insects have intimate associations with yeasts, but so do other animals. The nest of a Panamanian bird contains speckled eggs and is composed in large part of rhizomorphs, the dark, narrow-diameter filaments that are made up of ropes of microscopic thread-like somatic structures of fungi. *Nhu H. Nguyen*

▲ Bottom. Carmen Rodriguez (University of Georgia) and Nhu Nguyen (Louisiana State University), participants in the National Science Foundation Research Experiences for Undergraduates program, collect beetles at the Smithsonian Tropical Research Institute, Barro Colorado Island site, Panama. Students acquire many skills from collecting, isolating and purifying yeasts in culture to performing a number of molecular techniques. *Joseph V. McHugh*

◀ Left. Buds make the yeast. This photomicrograph of the type of a new species collected at the Smithsonian Tropical Research Institute, Barro Colorado Island, Panama, illustrates yeast buds. The name of the species, isolated from gut of a fungus-eating beetle, *Candida sinolaborantium*, commemorates the Chinese labourers whose lives were lost to build the transisthmian railroad that preceded construction of the Panama Canal. *Cennet Erbil*

◀ Right. *Polyporus tenuiculus*, a delicate wood-decaying basidiomycete is a favourite food of many beetles that harbour yeasts in their gut. *Nhu H. Nguyen*

Our need for a species concept became urgent when, in our first year of studying insect gut yeasts, we discovered almost 100 potentially undescribed taxa; then there were 200, 300...

species. Their original database, to which we and others have added hundreds of additional sequences, makes it possible to find close relatives of new yeasts using not only BLAST searches but also phylogenetic analyses for clade recognition and to provide a basis for delimiting species. It is common for gut yeasts to vary by as many as 30–70 bp in the D1/D2 region. Other yeasts may vary by only a few base pairs from a known strain, and these are more difficult, because the question of where to draw the species line arises. Kurtzman suggested that a substitution rate greater than 1 % in about 600 bp in the D1/D2 region be used to separate species – approximately 4 or more base pairs difference for ascomycete yeasts. The difference correlates well with data from mating tests in a study of biological species in yeasts that could be crossed. The 1 % solution, however, failed to distinguish *Saccharomyces cerevisiae* and *S. bayanus*, and other close relatives, and almost certainly provides a conservative estimate of species numbers as suggested for other fungi.

In practice, in our rush to characterize yeasts, we do not obtain sequences of several independent DNA regions for the novel yeasts. We do, however, use additional markers, e.g. microsatellites and the plastic physiological traits that are useful at low taxonomic level. These markers are more variable than sequences, and we almost certainly underestimate species numbers in order to handle the large numbers of novel isolates.

Needs for yeast systematics

We are fortunate to have a dense LSU rDNA database, and many literature resources are widely available for the study of yeasts. In addition to the required classic, *The Yeasts, a Taxonomic Study* (Kurtzman & Fell), with a fifth edition in preparation, an essential online resource, the BioloMICS database of the Centraalbureau voor Schimmelcultures (CBS) (www.cbs.knaw.nl/) based on the book, has characters of all species, an online identification system and up-to-date references. These resources available for yeasts are exceptional

among fungi, and yeast systematists are finding many new species that can be identified to near species level rapidly using DNA sequences – or can be recognized as undescribed. In fact rapid deposition of DNA sequences to GenBank has promoted collaboration among yeast workers with similar sequences in distant localities, including, in our experience, Panama, Brazil, Portugal and Belgium.

Although rDNA is well sampled for many species and about 20 complete genome sequences are available, more sequences, especially for protein-coding genes, are needed for basal yeasts to provide a well-supported predictive phylogenetic framework for organizing yeast taxa. Genomes will help to access additional appropriate DNA regions for use in systematics. Continued collecting from natural habitats will lead to the discovery of many more predicted novel yeasts. Additional sampling will also help to establish biogeographical limits of many species currently known from only few localities.

What more could we wish for to help characterize 300 yeast species? Our most immediate need is for rapid methods to determine yeast physiological profiles. We culture 1–3 isolates of every yeast we describe in about 100 different ways to determine physiological and morphological traits. This tedious characterization procedure is a part of the 'standard' description recognized by the community of yeast systematists, one that also gives value added data on potentially valuable biological organisms – especially at a time when discussions of petroleum alternatives are intense.

The elimination of the Latin diagnosis required for valid publication by the International Code of Botanical Nomenclature, would dramatically hasten the publication of our 200 novel yeast backlog. Time spent on translating a diagnosis to Latin or discussing whether a description should be in the nominative or the ablative case, could be used for more rapid publication of essential organisms in our environment!

How many fungi?

Search for and discovery of novel fungi were common themes of papers and entire symposia at the 8th International Mycological Congress held in Cairns, Australia, in late August. In our own presentation we suggested that known ascomycete yeast numbers had increased by almost 30 %, and we predicted that sampling of previously collected insect gut habitats in just Panama and the southern USA could easily yield another 100 species, something Dr Suh and I are close to achieving. Yeasts have been, and will continue to be, useful models in population studies that will lead to an understanding of mechanisms of speciation. Some degree of host and geographical specialization of nearly 300 insect gut yeasts and the vast regions left to sample for insect gut yeasts, indicates that these organisms will contribute significantly to increasing the numbers of ascomycete yeasts and indeed the total number of all fungi.

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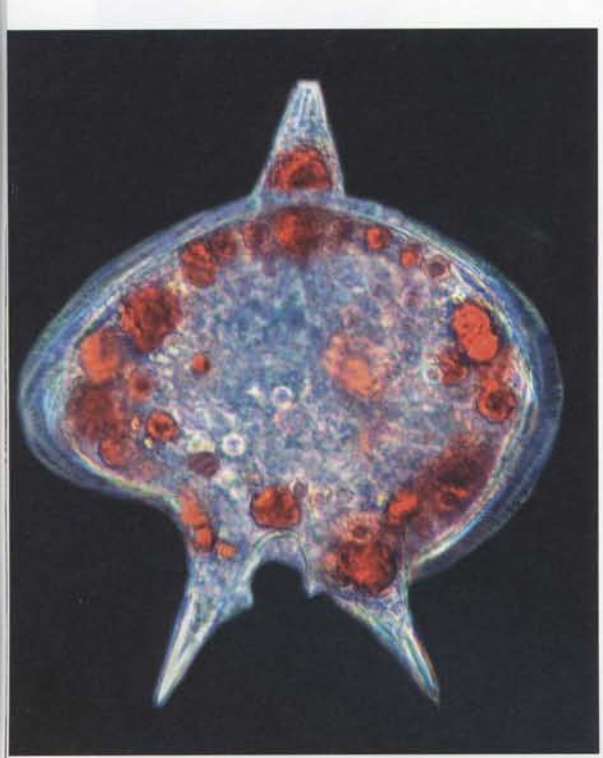
Protozoa: the most abundant predators on earth

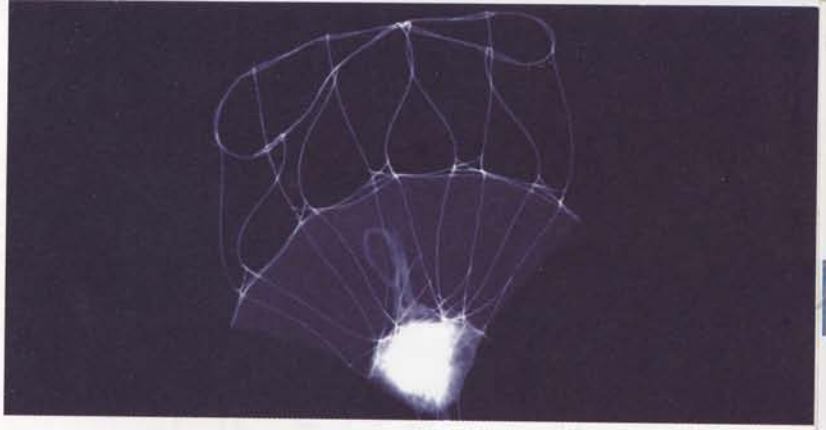


The most abundant predators on earth are not animals like lions or ants, but the microscopic, unicellular protozoa. When I was a student, protozoa were treated as one phylum in the animal kingdom, with just four classes: amoebae, flagellates, ciliates and the purely parasitic Sporozoa. Now they are recognized as a separate kingdom comprising 11 phyla. Ciliates remain as one phylum, Ciliophora, which also includes the highly specialized Suctoria that lack cilia in their sedentary adult stages, having evolved elaborate tentacles with explosive organelles for trapping prey. But amoebae and flagellates are abandoned as formal taxa, while Sporozoa have been drastically refined, by removal of microsporidia to Fungi and Myxozoa to Animalia, in a radical overhaul of protozoan systematics that lasted for three decades but is now settling down, after extensive lively controversies, into a period of broad consensus.

As a student in the 1960s I realized that electron microscopy showed protozoan cells as architecturally far more diverse than animal or plant cells. Classically, the boundary between protozoa and algae was fuzzy, with several groups claimed both by zoologists and botanists, in particular dinoflagellates, euglenoids, cryptomonads and chrysomonads, some of which are photosynthetic algae while others are predatory heterotrophs; a few, notably some dinoflagellates, that feed both ways were an especial problem for early classifications based just on nutrition and motility. The first major break from the simple 19th century fourfold division occurred 25 years ago when I established the kingdom Chromista for a mixture of algae and former protozoa and treated the remaining Protozoa as a separate kingdom, in which flagellates were split into several phyla (Fig. 1). By then it was widely accepted that chloroplasts originated by the enslavement of a cyanobacterium and that all eukaryote algae evolved ultimately from heterotrophic ancestors. We now know that the primary diversification of eukaryote cells took place among zooflagellates: non-photosynthetic predatory cells having one or more flagella for swimming, and often also generating water currents for pulling in prey.

Evolution of early eukaryotes

Cilia of ciliates and oviducts, and flagella of flagellates and sperm, are homologous structures that grow from centrioles with a beautiful 9-triplet microtubule structure; I here adopt the increasing tendency to refer to both as cilia. The traditional distinction that cilia are short and many, whereas flagella are long and few, is trivial and often fails, as does the idea of a distinct beat pattern. Cilia share no evolutionary relationship with the rotary extracellular flagella of bacteria, but are specializations of the eukaryote cytoskeleton that probably evolved at the same time as the nucleus and the enslavement of an alphaproteobacterium as the first



The kingdom Protozoa embraces an immense diversity of single-celled predators and numerous parasites causing diseases like malaria, sleeping sickness, and amoeboid dysentery. As **Thomas Cavalier-Smith** explains, they were the first eukaryote cells, giving rise to all higher kingdoms, and there has recently been a revolution in their high-level systematics.

mitochondrion. Thus the first eukaryote cell was a flagellate, very likely one with amoeboid tendencies, not a rigid pellicle – and probably only one cilium. Amoeboid protozoa arose several times from flagellate ancestors by evolving highly varied pseudopodia for creeping and crawling; some, notably the secondarily anaerobic mastigamoebae and *Multicilia* (both Amoebozoa) retained a cilium or cilia, but others lost them altogether or kept them only for wider dispersal, suppressing them during crawling/feeding.

The role of molecular systematics

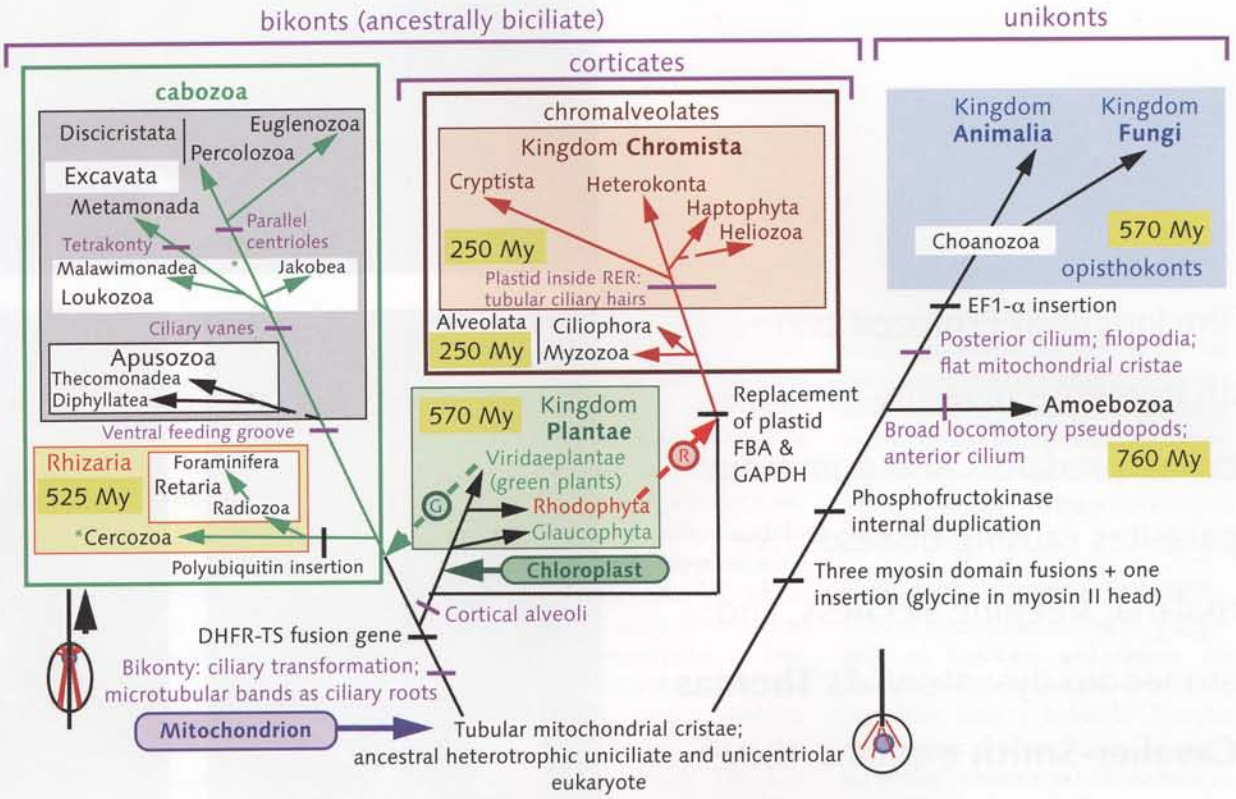
Some thoughtful 19th century protozoologists suspected that flagellates preceded amoebae, as just asserted. But until DNA sequencing and molecular systematics burgeoned in the 1980s and 1990s the often more popular idea of Haeckel that amoebae came first and flagellates were more advanced could not be disproved. Sequences enabled calculation of phylogenetic trees based on differing degrees of gene sequence divergence. Equally importantly, they reveal much rarer and more substantial discrete molecular changes, e.g. gene fusions, splitting of genes, insertion and loss of introns, gene duplications and indels (insertions or deletions). Such characters, treatable by the cladistic reasoning that morphologists used for centuries, are sometimes more decisive than sequence trees. Gene sequence trees excel in establishing close clusters of strongly related organisms, but sequence evolution is more complex and variable than sometimes thought, so is sometimes extremely misleading, yielding partially wrong topologies. The test of a good phylogeny and classification is that different independent lines of evidence yield congruent conclusions. We are approaching this happy state of affairs for protozoan large-scale systematics, though problems and controversies remain.

Diversification and diversity

The emerging picture (Fig. 1) shows the primary diversification of eukaryotes as involving changes in the development

▲ Transmission electron micrographs of choanoflagellate protozoa. Choanoflagellates feed on bacteria by trapping them with a collar of microvilli around the base of the cilium, much as do the remarkably similar internal feeding cells of sponges, the most primitive animals. It is probable that animals evolved from a choanozoan protozoan similar to a choanoflagellate. Top *Cosmoeca* sp.; lower left *Acanthoeca spectabilis*; lower right *Parvicorbicula* sp. *Dr Per R. Flood / Natural History Museum Picture Library*

◀ Dark field light micrograph of a live specimen of the predatory dinoflagellate *Protoperidinium depressum*. *Dr Per R. Flood / Natural History Museum Picture Library*



of cilia and structure of microtubular roots that attach them to the rest of the cell. Unikonts comprise animals, fungi and the protozoan phyla closest to them: Choanozoa (e.g. choanoflagellates) and Amoebozoa (typical amoebae with broad pseudopods, slime moulds like *Dictyostelium* and mastigamoebae), which all share the same myosin II as our muscles, which is absent from all bikonts. Bikonts comprise the plant and chromist kingdoms, three protozoan infrakingdoms (Alveolata, Rhizaria, Excavata), and the small zooflagellate phylum Apusozoa, whose closest relatives are unclear. Ancestrally bikonts had two diverging cilia: the anterior undulates for swimming or prey attraction; the posterior serves for gliding on surfaces in many groups (likely its ancestral condition), but secondarily adapted for swimming in others (or quite often was lost to make secondary uniciliates, not to be confused with genuine unikonts, which confusingly are sometimes secondarily biciliate). All major eukaryote groups except Ciliophora include severely modified organisms that lost cilia; some became highly amoeboid.

Among bikonts emphasis on pseudopodia is greatest in Rhizaria, comprising two phyla (Cercozoa, Retaria) in which pseudopods are typically very thin (filopodia: 'thread feet') and often branch or fuse together in elaborate feeding networks: especially well developed as nets (reticulopodia) in Foraminifera (predominantly benthic Retaria, the most abundant seafloor predators) and Radiozoa (major micropredators of oligotrophic oceans: acantharia and polycystine radiolaria). Cercozoa include flagellates, the peculiar chlorarachnean algae formed by the enslavement of a green alga (G in Fig. 1), amoebae with filopodia (many with shells that evolved independently of shells of some Amoebozoa) and amoeboflagellates; they are the most abundant predators in soils, yet were only recognized as a phylum in 1998; they also include parasites of commercially important shellfish (e.g. oysters) and crops (e.g. sugarbeet and cabbages).

Parasitism developed more strongly in phylum Myzozoa, named after their widespread feeding by myzocytosis (sucking out the interior of cells rather than engulfing them whole by phagocytosis). Sporozoa *sensu stricto*, e.g. malaria parasites, are all parasites and are grouped with some free-living sucking predatory zooflagellates as subphylum Apicomplexa, within Myzozoa together with Dinozoa (dinoflagellates and relatives), also often parasites. Myzozoa and Ciliophora comprise Alveolata, typically with well-developed cortical alveoli: smooth vesicles with skeletal and calcium segregating functions. Alveolates are sisters of kingdom Chromista, being jointly called chromalveolates. The ancestral chromalveolate flagellate had phagotrophic nutrition and chloroplasts surrounded by extra membranes arising from their origin by intracellular enslavement of a red alga (Rhodophyta; Fig. 1). Chromalveolates comprise chromophyte algae, whose chloroplasts bear chlorophyll c2, and many derived lineages that lost photosynthesis and sometimes plastids altogether, e.g. ciliates, oomycete chromists. Chromists differ from alveolates in that the phagocytic vacuole membrane around the enslaved red alga fused with the nuclear envelope, placing their plastids inside the rough endoplasmic reticulum. This contrasts markedly with kingdom Plantae, where chloroplasts lie in the cytosol and were never lost, even in chlorophyll-free plants.

The test of a good phylogeny and classification is that different independent lines of evidence yield congruent conclusions.

◀ Fig. 1. The eukaryote evolutionary tree. Taxa in black comprise the basal kingdom Protozoa. The thumbnail sketches show the contrasting microtubule skeletons of unikonts and bikonts in red. The dates highlighted in yellow are for the most ancient fossils known for each major group. Major innovations that help group higher taxa are shown by bars. Four major cell enslavements to form cellular chimaeras are shown by heavy arrows (enslaved bacteria) or dashed arrows (enslaved eukaryote algae). Four features of the tree need more research. (1) Are centrohelid Heliozoa (non-photosynthetic and non-flagellate predators that catch prey by slender radiating axopodia supported by microtubules) really sisters of Haptophyta; do they even belong in Chromista? (2) Are Apusozoa perhaps sisters of all other bikonts and not really early diverging excavates? (3) Was a green alga (G) really enslaved by the ancestral cabozoan rather than separately by Cercozoa and euglenoids (asterisks)? (4) Are Rhizaria and Excavata really sisters? *T. Cavalier-Smith*

Within excavates, the strictly non-amoeboid Loukozoa ('groovy animals') have a well developed ventral feeding groove with a scooped out, 'excavated', look that gives the infrakingdom its name; their mitochondrial genome retains more bacterial genes than in other eukaryotes. Percolozoa are predominantly amoeboflagellates (Heterolobosea, e.g. the human pathogen *Naegleria fowleri*), sometimes pure amoebae (having lost cilia and groove), rarely pure flagellates. Other excavates are largely non-amoeboid flagellates. Metamonada are secondary anaerobes, having converted mitochondria into hydrogenosomes or mitosomes, with a marked tendency to multiply cilia and/or nuclei, e.g. *Giardia*, *Trichomonas*. Euglenozoa include the seriously pathogenic trypanosomatids (e.g. sleeping sickness agents), the ubiquitous bodonid zooflagellates (the weeds of eukaryotic microbiology), and the euglenoids (with complex pellicular strips: phototrophs like *Euglena*, phagotrophs like *Peranema* or saprotrophs like *Rhabdomonas*).

Bikonts share a complex pattern of ciliary development with the anterior cilium being younger and transforming in the next cell cycle into a posterior cilium, often structurally and behaviourally different. This anterior-to-posterior ciliary transformation is unique to bikonts; it probably evolved in their common ancestor as an adaptation to gliding on surfaces, using the posterior cilium as a skid. Bikonts have two distinct posterior bands of microtubules as ciliary roots and ancestrally a structurally different anterior band. This cytoskeletal asymmetry was similarly adaptive, becoming secondarily more symmetrical in many algal lineages after they became planktonic and abandoned eating prey. Thus past nutritional history is reflected in cell structure of protozoa and their modern descendants. But the picture was confusing, because of repeated evolutionary losses of organelles, until molecular evidence helped sort it out.

In marked contrast to bikonts, unikonts ancestrally had only one centriole and no microtubular bands; instead a simple cone of microtubules anchored the centriole to the nucleus and the rest of the cell. I consider this simpler structure the ancestral state for eukaryotes that evolved together with the cell nucleus. In only slightly modified form this ancestral unikont pattern is still seen in zoospores of lower fungi, choanoflagellates, the best model for animal ancestors, and our own sperm – such is the force of stasis in cellular evolution, much of which we can reconstruct by studying those most fascinating of all organisms, the protozoa, our relatives and ancestors.

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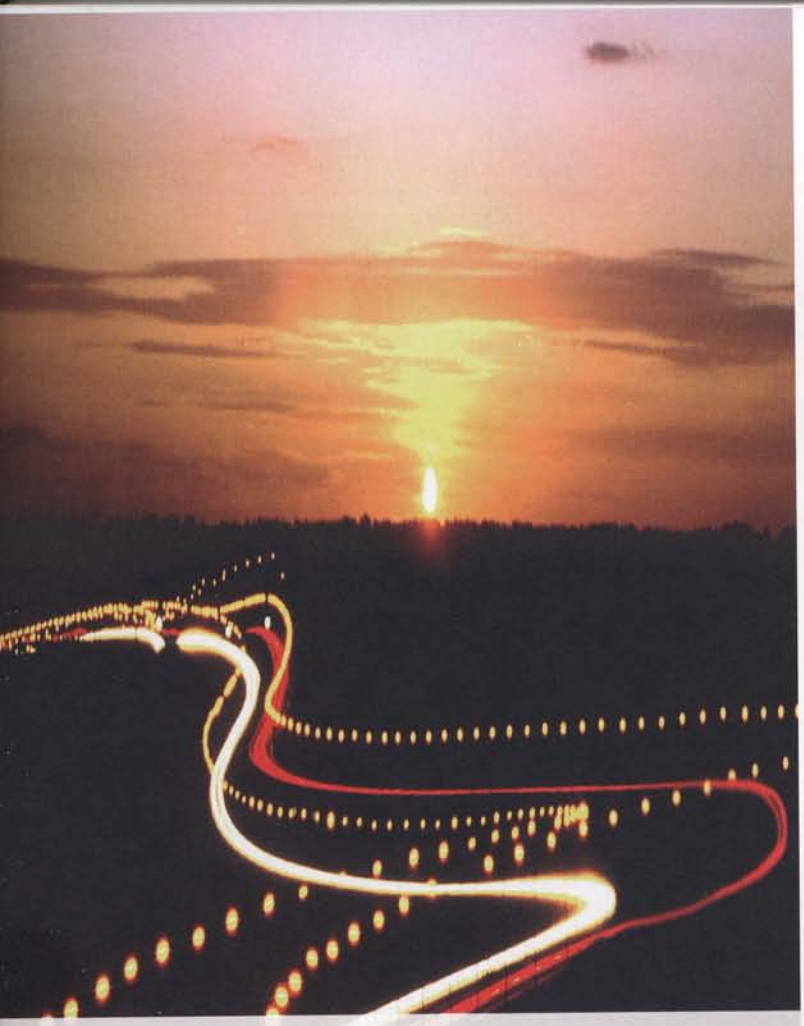


Disentangling the

Gemma C. Atkinson and **Sandra L. Baldauf** provide a guide to interpreting the complexities of phylogenetic trees

The exponential growth in publicly available molecular sequence data has created a gold mine of encoded information covering billions of years of evolution. One of the most immediate ways to make sense of these data is by the comparison of the sequences in phylogenetic analyses. Getting a simple tree is relatively easy and potentially very informative (if not exactly publishable). A deeper understanding of the methods can help you grow trees that are more reliable and reveal complex evolutionary histories.

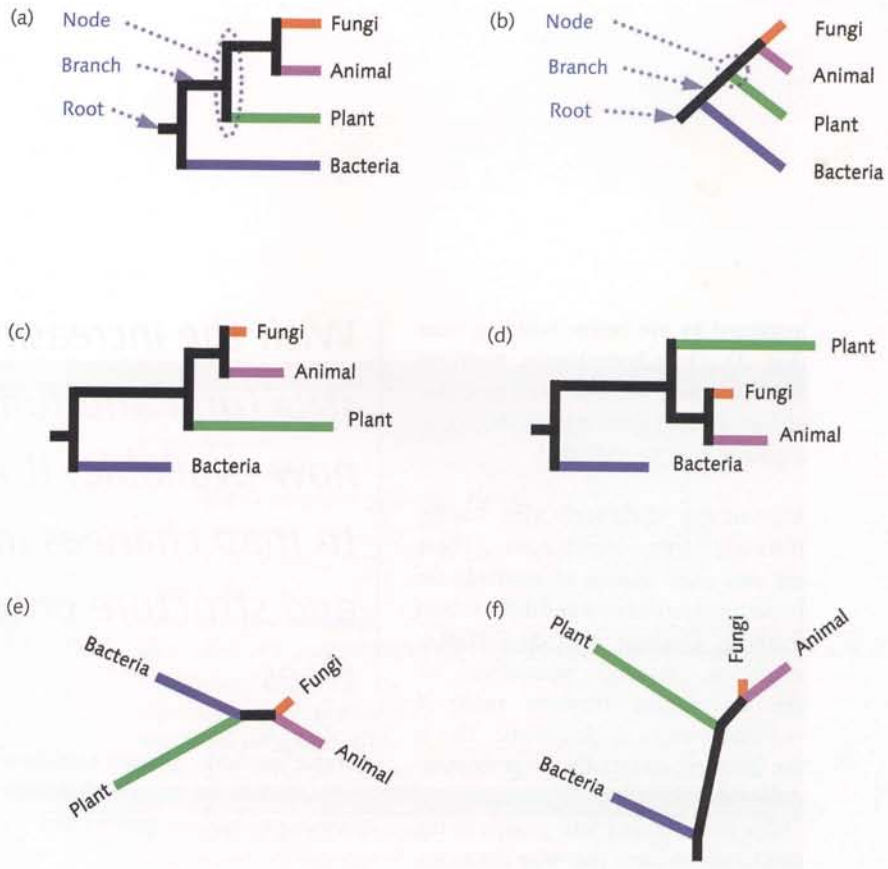
If it is relationships among organisms that you are interested in, it is becoming clear that a tree of organisms based on a single gene or its corresponding protein sequence may not necessarily represent the true history of the organisms in the tree. Rather, it tells the story of the evolutionary history of that particular gene while another gene a few bases away in the genome may have experienced quite a different evolutionary history. This is particularly true in bacterial genomes, where 'illegitimate' trading of genes is rampant (see below). Used with care, and perhaps a healthy dose of scepticism, phylogenetics offers powerful tools for tracing the often entangled evolutionary trails of genes. For bacteria, it is especially useful for revealing phenomena such as lateral gene transfer (LGT), and in all domains of life, gene duplications and, sometimes, true organismal relationships.

This article is intended as a brief tutorial on how to interpret phylogenetic tree diagrams and an introduction to the methods of preparing raw data and building a tree.

Throughout, we wish to emphasize that phylogenetics, like any other branch of science, requires informed judgement in the selection and application of methods to maximize the accuracy of the results.

Reading trees

Leaves, branches and nodes. Phylogenetic trees, or phylogenies, display deduced evolutionary relationships in the form of multiple branching lineages (Fig. 1). The sources of the sequences, or 'operational taxonomic units' (OTUs), are the tips or leaves of the tree. Nodes (junctions where two or more branches join), represent a divergence event (gene duplication or speciation) and also the hypothetical last common ancestor of all the branches arising from them. The further a node is from the tips, the further back in time the divergence happened, with the root of the tree being the most ancient node.



▶ Fig. 1. Equivalent phylogenetic trees. All these hypothetical trees have exactly the same topology and differ only in presentation style. (a, b) Rooted cladograms, which show branching order but no information on the amount of evolution on each branch; (c, d) rooted phylograms, which show branch lengths proportional to evolutionary distance; and (e) unrooted and (f) rooted radial trees. Note: the node height in trees (a), (c) and (d) is purely a device for even spacing of branches. *G.C. Atkinson*

◀ Entwined trails of car and road lights, representing the tangled trails of evolution. *Brand X Pictures / Punchstock*

trails of evolution

Orthologues and paralogues. All sequences that share a common ancestor are homologues. Homologues come in several different flavours (Fig. 2). Orthologues are the direct descendents from a single ancestral sequence and are duplicated along with the rest of the genome in each generation. Paralogues arise by gene duplication, thus giving rise to multigene families.

Xenologues. Phylogenetic trees are one of the best means of detecting LGT, which is rampant in bacteria and archaea, and also occurs at a lower, if largely unknown, frequency in eukaryotes. Xenologues are homologues that have undergone LGT and can be identified in phylogenetic trees by their tendency to nest with sequences from the donor's lineage (Fig. 2b).

Growing your own tree

Digging in the databases. A huge, exponentially growing amount of nucleotide and protein sequences is stored in public sequence databases (Table 1), the main ones of which are updated against each other daily. These are usually the first port of call when assembling or augmenting a dataset. As annotations are unreliable, the best way to find homologous sequences is by doing BLAST or FASTA sequence similarity searches. These look for matches to a user-provided query sequence in one or many sequence databases (Table 1).

The alignment: the foundation from which a tree grows. Once you have found your sequences, the next step is to align them (Table 1). A phylogenetic tree is only as good as the alignment it is built on. Multiple sequence alignment programs have continuously improved over the years, but they still tend to perform badly in regions of poor sequence conservation. Here, the human eye is often better at recognizing homologous patterns. Therefore, all alignments should be

inspected by eye before building trees (Fig. 3). Only homologous positions should be used to build trees; gaps and ambiguously aligned regions should, as a general rule, be excluded.

With the increasing amount of structural and functional information now available, it is becoming possible to map changes in protein function and structure onto evolutionary trees

Minimizing distances and hiking through tree landscapes. There are two main classes of methods for building trees: distance and discrete data methods. Distance methods (UPGMA, neighbour joining) summarize all the information between pairs of sequences into a single statistic. This is the distance, essentially the percentage difference, between two sequences. OTUs are clustered into groups in the tree based on these pair-wise distances. Parsimony, maximum likelihood (ML) and Bayesian inference methods are discrete data methods as they treat each column in the alignment as a discrete data point. These methods search 'tree-space', a probabilistic landscape of hills and valleys made up of all possible trees and their 'fitness', in a quest to find the tree or set of trees that best fit the data.

All tree building methods have their strengths and limitations. Therefore, it is a good idea to repeat analyses with multiple methods. Greater confidence in results can be gained if different methods produce congruent trees.

Modelling evolution. All phylogenetic programs assume some evolutionary model to explain amino acid or nucleotide substitution patterns. These are used by the various phylogeny programs to attach weights to different classes of substitutions, rather than assuming all mutations are equally likely. This is important for accurate trees. For example, a mutation that results in an amino acid substituting for another with similar chemical properties tends to have a minimal effect on the function of the protein. Such substitutions are frequent and indicate less evolutionary time than changes at slowly evolving sites; this is taken into account by the gamma rate correction.

Where is the root? Almost all phylogenetic programs produce unrooted trees from molecular data. However, a root is essential for establishing the order of divergences in a tree. Often, the first diverging sequence in a dataset is unknown. In this case, an outgroup of one or more OTUs can be included

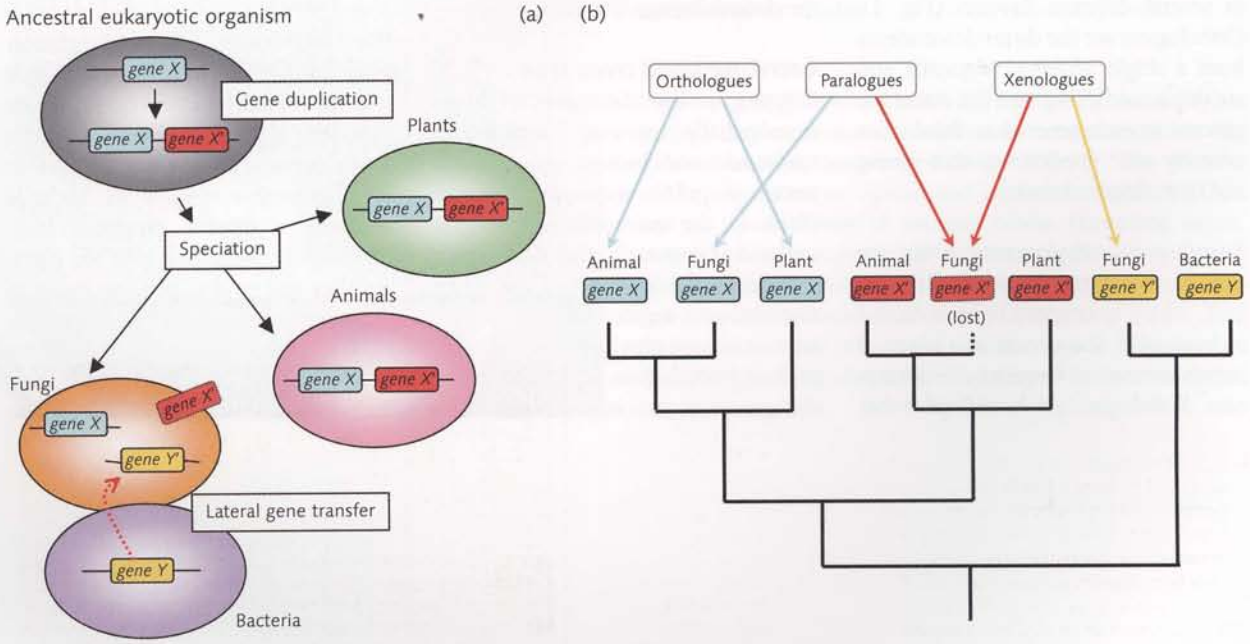
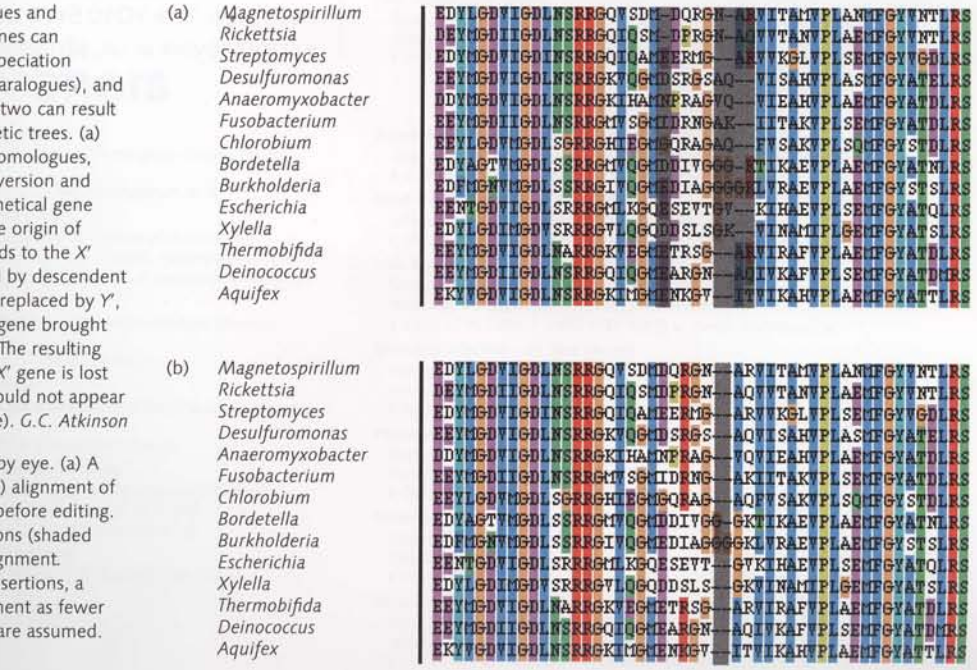


Table 1. Phylogenetic resources

Online sequence acquisition		
Major databanks	DDBJ	www.ddbj.nig.ac.jp/
	EMBL	www.ebi.ac.uk/embl/
	NCBI	www.ncbi.nih.gov/
	Uni-Prot	www.ebi.uniprot.org/index.shtml
Major genome centres	TIGR	www.tigr.org/
	Sanger	www.sanger.ac.uk/
	JGI	www.jgi.doe.gov/
Genome project portals	GOLD	www.genomesonline.org/
	Eukaryote Genome Portal	www-users.york.ac.uk/~ct505/PhD_Project5/Eukaryote_Homepage.htm
Keyword searches	SRS	http://srs.ebi.ac.uk/
	Entrez	www.ncbi.nlm.nih.gov/Entrez/
Homology searches	BLAST	http://ncbi.nih.gov/BLAST/
	FASTA	www.ebi.ac.uk/fasta33/index.html
Multiple sequence alignment		
	ClustalX	ftp://ftp.ebi.ac.uk/pub/software/
	Muscle	www.drive5.com/muscle/
	T-Coffee	www.ch.embnet.org/software/TCoffee.html
	Bioedit	www.mbio.ncsu.edu/BioEdit/bioedit.html
Phylogenetic analysis		
	PAUP*	http://paup.csit.fsu.edu/
	PHYLIP	http://evolution.genetics.washington.edu/phylip.html
	PHYML	http://atgc.lirmm.fr/phyml/
	MrBayes	http://mrbayes.csit.fsu.edu/
	GARLI	www.bio.utexas.edu/grad/zwickl/web/garli.html
	MEGA	www.megasoftware.net/
	TreeView	http://taxonomy.zoology.gla.ac.uk/rod/treeview.html
	RAxML	www.ics.forth.gr/~stamatak/index-Dateien/Page443.htm
Comprehensive list		http://evolution.genetics.washington.edu/phylip/software.html

◀ Fig. 2. Orthologues, paralogues and xenologues. Homologous genes can arise by vertical descent or speciation (orthologues), duplication (paralogues), and LGT (xenologues). The latter two can result in non-cannonical phylogenetic trees. (a) Genes *X* and *Y* are ancient homologues, with *X* being the eukaryotic version and *Y* the bacterial one. A hypothetical gene duplication event prior to the origin of plants, animals and fungi leads to the *X'* paralogue, which is inherited by descendent species. In one lineage, *X'* is replaced by *Y'*, a xenologous version of the gene brought about by LGT of gene *Y*. (b) The resulting phylogeny. Since the fungal *X'* gene is lost in this example, this gene would not appear in the phylogeny (dotted line). *G.C. Atkinson*

▶ Fig. 3. Editing an alignment by eye. (a) A section of a CLUSTAL X (Table 1) alignment of bacterial protein sequences before editing. Five columns contain insertions (shaded columns). (b) The refined alignment. Only two columns contain insertions, a more parsimonious arrangement as fewer insertion or deletion events are assumed. *G.C. Atkinson*



in the dataset. This is essentially an external point of reference to identify the oldest node in the tree, which is the outgroup's closest relative. For example, for a tree of mammalian genes, one could use the orthologus gene from a marsupial as the outgroup.

Statistical tests for support – how strong is that branch?

There are various ways to determine how 'strong' a tree is, that is, how much better it is than other possible trees. The most commonly used method is bootstrapping. This involves phylogenetic analyses of multiple random subsamples of your dataset. The bootstrap support for each branch corresponds to the percentage of analyses where that branch appears.

Bayesian inference is becoming increasingly popular in molecular phylogeny. Instead of bootstrapping, this discrete data method uses all the trees it encounters in the tree space to calculate the posterior probability for each branch in a consensus tree of all encountered trees. This is fundamentally very different from bootstrapping and posterior probability and bootstrap support values are not directly comparable. Generally, a bootstrap percentage greater than 70 % can be taken as good support, but only probabilities greater than 95 % are reasonable support for Bayesian inference trees.

Fatal attraction: the curse of long branches.

The rate of molecular evolution is not uniform across a tree; some OTUs may have acquired more mutations than others over time and therefore grow longer branches in the tree of their evolutionary history. Tree-building programs have problems with such branches, and tend to group long-branched OTUs together due to spurious sequence similarities. This is the notorious 'long-branch attraction' or LBA. Likelihood methods are on the whole less likely to be duped by variations in evolutionary rate, and the use of more accurate evolutionary models can give any algorithm a helping hand. However, likelihood methods are not beyond failure and there is no perfect model of evolution. Therefore, the easiest remedy for LBA is often to include intermediate sequences to break up long branches. Alternatively, if the long branches are not essential for the questions you are asking of the tree, it is often best just to leave them out.

Conclusions

Phylogenetics allows us to piece together clues left by chance to retrace evolutionary history, but the clues are patchy as most taxa and many genes are extinct. Because of this, phylogenetic methods rely on the experience and judgement of the researcher for the quality of the results they deliver, and knowledge of how to interpret trees is essential for making sense of the patterns within them. The rewards are great, however, as retracing the evolution of a gene with phylogenetic analysis can reveal complex and often surprising stories of molecular history. With the increasing amount of structural and functional information now available, it is becoming possible to map changes in protein function and structure onto evolutionary trees. This will allow us to begin to ask not just how organisms work, but why they do the sometimes seemingly very strange things that they do.

Gemma C. Atkinson and **Sandra L. Baldauf**
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Recent educational resources about health and disease

For the younger reader...

Why Must I Wash My Hands?
Why Must I Brush My Teeth?

These very basic questions are comprehensively answered in two books published by the Community Practitioners and Health Visitors' Association. The books are packed full of information and cover far more material than the titles would suggest, including the structure of teeth, healthy eating, history of soap and toothpaste, food safety and personal hygiene. The text is simple and the illustrations include lots of photos of children which help engage the younger reader. Each spread has a main theme and ends with points to remember. They also include some fascinating facts related to the theme. I had a slight problem navigating the pages until I became used to the style and use of font to distinguish the narrative from the factoids. As a microbiologist, I was keen to see how microbes were represented and on the whole was impressed with the books, apart from the description of bacteria as 'types of germ' in *Why must I brush my teeth?*, although the author did point out that many bacteria are harmless or useful to humans.

Since the books are in a style aimed at primary school children, I asked my daughters for their point of view. Eleanor (aged 10) thought the books were attractive and full of useful information, but felt that the style was a bit too childish for her age group and suggested that they would be most suitable for children in years 3 and 4. Isobel (age 6½) was not able to read the books for herself, but enjoyed listening to me read and looking at and discussing the illustrations. I think the books would make a useful addition to any primary school library and would support the science and PSHE teaching at KS1 and KS2. Both books cover information relevant to the micro-organisms unit in the KS2 Specifications, but the style might be off-putting to a more advanced reader.

Jane Westwell
SGM External Relations Office

Why Must I Wash My Hands?
Jackie Gaff, Cherrytree Books (2005)
£9.99, pp. 32, ISBN 1-84234-259-2

Why Must I Brush My Teeth?
Jackie Gaff, Cherrytree Books (2006)
£9.99, pp. 32, ISBN 1-84234-258-4

For Key Stage 3+...

Milestones in Modern Science – The Discovery of DNA

The scope of this small book is much wider than indicated by its title, perhaps chosen for consistency with others in the series. It is attractively presented, clearly written by an experienced science writer, not overburdened with text, and generously illustrated with attractive photographs and diagrams and information boxes. This provides an inviting balance for the intended readership, KS3 upwards.

There is a brief introduction to DNA and genetics, followed by five other chapters dealing with a history of genetics, the discovery of DNA and its structure, protein synthesis and mutations, applications to genetics, and future prospects. There is also a

timeline, glossary, sources of further information (websites and books) and an index.

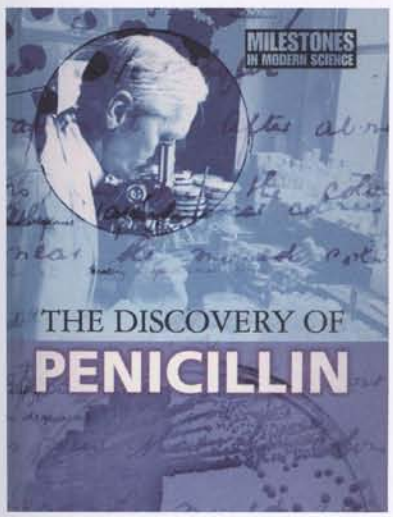
The book is appropriate for a school library but where resources are scarce it is an expensive purchase when a similar coverage and more pages can be obtained elsewhere for little more than half the price.

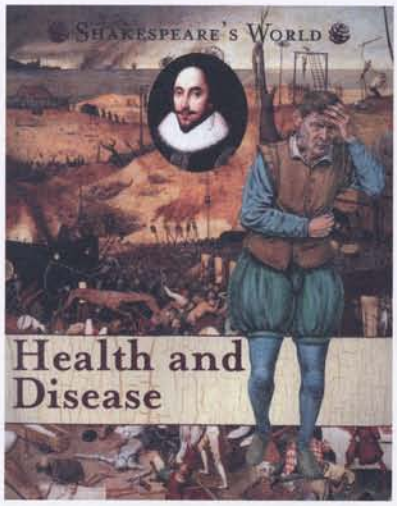
John Grainger
Chairman, MISAC

Milestones in Modern Science – The First Polio Vaccine

Milestones in Modern Science – The Discovery of Penicillin

Somewhat surprisingly these titles are written by Guy de la Bédoyère, who is more commonly associated with archaeological digs on TV, but nevertheless are very well researched and highly readable. Each book covers far more than its title suggests, ranging over different aspects of the history of medical microbiology whilst bringing the subject bang up-to-date. Each introduction summarizes the book in two pages and probably gives enough facts for the average student to understand the topic and even do their homework. However, another 34 pages packed with interesting information follows, complimented by a timeline, glossary and index.





The further reading includes some recent, quite challenging books, as well as useful websites.

The chapters in the polio volume are headed: early experiments with vaccines, the polio virus, Salk's investigations, the vaccine works – field tests and mass trials, new vaccines, polio today. Chapters in penicillin book are: bacteria and disease, the path to penicillin, making penicillin work, penicillin for all, and penicillin today.

I really liked the layout, which is easily navigated and bucks the current trend in educational books for a lurid scattergun design, whilst still using colour-coded boxes about 'Key People' and 'Facts' to break up the text. I was particularly impressed by the picture research, which has produced some unusual and highly relevant illustrations alongside the diagrams. I suspect that this may be a factor in the cost of the books. Good quality science photographs do not come cheap!

In my former life as a school librarian, I would have snapped up these books; they meet all the selection criteria. Their content is excellent, presents a balanced view and does not shy away from the controversy, uncertainty and personality clashes that make up real science.

Janet Hurst
SGM External Relations Office

The Discovery of DNA
Camilla de la Bédoyère,
Evans Brothers Ltd (2005)
£14.00, pp. 48, ISBN 0-23752-740-5

The First Polio Vaccine
Guy de la Bédoyère,
Evans Brothers Ltd (2005)
£14.00, pp. 48, ISBN 0-23752-738-3

The Discovery of Penicillin
Guy de la Bédoyère,
Evans Brothers Ltd (2005)
£14.00, pp. 48, ISBN 0-23752-739-1

Health and Disease
Kathy Elgrin, Cherrytree Books (2005)
£11.99, pp. 32, ISBN 1-84234-190-1

Health and Disease

Here is a book with a strange concept. It is one of a series, *Shakespeare's World*. The book covers a range of topics and on each page there is a quotation from the great bard with an explanation of what this teaches us about life in Tudor times. Of course there is no microbiology, because no-one knew that unseen life forms were the cause of infections, but the doctors of the day were beginning to understand the scientific reasons for illness. The book explains this quite clearly.

Two-page spreads cover health and hygiene, theories about health, common complaints, surviving birth and childhood, plague, doctors, healers, cures and remedies, herbs and herbals, madness, hospitals, wounds and operations. I was quite surprised to see how often Shakespeare refers to doctors and medicine in his plays. The book is attractively laid out and beautifully illustrated. It is clearly written and appears to have been well researched. I really enjoyed reading it. However, I am not sure who it is for, or how it would be used in school as it crosses over history, English literature and science.

Janet Hurst
SGM External Relations Office

gradline

Gradline aims to inform and entertain members in the early stages of their career in microbiology. If you have any news or stories, or would like to see any topics featured, contact **Jane Westwell** (e j.westwell@sgm.ac.uk).

Research and development in the biopharmaceutical industry

▲ David Glover

The biotech industry has been slow to mature, but is finally coming of age. The number of biopharmaceuticals being approved for human therapy is increasing year on year and many traditional pharmaceutical companies are turning their attention to this area.

Biopharmaceuticals are typically complex molecules that are expensive to manufacture. Their increased use in human therapy is placing a large financial burden on healthcare providers. A high profile example is Herceptin (trastuzumab; Genentech/Roche), a monoclonal antibody indicated for the treatment of breast cancer, which has been the subject of a public campaign to make the drug widely available on the NHS and a debate over its affordability. It will be up to bioprocess scientists to develop low-cost manufacturing technologies to ensure biopharmaceuticals can be made widely available whilst maintaining the highest quality standards.

Sustaining the biologics growth, particularly as product pipelines mature, will require expansion of bioprocessing capabilities. A recent report to government by the Bioscience and Inovation Growth Team (BIGT) on maintaining the UK's competitive position in bioscience identified the need to build a strong bioprocessing sub-sector as one of six major recommendations (www.bioindustry.org/bigtreport/).

***David Glover**, UCB Celltech*

Gusto / Science Photo Library

Biopharmaceutical

A therapeutic agent produced by organisms using recombinant DNA technology. These include monoclonal antibodies, recombinant proteins, therapeutic vaccines and gene therapy products. Biopharmaceuticals are a subset of biologics.

Biologics

Medicinal products manufactured using living organisms, tissues and cells. Biologics include prophylactic vaccines, blood products, toxins and stem cell therapies in addition to biopharmaceuticals.

Bioprocessing

A collection of techniques used in the development and manufacturing of biologics, from lab-scale development through to large-scale manufacturing, their formulation and delivery. These techniques include molecular biology, microbial fermentation, mammalian cell culture, transgenics, purification, and analysis. Personalized medicines, such as certain stem cell therapies, also require bioprocessing.

Profile

Name David Glover
Age 39
Present occupation Director, BioProcess R&D, UCB Celltech
Previous employment 2005–present, UCB Celltech; 2003–05, GlaxoSmithKline; 1997–2003, Celltech; 1991–95, University of Birmingham; 1990–91, BioExcellence; 1988–89, Hoechst Animal Health
Education University of Birmingham, PhD Biochemistry (1997); Sunderland Polytechnic, BSc Applied Biology (1990)

Q What influenced your decision to do a PhD?

The early part of my career was very much influenced by the Spinks report and the euphoria of the 1980s that surrounded the predicted impact biotechnology would have on our lives. I was fascinated by the concept of making a new generation of 'drugs', or 'biopharmaceuticals', in genetically engineered organisms. The applied and multi-disciplinary nature of biotechnology appealed, but I particularly wanted to contribute to further understanding of this emerging science. Thus it seemed obvious I should study for a PhD, out of fundamental interest and to equip me for a career in the biotech industry.

Q How did you choose your project?

I always intended doing a PhD, but worked in industry for a year before returning to academia. I selected a project that was multi-disciplinary in nature, being run jointly between the Schools of Biochemistry and Biochemical Engineering at the University of Birmingham. I was using fermentation and physiological profiling tools to study the regulation of gene expression by environmental factors in a yeast system. The interest to the field of biochemistry was further understanding of control of gene expression *per se*, whereas the biochemical engineers were looking for real systems to study the impact of poor mixing on cell physiology in large fermenters.

Q How easy was the transition to research in the commercial sector after your PhD?

Transition to the commercial sector was not as difficult as might be expected. Explaining what my PhD was about to lay persons (my parents, for instance!) and why it was relevant to the real world was always difficult. Suddenly I was able to relate what I did to something tangible and of benefit to mankind – I could refer to familiar diseases and how the drugs I worked on could make a difference. Of course, the need to deliver to strict timelines and remain focused on the goals was a bit of a culture shock. In industry it's not always possible to further explore that interesting result, particularly if this means departing from the main project. However, the buzz from working on something that may ultimately help patients is great.

Q What is rewarding about your job?

The UK has long been associated with innovative research in bioscience and medicine. However, it is bioprocessing that takes ideas for medicines from the lab to patients via commercially viable expression and manufacturing technologies. Being responsible for making sure a new drug gets to all the patients, at the right time, and to the highest quality standards to ensure their safety is highly rewarding.

Q Can you describe a typical day?

The work varies considerably from day to day. One day I might be reviewing a technology initiative for a novel drug design or a new protein expression system, the next I could be involved with a regulatory filing to gain approval for our most advanced drug. In between these activities, I could be involved in the selection of antibody-based drugs; development of fermentation and purification processes for their production; analysis for quality attributes; scale-up and manufacture for clinical trials and interaction with the regulatory agencies to ensure patient safety and approval of plans.

Q Do you have any advice to anyone planning a research career in the biopharmaceutical industry?

Implementation of the BIGT recommendations will see increased opportunities for training in the biosciences. For example, the research councils and industry have formed the Bioprocessing Research Industry Club (BRIC) to steer and fund industrially relevant academic research in bioprocessing (www.bbsrc.ac.uk/science/initiatives/bric). I would advise those interested in a career in the biopharmaceutical industry to embark on training of a multi-disciplinary nature, such as that provided under the BRIC programme. Whilst expertise in a particular area is required, we like our scientists to have an appreciation of where and how their expertise interfaces with other areas needed to discover and develop new drugs.

Q How do you see your future?

We face the challenge of developing the infrastructure to attract and train new bioprocess scientists to support the growth of both my own company and the wider UK bioscience sector. I see my future playing a role in both.

Further information:

www.bioprocessuk.org – the DTI-funded knowledge transfer network dedicated to the advancement of the bioprocessing sector in the UK. The website contains information on training, careers and jobs in the UK.

www.bioindustry.org – The BioIndustry Association is the trade association for the UK's bioscience sector, representing the industry to stakeholders – from patient groups and politicians to the media and financial sector.



▶ Recipients of SGM Vacation Studentships in 2005. Left to right: Alison Thornton, Calum Davis, Hannah Trewby, Helen Ewles, Janet Smith, Joshua Willoughby, Lee Wiersma.

Vacation work

Education Officer **Sue Assinder** reviews some of the reports written by the recipients of SGM Vacation Studentships in 2005.

An award allows a student to conduct a research project in some aspect of microbiology during the summer vacation prior to their final year of study. Around 50 awards are made annually. At the end of the project, the student submits a brief report of the research. A selection of the 2005 reports are summarized below. They illustrate the diverse nature of the projects and also how the students viewed their brief encounter with microbiological research. See p. 146 for details of the 2007 scheme.

Alison Thornton worked with Dr Evelyn Doyle, Department of Industrial Microbiology, University College Dublin. Her project was concerned with the bioremediation of sheep dips containing synthetic pyrethroids. These compounds are highly toxic to aquatic creatures and potentially serious contaminants of water courses. Alison examined the capacity of a naturally occurring soil *Pseudomonas* to degrade the synthetic pyrethroid most commonly used in sheep dip, cypermethrin. The level of cypermethrin degradation observed by the bacterium in this study is the highest reported to-date for a pure culture of a bacterium.

> '*The project allowed me to work with some highly skilled scientists who guided and advised me throughout. The work taught me skills that will be useful regardless of the field I choose in the future.*'

Calum Davis worked with Dr Gail Preston, Department of Plant Sciences, University of Oxford, on the effects of metal hyper-accumulation on plant disease resistance. All plants require heavy metals, such as Zn and Ni, to function properly, although high concentrations are usually toxic. However, some plants tolerate and even actively accumulate high concentrations of heavy metals. The metal-hyper-accumulating plant *Thlaspi caerulescens* was grown on a range of Zn concentrations and then inoculated with the plant pathogen *Pseudomonas syringae* pv. maculicola. Bacterial growth was inhibited in plants treated with higher Zn concentrations. The results suggest that the increased disease resistance of metal hyper-accumulating plants could be due to a direct toxic effect of metals on pathogens and explain the benefit of the energy-costly process of metal hyper-accumulation.

> '*The studentship proved an invaluable experience, not only in allowing me to carry out research over the summer, but also in giving me a taste of what research is like. I now know that I would like to continue in biological research.*'

Hannah Trewby worked with Dr Eirwen Morgan, Institute for Animal Health on the identification of *Salmonella* colonization factors in chicks. *Salmonella enterica* serovar Typhimurium produces enteritis in a wide range of animals, including man. The introduction of *Salmonella* into the food chain via infected chicken carcasses and eggs is a major cause of human food poisoning. The mechanisms *Salmonella* uses to colonize poultry are therefore of key public health importance and also impact on poultry welfare and the economics of the industry. Hannah used signature-tagged mutagenesis (STM) to screen a bank of random *Salmonella* Typhimurium transposon insertion mutants for their ability to infect 1-day-old chicks. Her results confirmed previous findings that genes in the *Salmonella* Pathogenicity Islands SPI-1 and SPI-2 are needed



for virulence. She identified many mutants that cannot cause disease in 1-day-old chicks but which are known to colonize calves and 14-day-old chicks. These mutants must therefore have transposon insertions in genes which could influence host specificity.

> *'As a vet student it was particularly interesting to see what goes on to achieve the science that vets tend to accept without question. Also interesting was the mental U-turn needed to go from thinking in terms of the whole animal to considering disease from the microbial perspective.'*

Helen Ewles worked with Dr Adam Roberts, Eastman Dental Institute, UCL, to characterize a novel tetracycline resistance gene, *tet(32)*, isolated from human mouth bacteria. Antibiotic resistance is an ever-increasing problem. Oral microflora harbouring novel tetracycline resistance genes could play an important role in spreading resistance to pathogens. A fully characterized gene would provide information on resistance mobility, and the method of tetracycline resistance. Helen's investigations showed that *tet(32)* is not mobile and that it is inducible by tetracycline. She also amplified part of the *tet(32)* gene, generating new sequence data. Further studies of *tet(32)* may aid the development of new drugs and possible methods to overcome resistance.

> *'I thoroughly enjoyed my summer studentship. It helped me enormously with preparing for my third year project, as well as confirming my desire to pursue a career in research.'*

Janet Smith worked with Dr Brian Gibson in the Brewing Yeast Research Lab at Oxford Brookes University on the role of glutaredoxins in protecting brewing yeast against peroxide stress. Oxygen is essential to maintain a viable yeast culture during fermentation, but the accumulation of harmful oxygen derivatives, known as reactive oxygen species, can damage yeast cells. Glutaredoxins are small proteins found in *Saccharomyces cerevisiae*, which are known to protect cells from these damaging effects. They are encoded by genes *GRX1* and *GRX2*. Janet showed that mutant *S. cerevisiae* strains lacking one or other of these genes were less tolerant to exposure to hydrogen peroxide than the wild-type strain and that *GRX2* accounts for the majority of glutaredoxin activity in yeast. The relative sensitivities to peroxide stress of a wild-type strain and four brewing strains were also investigated. Of the brewing strains, one ale strain was particularly sensitive to hydrogen peroxide, whereas the sensitivity of the lager strains was comparable to the wild-type strain. These results were presented at a malting and brewing seminar in Belgium and have recently been published (Gibson, B.R. & others, 2006; *Cerevisia – Belg J Brew Biotechnol* 31, 25).

> *'Doing this project gave me the chance to spend more time in the lab than would normally be possible in an undergraduate degree. Because I enjoyed it so much, I'd like to work in clinical science or research in the future.'*

Joshua Willoughby worked with Professor Geoff Turner, University of Sheffield, on whether actin cables or microtubules direct the movement of peroxisomes in a filamentous fungus. Peroxisomes oxidize specific organic substrates in a reaction that produces hydrogen peroxide. They are important organelles for fatty acid metabolism and some of the steps of antibiotic synthesis in fungi. In humans, peroxisomes make lipids which insulate nerve cells, so peroxisomal defects can result in severe neurological diseases. A related organelle is essential for pathogenesis in the economically significant rice blast pathogen *Magnaporthe grisea*. Joshua used a strain of the fungal model organism *Aspergillus nidulans* in which green fluorescent protein was fused to the peroxisomally located enzyme isocitrate lyase. Peroxisomes were easily seen in live hyphae using UV light microscopy. The drugs benomyl and cytochalasin were used to disrupt microtubules and actin, respectively. Both affected fungal growth, but neither drug alone appeared to prevent migration of peroxisomes into hyphae.

> *'The studentship had a strong positive influence in the decision to do a PhD.'*

Lee Wiersma worked with Professor Stuart Siddell, University of Bristol. Her project was on feline infectious peritonitis (FIP), a condition caused by FIPV, a coronavirus. Up to 40% of UK cats are thought to be infected with it and 5–10% of them develop FIP, which is invariably fatal. Lee aimed to express the nucleocapsid (N) protein of FIPV, which is one of the structural proteins. She cloned the N gene into a bacterial plasmid designed for expression in bacteria. The bacteria with the expressed protein were lysed and the protein was purified by affinity chromatography. The resultant N protein was of sufficient purity and quantity to be used in the production of monoclonal antibodies (mAb). These mAbs will be useful tools for further study of the molecular cellular biology of the virus with the aim of developing an effective vaccine against FIP.

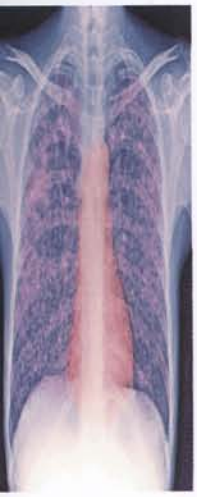
hotoffthepress

Science writer **Meriel Jones** takes a look at some recent papers in SGM journals which highlight new and exciting developments in microbiological research.

New approach to TB control using granulysin

Liu, B., Liu, S., Qu, X. & Liu, J. (2006). Construction of a eukaryotic expression system for granulysin and its protective effect in mice infected with *Mycobacterium tuberculosis*. *J Med Microbiol* **55**, 1389–1393.

Tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, kills around 3 million people each year. Even more worryingly, resistance to existing anti-TB drugs is increasing, so new therapies are needed. Researchers at the School of Medicine in Wuhan University in China have been exploring whether a peptide antibiotic made by some human cells can be developed as an effective treatment. Granulysin is produced by human cytotoxic T lymphocytes and natural killer cells, and acts in combination with a second pore-forming protein called perforin and proteinases called granzymes to destroy cancerous and virus-infected cells within the body. However, in laboratory tests, granulysin can kill or inhibit many bacteria, including *M. tuberculosis*, fungi and parasites on its own.



The researchers have demonstrated that human granulysin provides protection to mice infected with *M. tuberculosis* when it is made by their own cells. To do this the researchers isolated the sequence for the granulysin gene and inserted it into an expression plasmid, a circular piece of DNA containing all the instructions to allow any animal cell to synthesize granulysin. They checked that this worked using a cell culture, and then injected mice with the granulysin expression plasmid. Some of the mice were then infected with *M. tuberculosis*. After a month the mice had the sort of inflammation and lesions that occur in tuberculosis. However, these symptoms were much milder in the mice immunized with the granulysin expression plasmid. There were significantly fewer bacteria in these mice as well, with many bacteria showing signs of damage. This is the first evidence that a granulysin expression vector can protect against *M. tuberculosis* infection and suggests a new approach to TB control.

► Left. Alternatives to fossil fuels are a challenge for the 21st century as demand steadily increases, but reserves become depleted. *Jeremy Walker / Science Photo Library*

► Right. Hibiscus flower from Rarotonga, with nitidulid beetles (*Aethina* sp.). *André Lachance*

◄ Coloured X-ray of a patient's chest showing disseminated tuberculosis (TB) in the lungs. The lungs contain lesions (tubercles, pink) consisting of infected dead tissue. *Du Cane Medical Imaging Ltd / Science Photo Library*

SARS in horseshoe bats

Ren, W., Li, W., Yu, M. & others (2006). Full-length genome sequences of two SARS-like coronaviruses in horseshoe bats and genetic variation analysis. *J Gen Virol* **87**, 3355–3359.

Severe acute respiratory syndrome (SARS) took the medical world by surprise when it was first reported as a new disease in Asia in February 2003. There was concern because of its rapid worldwide spread and high mortality rate, with an estimated 774 deaths among the 8,098 people who contracted SARS in the 2003 outbreak. Rapid work identified the causal agent as a coronavirus. Although no new cases have been recorded since late 2004 among the public, researchers still want to know more about where the virus came from.

Researchers from China have already detected virus from the SARS cluster coronaviruses in at least five horseshoe bat species from the genus *Rhinolophus*, suggesting that bats might be the natural home of the virus. The sequence of all of the genes of some of these group 2b coronaviruses (G2b-CoV) is now known, and is similar to that of viruses isolated from humans and civets in 2003. The researchers have now characterized two additional G2b-CoV genome sequences from bats and have compared all of the genome sequences to see whether this gives clues about why the virus was so virulent or how it was transmitted between different animal hosts.

The bat Gb2-CoV isolates have an identical genome organization and share an overall identity of 88–92 % among themselves, and between them and isolates from civets or humans. However, there is considerable diversity in their detailed DNA sequences, even though researchers think that they all evolved from a common ancestor of the SARS virus. The researchers came up with different relationships among the strains depending on which gene sequences they used. This often indicates that there has been recombination between viruses in the past, although more sequences would be needed to prove this. One isolate from bats, Rf1, had a unique feature that might make it an evolutionary intermediate between the virus that infects bats and strains found in people. Further tests suggested that the viruses in bats had evolved independently for a long time, whereas the ones from humans and civets had recently undergone strong positive selection, reinforcing the idea that something has recently allowed the virus to cross between species.

Microdiesel from *E. coli* – an alternative to fossil fuels?

Kalscheuer, R., Stölting, T. & Steinbüchel, A. (2006). Microdiesel: *Escherichia coli* engineered for fuel production. *Microbiology* **152**, 2529–2536.

Practical alternatives to fossil fuels are a challenge for the 21st century. Not only are the number of exploitable oil reserves around the world decreasing as demand increases, but using this non-renewable resource also contributes to global warming. Among the solutions is biodiesel, an alternative to petroleum-based diesel made from plant oils. Its major drawback is that the acreage of oil crops like oilseed rape, soybeans and oil palm needed to meet the world's current demands would leave little space for food crops. However, most of the carbon in plants is within structural materials like cellulose and starch rather than the seed oils. Biodiesel would be much more practical if it could be made from these chemicals. A second problem is that biodiesel is currently produced by converting the plant oils to fatty acid methyl esters (FAMEs) using methanol, which is both toxic and derived from non-renewable natural gas. Fatty acid ethyl esters (FAEEs), synthesized using ethanol that can be produced biologically, have similar fuel properties to FAMEs, but are more expensive to make by chemical synthesis.

Researchers at the Westfälische Wilhelms-University in Germany have taken a lateral approach to these problems to see whether bacteria can help. Their solution has demonstrated that bacteria can be designed to make 'Microdiesel', resembling biodiesel. The researchers brought together genes from three different bacteria. They had been working with the species *Acinetobacter baylyi* that makes fats to store within its cells. The key enzyme (WS/DGAT) in this biosynthesis turns out to work well with a remarkably broad range of substrates, including many never encountered in nature. This enzyme therefore might make Microdiesel if it was supplied with suitable materials. Unfortunately, *A. baylyi* cannot synthesize suitable amounts of ethanol. The solution was to add two genes from another bacterium, *Zymomonas mobilis*, to the microbiological workhorse *Escherichia coli*, giving it the capacity to synthesize enough ethanol. Adding the gene for WS/DGAT from *A. baylyi* as well created genetically modified bacteria that could churn out up to 26 % of their dry weight as FAEEs, once supplied with sugars and fatty acids.

Higher efficiency and yields would be needed in a practical industrial process, as well as the ability to use crude plant materials as substrates. However, the versatility within bacterial metabolism means that this may well be possible using the right combination of genes and bacteria. The German researchers have shown that the concept works and have opened up the way for further developments.

Novel yeast distribution in insects

Lachance, M.-A., Bowles, J.M., Wiens, F., Dobson, J. & Ewing, C.P. (2006). *Metschnikowia orientalis* sp. nov., an Australasian yeast from nitidulid beetles. *Int J Syst Evol Microbiol* **56**, 2489–2493.

Nitidulid beetles live on fruit, flowers, leaves and other discarded parts of plants, and can be a pest in dried fruit. However, three of these small beetles have posed an interesting question about species distribution, not of beetles, but of yeasts. The beetles were collected from roadsides separated by 11,000 km of land and ocean in cool regions of Rarotonga in the Cook Islands and the Cameron Highlands in Malaysia. A novel species of yeast, *Metschnikowia orientalis*, was discovered in their droppings. Remarkably, *M. orientalis* has not been detected before in extensive collections from habitats in Australia, New Caledonia and Fiji. Researchers from Canada have been surveying the insect-borne yeast flora of these areas. The only tenuous clue linking *M. orientalis* to these two locations is the low maximum temperature at which the yeast will grow. This must not exceed 30–31 °C, which could limit the number of suitable habitats in these tropical regions.

The researchers suspected that they had a novel species when they recovered several strains of a yeast that would apparently only reproduce asexually from two nitidulid beetles in Rarotonga in 1999. In 2005 they found a single isolate from an insect on roadside flowers in Malaysia that had similar growth and DNA characteristics to the Rarotonga yeasts. The only way to distinguish this novel species from other yeasts is to either examine DNA sequences or test for the production of sexual spores once possible isolates are mixed with authentic strains of each mating type.

The question of why the strains were found so far apart, and where others may turn up, is still open.

Science Teaching in Schools Enquiry


Andrew Lambert Photography / SPL

Sue Assinder (SGM Education Officer) gave oral evidence on behalf of the Biosciences Federation (BSF) to the recent House of Lords Select Committee enquiry on Science Teaching in Schools, alongside representatives from the Royal Society, the Institute of Physics and the Royal Society of Chemistry. This enquiry was particularly concerned with the decline in the number of A-level entries in the sciences and focused on the role that teachers and teaching methods can play in reversing that decline.

Sue told the enquiry that the BSF was keen to see an improvement in the take-up of all sciences at A-level. Insights from chemistry and physics are increasingly important for bioscience research and the decline in their popularity threatened to hinder developments in the biosciences. If the recent Government targets to increase the number of pupils taking science A-levels were to be met, it was important to focus on pupils' engagement with the sciences, not just their levels of achievement. Pupils would be persuaded to continue to A-level only if they were excited about science and appreciated its relevance to them. Members of learned societies like the SGM potentially had an important part to play in enthusing school children about science and many of them already did so. However, a major problem was that school outreach work carried out by university academics was often not highly valued by their institutions.

In a wide-ranging discussion, other issues raised included the perception that GCSE and A-levels in the sciences are more difficult than in other subjects, the problem of gender imbalance in the physical sciences, the poor quality of careers advice offered by some schools and the current shortage of specialist physics and chemistry teachers. The issue of Health and Safety in practicals was also discussed, giving Sue the opportunity to highlight the important role that the SGM plays in giving advice and training to teachers about safe microbiology practical work.

It is always difficult to judge the impact of these sorts of activities. However, the Lords had clearly done their homework, including visits to schools, and appeared interested in the evidence presented to them. The Committee is expected to publish its report by the end of the year.

Sue Assinder (e s.assinder@bangor.ac.uk)

microbiology awareness campaign

MAC

The Society's campaign to raise the profile of microbiology to Government and other opinion formers continues. In November we will once again have a stand at the Royal Society of Chemistry's Scottish Parliament Day, where we will be trying to persuade MSPs and their advisers of the importance of hand hygiene. We will also be distributing literature such as the popular one-page briefings on topical microbiological issues. The latest two briefings feature Measles and *Clostridium difficile*, adding to MRSA, Malaria and TB. Plans are also in hand for future events at Westminster and in Ireland.

Early-career researchers are told *stand up for science!*

It can often seem that the public's perception of science is of men in white coats working away behind closed doors determined to alter lives in unwelcome ways. This detachment of scientists has led to recent debates over scientific issues being alarmingly misinformed. Sense about Science, which works with scientists to promote evidence and good science in public discussion of topical and controversial issues, has been running Voice of Young Science (VoYS) media workshops since 2004. These workshops enable early-career scientists to voice concerns about talking to the media and provide an opportunity to form views on how science is portrayed and communicated, and to question people on the 'frontline' directly.

What was apparent in the workshops was the enthusiasm of early-career scientists to get involved in promoting good science, but uncertainty as to how to go about it. With that in mind, our VoYS writing team, made up of early-career scientists determined to resolve this problem, has compiled a short-guide to the media; *Standing up for Science*. This colourful guide contains interviews with scientists and journalists to give an insight into how the media reports science and gives practical tips on how they can get more involved in public debates about science.

SGM is one of our partners. Janet Hurst, Deputy Executive Secretary, highlights why they wanted to be involved: '*With so many scientific issues in the headlines affecting not only our own well-being but that of the planet, it has never been more important for scientists to interact pro-actively with the media ... SGM is therefore delighted to support this guide, which seeks to encourage early-career scientists to communicate enthusiastically and clearly to the media about their work.*'

Television, radio, newspapers and the internet reach into the home and the workplace throughout the country. It is a fact of 21st century life that the media is inescapable and, like it or not, most scientists will find that they are approached by journalists at some point in their career to talk about their own or someone else's research. Whilst this may be a daunting prospect, it needn't be a negative experience; speaking to the media allows them to share their enthusiasm for their subject, and gives the opportunity to influence public opinion and public policy.

The concerns dealt with in the guide go from why not all science journalists have science backgrounds, to the fear of misrepresentation in the media and the fear that when simplifying the language for the interested lay person you will end up saying something that sounds inaccurate, stupid or both. Everyone interviewed agreed that these problems were easily avoidable and all the interviewees, scientists, journalists and press officers alike, expressed their optimism at the part which early-career scientists can play in the communication of science, forming the perfect bridge between the public and the professor.

Standing up for Science is the culmination of months of hard work fuelled by a belief that with a helping hand, early-career scientists really can stand up and make a difference. It is by no means a definitive guide to all dealings with the media, but it gives individuals the knowledge, tools and inspiration to go out and stand up for science themselves.

A copy of *Standing up for Science* is enclosed with every SGM Postgraduate Student Member's copy of *Microbiology Today*. It is available on our website, **www.senseaboutscience.org/VoYS**, where hard copies can also be freely ordered. Here you can find other ways to get involved in the VoYS network. Contact Frances Downey at **fdowney@senseaboutscience.org** or **0207 478 4380** for further information.

Sarah Anderson and **Frances Downey**

reviews

If you would like your name to be added to our database of book reviewers, please complete the book reviewer interests form on the SGM website. A classified compendium of reviews from 1996 to the present is also available on the website.

Manual of Molecular and Clinical Laboratory Immunology, 7th edn

Edited by B. Detrick, R.G. Hamilton & J.D. Folds
Published by American Society for Microbiology (2006)
US$179.95 pp. 1374
ISBN 1-55581-364-X

Most clinical immunology laboratories will have at least one previous edition of the *Manual of Clinical Laboratory Immunology* from ASM Press somewhere on their shelves (we have three – a 2nd, a 3rd and a 6th). As with similar tomes from the ASM, such as the *Manual of Clinical Microbiology*, this book aims to be a definitive reference text for its target laboratories. Certainly, a description of every immunological test we perform can be found within its pages together with a number of alternative techniques.

For textbooks of this kind to remain relevant and fulfil their remit, it is essential that they evolve with current trends in laboratory practice. To achieve this, the manual has been updated to a new edition approximately every 5 years since the first edition was published in 1976. Furthermore, the quantity and complexity of molecular biology contained within this 7th edition is considerable in comparison to previous editions, a fact that the editors obviously wished to emphasize by the inclusion of 'Molecular' in the title. Rather than appending a few chapters solely concerned with molecular techniques, each section and chapter has been expanded, rewritten and reorganized to include the relevant material.

Given the change in title and new direction this textbook series is taking, the temptation may have been to veer too far away from non-molecular techniques. However, the authors and editors seem to incorporate the expanding application of molecular techniques without ignoring traditional non-molecular techniques that remain standard in many laboratories and will continue to be used for some time.

Aside from the new molecular emphasis, additional chapters have been included concerning topics that have come to prominence since the publication of the last edition in 2002, examples being chapters on *Bacillus anthracis* from its potential use as an agent of bioterrorism and coronaviruses, the causative agent of SARS.

As with many collaborative works there is wide variety in the language and prose used in different chapters, some are definitely more reader-friendly than others. This is to some extent due to the subject matter of each section and chapter and an expert eye would probably appreciate the specifics of their chosen field.

The indexing is as comprehensive as it needs to be, making navigation through the 1,340 pages straightforward and allowing quick access to specific topics. This feature cannot be overlooked given the competition from online- and DVD/CD-ROM-based reference material, where search facilities are of great benefit.

As one would expect with well-established reference material of this type, the scientific accuracy cannot be disputed as each chapter is extensively referenced to include up-to-date original research and review articles. Each topic is introduced and discussed in depth and then followed by detailed descriptions of the techniques, discussion of specific applications and guidelines on test selection for individual clinical situations and interpretation of findings.

Only the keenest of students and other individuals will consider purchasing a personal copy. In any case the most benefit from reference volumes of this type is probably obtained when used in the actual laboratory setting, whether clinical or academic, so that the information can be put directly in the context of patient care or research. The brief final section concerns laboratory management, indicating that the book is targeted at institutions and laboratory heads.

Immunology laboratories currently relying on a previous edition as a primary reference manual would definitely benefit from purchasing this extensively revised edition, which will more than satisfy their current needs. Furthermore, the increased emphasis on molecular biology in this edition should guarantee its relevance for several years to come, at least until advancement of the field necessitates an 8th edition.

***Martin Stearn**, Royal Brompton Hospital*

Emerging Foodborne Pathogens

Edited by Y. Motarjemi & M. Adams
Published by Woodhead Publishing Limited (2006)
£150.00/US$270.00 pp. 634
ISBN 1-85573-963-1

Despite major advances in food safety and public health microbiology, food-borne disease remains a leading cause of illness worldwide and, because of multifactorial influences, new food-borne pathogens will continue to emerge and old ones re-emerge. This well-written book brings together current knowledge and concepts providing a good introduction and overview of emerging food-borne pathogens, covering evolution of

pathogens, surveillance, industrial microbiology and risk assessment. The second part of the book, with a more conventional layout, consists of chapters on individual emerging pathogens and is comprehensive in its coverage. Overall, I found the book highly informative, up-to-date and easy to read, providing essential information for all food microbiologists as well as food scientists with an interest in food safety. All scientists working with food pathogens or involved with food safety would benefit from having access to a copy.

***Kathie Grant**, Health Protection Agency*

The Enterobacteria, 2nd edn

By J.M. Janda & S.L. Abbott
Published by American Society for Microbiology (2005)
US$119.95 pp. 426
ISBN 1-55581-342-9

The *Enterobacteriaceae* are a hugely significant and increasingly diverse family of pathogenic bacteria. The book is an outstandingly authoritative and detailed reference source on the phenotypic, and to some extent genotypic, characterization and identification of these organisms. As such it is excellent value for the clinical laboratory or medical microbiologist. Yet, it neglects totally the paradigm-shifting insights that are now flowing fast and freely from whole-genome sequencing of organisms within the family, for which there are now about 40 complete or near-complete genome sequences. These data are totally changing our concept of the species, among both the *Enterobacteriaceae* and bacteria in general. In a nutshell, the book is a fantastic resource for the science that represents the culmination of the era of classical microbiology, but contributes little to the increasing needs for post-genomic re-evaluation of enterobacterial diversity and phylogeny, where the intellectual challenges for the future lie.

***Charles Penn**, University of Birmingham*

OIE/FAO International Scientific Conference on Avian Influenza

Edited by A. Schudel & M. Lombard
Published by S. Karger AG (2006)
US$236.50 pp. 278
ISBN 3-80558-031-2

Avian influenza has had an enormous economic and social impact on affected countries, and the risk of this animal disease evolving into a human pandemic is of global concern.

In order to effectively combat the disease, veterinary and human health organizations need to work together. This book highlights and discusses important areas of research and collaboration, for example, the implementation of more surveillance and epidemiological studies in migratory birds to understand dissemination of highly pathogenic avian influenza (HPAI), as well as increasing awareness of pathogenesis, symptoms and disease signs of HPAI. There are comprehensive discussions of ecology, epidemiology, pathogenesis, human health implications, diagnostics and the control of avian influenza, including vaccination.

This book has extensive background information about all areas of avian influenza and would be ideal for anyone looking for a summary and overview of the current situation for avian influenza, and how organizations such as OIE/FAO and WHO are implementing ways to control and combat outbreaks and spread of the disease.

***Ruth Harvey**, University of Oxford*

Reviews on the web

Reviews of the following books are available on the website at www.sgm.ac.uk/pubs/micro_today/reviews.cfm

Salmonella *Infections Clinical, Immunological and Molecular Aspects*
The Bacteriophages, 2nd edn
Applied Multilevel Analysis
Vaccine Cell Substrates 2004
Influenza Virology Current Topics
Principles of Gene Manipulation and Genomics, 7th edn
Microbial Subversion of Immunity: Current Topics
The Identification of Fungi
Compendium of Bean Diseases
Compendium of Turfgrass Diseases, 3rd edn
Gene Cloning & DNA Analysis An Introduction
Viruses vs. Superbugs A Solution to the Antibiotics Crisis?
Biodiversity and Ecophysiology of Yeasts
Clinical Applications of PCR, 2nd edn
Experimental Design for the Life Sciences, 2nd edn
Gram-Positive Pathogens, 2nd edn
Bacterial Wilt Disease and the Ralstonia solanacearum *Species Complex*
PCR Troubleshooting: The Essential Guide
Fungi in Biogeochemical Cycles
Bacterial Cell-to-Cell Communication Role in Virulence and Pathogenesis
Disease Ecology Community Structure and Pathogen Dynamics
Introduction to Glycobiology, 2nd edn
Immunology and Immunotechnology
Food Spoilage Micro-organisms
New and Emerging Proteomic Techniques
Evolution of Microbial Pathogens
Essential Bioinformatics
Reverse Transcriptase Inhibitors in HIV/AIDS Therapy
Emerging Issues on HPV Infections from Science to Practice
Basic Biotechnology, 3rd edn
Risky Trade: Infectious Disease in the Era of Global Trade
Bacterial Flora in Digestive Disease Focus on Rifaximin
The Biology of Vibrios
Brewing Yeast and Fermentation
Outbreak: Cases in Real-world Microbiology
Molecular Diagnostics: Current Technology and Applications
Agrobacterium *Protocols, 2nd edn, Volume 1*

obituary

Sidney Reuben Elsden (13.04.**1915**–29.4.**2006**)

Sidney Elsden, who has died at the age of 91, was Head of the Microbiology Department at the University of Sheffield from 1949 to 1965 and Director of the Agricultural Research Council's Food Research Institute from 1965 to 1977. In addition to his experimental contributions to biochemical microbiology, he was responsible for leading the establishment and early development of both these laboratories. Elsden was an original member and then an Honorary Member of the SGM, serving on Council from 1963 to 1967 and as President from 1969 to 1972. In 1967 he was awarded the Marjory Stephenson Memorial Lecture.

Sidney Elsden spent his early years in Cambridge and, after attending the Cambridge & County High School for Boys, entered Fitzwilliam House, where he held a Goldsmiths' Company Exhibition, and graduated in 1936 with a double first in the Natural Sciences Tripos. Following a year of research in the Cambridge Biochemistry Department under Dr Marjory Stephenson, who kindled his lifelong interest in micro-organisms, he was appointed to a lectureship in the Physiology Department of Edinburgh University. In 1941 he gained his PhD from Cambridge University for biochemical work on bacteria and on muscle tissue. His long association with the Agricultural Research Council (ARC) began in 1942 when he joined their Unit of Animal Physiology in Cambridge. Here he devised an innovative method for the separation of short-chain fatty acids on silica gel columns and used it to investigate their microbial production in ruminants.

In 1948 Elsden was appointed Senior Lecturer in the University of Sheffield, heading a biochemistry-based sub-department within the Bacteriology Department, and a 1-year postgraduate course in microbiology was introduced in 1950. At that time, the commercial production of biochemical apparatus was in its infancy, but Elsden's foresight and the skills of the Medical Faculty's workshop placed the department in a strong position to exploit new techniques, such as the Hughes press and other methods for the production of bacterial cell-free extracts, radioactive tracers, continuous culture methods and gas–liquid chromatography.

Elsden's research was many-faceted. He was adept at connecting a fistula to a sheep's rumen and he could thus easily obtain rumen contents with their plethora of micro-organisms. Many of these are fastidious anaerobes and, having been exposed to the methods of C.B. van Niel, Elsden was well equipped to isolate and manipulate such bacteria. One of his isolates was an hitherto unknown, large, Gram-negative, non-sporing, anaerobic coccus which produced C1–C6 fatty acids. This organism was eventually adopted as the type species of a new genus and appropriately named *Megasphaera elsdenii*.

In 1952, Sheffield University created a separate Department of Microbiology with Elsden as its head and the ARC then appointed him Honorary Director of a Unit for Microbiology which they established within the new Department. The research programme expanded steadily and embraced photosynthetic bacteria, growth yields in relation to ATP generation, bacteriophage and bacteriocins as well as numerous aspects of anaerobes. In 1959, the West Riding of Yorkshire endowed a Chair of Microbiology with Elsden as the first incumbent. The Department's increasing reputation attracted a steady flow of postgraduate students and visiting workers, many of them from overseas countries, including Australia, Norway, Sri Lanka and the USA.

In 1965, Elsden started the second phase of his professional career by accepting the Directorship of the new ARC Food Research Institute to be built in Norwich. His careful planning and excellent rapport with the architects resulted in a spacious and elegant building with a good balance of standardized laboratories and specialized areas for services and large equipment. The layout subsequently proved easy to adapt to meet changes in the research programme and safety regulations. The new institute was intended to bring together several of the ARC's staff from research groups in Sheffield, Cambridge, Aberdeen and Ditton in Kent. To facilitate the integration of these diverse groups, a nucleus of staff from each was built up at a temporary laboratory in Norwich in

the years immediately before the new building was occupied in 1968. The Institute was sited close to the newly founded University of East Anglia (UEA) which had a strong biological ethos and a close association developed between the two.

Following the Rothschild Report of 1971–72, the research programme was increasingly subjected to more centralized control, with a large part of the Institute's budget coming from work contracted by MAFF. In 1975 a Director's Advisory Board was set up to improve contact with the food processing industry. Major changes followed, including the introduction of work on nutrition and the transfer of work on poultry meat to the Meat Research Institute at Bristol. These changes were well under way by the time Elsden retired in 1977.

Inevitably, Elsden became increasingly involved in a wide range of committee and advisory work relating to food research and biology, both locally and nationally. Despite these pressures he occasionally managed to spend time working with his assistant on properties of clostridia relating to their classification. After retirement, he continued with his scientific interests for a few years at UEA before reverting to his long-standing hobbies of gardening, fishing and cooking. In 1985, the University of Sheffield recognized his distinction as a microbiologist and as Director of the Food Research Institute by awarding him the degree of Doctor of Science, *honoris causa*.

Sidney Elsden had a lasting interest in new techniques and was keen to ensure that his staff were well equipped in that respect. His attitude to his colleagues was forthright, but encouraging and sympathetic, particularly with regard to personal problems. Early in his career his own domestic life had been clouded by the sudden death of his first wife, Frances. His second wife, Erica, whom he married in 1948, survives him, together with their twin sons. Despite increasing frailty in his final years Sidney retained a lively and good-humoured outlook until his death shortly after his 91st birthday. He leaves behind a wealth of happy and grateful memories among those who were privileged to work with him.

John L. Peel
Norwich

Bernard A. Fry
Sheffield

comment

microbiologybytes

microbiologybytes.wordpress.com

New and exciting ways of disseminating information to the public and students are taking off. Thanks to the increasing popularity of devices such as mp3 players, **Alan Cann** believes that podcasting and other Web2.0 technologies offer a great opportunity to promote microbiology.

Podcasting is the distribution of multimedia files over the internet. The files can be downloaded and viewed or listened to on a computer or a mobile device such as an mp3 player or mobile phone. It is not necessary to have an iPod to listen to podcasts – any computer or mp3 player will do. Unlike other file downloads, a key feature of podcasting is the ability to subscribe through RSS (Really Simple Syndication) feeds. There is a wide range of software available to manage and listen to podcasts, most of which is free. Once the software is installed, listeners subscribe to the podcast feed which tells them when new podcasts are available and automatically downloads. Alternatively, it is possible to manually download the files to a computer by clicking on the episode links on the podcast homepage.

Podcasting has grown enormously in the last few years, but is set to move into the mainstream as a major source of information during the next year with the current investment by the BBC and other broadcasters and the features included in Microsoft Windows Vista, due to be released in January 2007 (e.g. RSS support in Internet Explorer). Podcasting currently has a young demographic, predominantly used by the age 18–24 age group. Many students and potential students now turn to the internet as their first source of information. Podcasts are optimally placed as the medium of choice to reach this group with current and engaging information about microbiology.

After experimenting with podcasts for University of Leicester students for the past year, I began producing a regular public microbiology podcast in April 2006 – **microbiologybytes.wordpress.com**. More than 3,500 people from over 50 countries downloaded a *MicrobiologyBytes* podcast in September, and the number of listeners is doubling each month.

After being featured recently in the *Science* 'NetWatch' column, the Apple iTunes Store 'New and Notable Podcasts' and Wordpress.com's 'Blog of the Day', this exciting new project has just received sponsorship from the SGM in the form of a Public Understanding of Science grant.

Unlike other university podcasts, *MicrobiologyBytes* is not simply recycling of lecture content in audio format. Its content is tailor-made to engage the largest possible audience by presenting current topics in microbiology in a form that everyone can understand. Over a period of time, the content of the podcasts covers the whole field of microbiology. Recent topics include:

> *Is there a role for SV40 in human cancer?*
> *Quorum sensing in bacteria*
> Escherichia coli *in spinach*
> *How HIV causes AIDS*
> *Plague: from the 14th to the 21st century and still going strong*
> *Extreme drug-resistant tuberculosis (XDR-TB)*

There is a tremendous storehouse of knowledge locked up in universities. New technology such as Web2.0 – the read-write internet – allows us to share this by blogging and podcasting. The aim of *MicrobiologyBytes* is to bring people the latest news from the forefront of biomedical research in a form that everyone can understand. Obviously, the hope is that this will also attract more students to undertake microbiology degrees, but it is not reasonable to expect that someone who listens to *MicrobiologyBytes* in, say, Mexico, will turn up on our doorstep wanting to study for a degree. It's all about the conversation we as academics should have with the public.

▲ iPod nano. *Gusto / Science Photo Library*

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Further reading

Wikipedia: Podcast. **en.wikipedia.org/wiki/podcast** (last accessed: 3.10.06).

Cann, A.J. Really Simple Syndication. *Teaching Bioscience Enhancing Learning Series – Effective Use of Technology in the Teaching of Bioscience – UK Higher Education Academy Centre for Bioscience*. **www.bioscience.heacademy.ac.uk/publications** (last accessed: 3.10.06).

Podcasting gains an important foothold among US adult online population. **www.nielsen-netratings.com/pr/pr_060712.pdf** (last accessed: 3.10.06).

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