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The second AFRC PMB *Arabidopsis* Newsletter

March 1990

Vienna Coach

Many of you, we hope, will be pleased to know that the AFRC PMB *Arabidopsis* coach trip to the Fourth International Conference on *Arabidopsis* Research in Vienna is on. A 47-seat "executive" coach has been booked and a deposit should have been paid by the time you read this. As mooted in the first newsletter, for *all* those in receipt of an AFRC PMB *Arabidopsis* grant (or employed on one) this is *free*. Anyone else is welcome, indeed encouraged, to come along, as the coach is not yet fully booked, but non-grant holders will have to pay cost price. This will be £72, including the ferry crossings for the return trip. Details have yet to be finalised, but the following is arranged: the coach will leave Norwich at 9a.m. on Thursday 31st May and, after picking up passengers in London, will drive overnight non-stop to Vienna, to arrive on Friday morning. Departure for the return journey will be at 3p.m. on Wednesday 6th June. These days of travel are different from those previously suggested, but they will give participants Friday night to recover before the conference starts on the Saturday and, on the Wednesday, will allow for a little sightseeing or for recovering from the conference closing-party. The coach will arrive back mid-afternoon on Thursday 7th. This should leave enough time for those travelling to elsewhere in the U.K. to reach their destinations on the same day. The following people have already been booked onto the coach:

Sue Albini, Justin Ainscough, Tom Ashfield, Luis Balcells, Ian Bancroft, Christine Barber, Gerda Cnops, Louise De Villiers, John Gray, Nic Harberd, Pat Heslop-Harrison, Mary F[illegible]'sworth, Emily Lawson, Ottoline Leyser, Clare Lister, Bernard Mulligan, Claire Nall, Helen North, Kevin Pyke, Renate Schmidt, Trudi Schwarzacher, Rosalind Slatter, June Swinburne, Katherine Weiss, Zoe Wilson, Tania Young.

If you definitely want to travel on the coach but are not on the above list, fill in the form at the bottom of this page, and send it off a.s.a.p. (Similarly, if you are on the list, but do *not* want to go, complete the form too.) Please note that if you have a place and then cancel within a month of departure you will still have to pay the cost price of £72 unless you find a suitable replacement.

continued on page 2...

In This Issue:

Plus – attached

AFRC PMB *ARABIDOPSIS* COACH TO THE VIENNA CONFERENCE

NAME: ..... FAX/Tel: .....

ADDRESS: .....

O YES, I am going on the coach to Vienna..... If yes: O I am funded by an AFRC PMB *Arabidopsis* grant.

O NO, I am *not* going on the coach to Vienna O I am *not* funded by an AFRC PMB *Arabidopsis* grant.

(tick relevant boxes)

Please photocopy this form and give it to all who may be interested. FAX completed forms to David Flanders, 0603-56844

The photograph (above) shows the participants at the First Annual AFRC PMB *Arabidopsis* Meeting, held at the John Innes Institute last December.

...continued from page 1

Please also remember that all attendants will have to find their own or alternative sources of funding for the costs of registration, accommodation, etc. More information will be sent a.s.a.p. to those going on the coach.

Registration and further details about the conference can be obtained from the Conference Secretary:

Kathrin Peuker, Dept of Cytology and Genetics
Institute of Botany, Rennweg 14 A-1030 Vienna
Tel: 010-43-222-78 71 01; FAX: 010-43-222-78 71 94

The deadline for early registration is the 31st March and so a copy of both the registration and model abstract form is attached to this newsletter. Early registration costs 2,000AS or 1,000AS for students (there are about 20AS to the pound). Accommodation enquires and bookings should be directed to either Frau Bauer or Beatrix, Tel: 010-43-222-58 80 01 11. A single room in a simple pension costs 330AS (350 with shower) and a double is 460 (520 with shower). A single, with toilet and shower, in a higher quality pension costs 680AS and a double costs 960AS. All prices are per night, and include breakfast.

MOLECULAR BIOLOGY OF PLANT DEVELOPMENT

From Gareth Jenkins...

The SEB's Symposium on the Molecular Biology of Plant Development, to be held in Glasgow from 28-31 August 1990, includes several *Arabidopsis* talks:

M. Koorneef: Developmental mutants
J. Chory: The *Arabidopsis det* mutant
E. Grill: Development of a system for efficient chromosor walking in *Arabidopsis*
C. Dean: Development of a gene tagging system in *Arabidopsis*
D. Marks: Molecular genetic analysis of trichome development in *Arabidopsis*
J. Bowman: Flower morphogenesis genes in *Arabidopsis*
T. Bleecker: Ethylene response mutants and genes in *Arabidopsis*

In total, there will be approximately 30 speakers (others include: B. Goldberg, I. Sussex, M. Van Montagu, and A. Cashmore) and a poster session. So, if you can't afford Vienna, why not be an *Arabidopsis* groupie in Glasgow? Further details of the meeting will be available at the Warwick SEB meeting and will be circulated to SEB members. Details can also be obtained from the SEB, Burlington House, Piccadilly, London WIV OLQ.

From Sue Albini...

Synaptonemal complex spreading, an ultrastructural approach to chromosome analysis in *Arabidopsis thaliana*.

S.M.Albini, G.H.Jones and J.S.Parker; School of Biological Sciences, University of Birmingham, P.O. Box 363, Edgebaston, Birmingham B15 2TT.

Since this research project started at the begining of January, lines of investigation have been gathering momentum. Firstly, our method of preparing plant synaptonemal complexes (SCs) is being adapted to examine *Arabidopsis* SCs and secondly, conventional methods of preparing mitotic and meiotic chromosomes are being investigated. The basic method of producing plant SCs has been scaled down to cope with the tiny *Arabidopsis* anthers. At prophase 1 of meiosis, buds are approximately 0.3mm long and ers 0.1mm. One anther is removed and stained and squashed to check the meiotic stage; the remaining five anthers are dissected out and processed to produce SC preparations. So far the preliminary experimental runs have shown that surface spread nuclei containing SCs are being isolated and preserved. The next step is to adapt the treatment of the nuclei so that yield and quality are improved. In parallel with the SC studies, conventional chromosome analysis is being attempted. Using floral parts as a source of actively dividing cells, mitotic metaphase spreads of the chromosomes have been prepared. From these, the chromosomes can be counted and a more detailed karyotype analysis will be attempted.

RSHALLDF@UK.AC.BHAM.VAX1

From Ken Buck...

A novel approach to the isolation of origins of plant DNA replication using *Arabidopsis* as a model system.

T.D. Jones and K.W. Buck; Dept. of Biology, Imperial College, London SW7 2BB.

The project commenced on 1 January 1990. The first few stages of a multistage construction to produce a vector containing overlapping parts of a hygromycin phosphotransferase gene into which an origin of DNA can be inserted have been completed. It is anticipated that when integrated into the plant chromosomal DNA, intramolecular recombination will occur to generate a circular, replicating plasmid containing a functional hygromycin phosphotransferase gene.

From David Coates...

Molecular biology of the regulation of the plasma membrane calcium transporter in *Arabidopsis* and *Zea*

David Evans, Brian Cox (Plant Sciences, Oxford) and David Coates (Leeds).

So far, we have been able to start work on only half of the project due to staffing problems, which should shortly be resolved. Dr Joy Boyce (Oxford) has been appointed to obtain both the cDNA and genomic sequence of the plasma membrane calcium transporter in *Zea* and *Arabidopsis*, and has commenced by using monospecific antibodies to screen a λgt11 cDNA library from *Zea*. So far we have a number of exciting looking positives of up to 2kb, which we are about to sequence. We will then compare these sequences with sequences of the mammalian plasma membrane calcium pump to look for homology, before using them to probe both cDNA and genomic libraries and for developmental studies. We are eagerly waiting to get on with the parallel biochemistry in *Zea* -- namely reconstitution of the calcium pump and study of its regulation by a variety of regulators; hopefully this will be underway before our next report.

PAB6DC@UK.AC.LEEDS.BIO.VAX

From George Coupland...

A two-component transposon tagging system in *Arabidopsis*.

L. Balcells, J. Swinburne, K. Ingle, S. Scofield, J. Jones and G. Coupland; IPSR (Cambridge lab.) and Sainsbury lab. (Norwich).

The aim of this project is to construct a high efficiency transposon-tagging system based on the maize transposon *Ac*. Several groups have observed low frequencies of *Ac* transposition in *Arabidopsis*, and we are testing whether these low frequencies are a consequence of a low rate of transcription from the *Ac* promoter. Previous experiments have shown that *Ac* encodes one 3.5kb transcript which is present in low abundance ($3x10^{-7}$ of all mRNA) in *Ac*$^+$ maize lines (Kunze *et al., EMBO J.* 6, 1555-1563). The protein encoded by this transcript promotes transposition of internally deleted elements (*Ds* elements) which are not capable of autonomous transposition (Coupland *et al., EMBO J.* 7, 3653-3659). We assume that this product is the only *Ac*-encoded protein (the transposase) required for transposition. The transposase gene is unusual in that it contains a 650bp untranslated leader sequence. Methylation of a CpG-rich region at the 5' end of the untranslated leader correlates with inactivity of the element in maize (Kunze *et al., Mol. Gen. Genet.* 214, 325-327).

We have made several promoter fusions to the transposase gene in order to increase the abundance of the mRNA. In all of these constructs we have deleted one terminus of *Ac* so that they will express transposase, but will not transpose. We have inserted the promoters at two locations within the untranslated leader. One of these locations is directly adjacent to the ATG of the transposase open reading frame, while the second is 250bp from the ATG, but downstream of the heavily methylated CpG-rich region. Directly adjacent to the ATG, we have inserted the nopaline synthase promoter, the octopine synthase promoter and the CaMV35S promoter. Within the leader we have inserted the nopaline synthase promoter, the CaMV35S promoter, the inducible soybean heat-shock promoter, the *Ac* promoter containing two copies of the CaMV35S promoter 300bp upstream of the start and finally an unmodified *Ac* promoter.

So far, five of these fusions have been introduced into *Arabidopsis* by use of the root transformation protocol and selection with kanamycin. Seeds have been harvested from many of these transformants, and some of them have been sown on kanamycin-containing medium to identify those plants which have inherited the T-DNA. No kanamycin resistant plants were detected among the progeny of approximately 25% of our putative transformants, suggesting they were not transformed. Most of the remaining plants show a segregation of approximately $3km^r$ seedlings:$1km^s$ seed-

ling among their progeny, indicating insertion of the T-DNA at one genetic locus. We have now crossed kanamycin-resistant plants harbouring our transposase fusions to plants homozygous for a *Ds* element inserted within a streptomycin-resistance gene. These crosses have been performed for four of our transposase fusions, and hybrid seed has been obtained. To test the efficiency with which our fusions promote transposition of the *Ds* element, we will plate these seed on streptomycin-containing medium and measure the number of clones of streptomycin resistant cells which appear on the cotyledons of the germinating seedlings. These clones will be a consequence of *Ds* excision from the streptomycin resistance gene (Jones *et al., Science* **244**, 204-207). These results will be available soon!

Isolation of the flowering-time gene *fg*

K. Ingle and G. Coupland. IPSR (Cambridge lab.), Norwich.

We have initiated a chromosome walk from *tt*-4, which encodes chalcone synthase and is located on chromosome 5 at position 14.4cM, to *fg* which is located at position 16.4cM. Jo Putterill, a post-doc from Auckland, will join us on this project in June. We will write a report of our progress for the next newsletter. At the moment we are busy packing up our lab in preparation for our move from Cambridge to Norwich.

From Simon Covey...

***Arabidopsis* genes involved in cauliflower mosaic virus pathogenesis.**

S.N. Covey; A.J. Maule, C. Greif and A. Bannister; John Innes Institute, Norwich.

The purpose of this project is to screen *Arabidopsis* variants to identify differential responses to cauliflower mosaic virus (CaMV) infection which we can eventually trace to mutant plant genetic loci. During the first three months of the project, we have been optimising growth and inoculation conditions to minimise variations in symptomatic responses due to these factors. We have also been analysing viral gene products and replication intermediates which accumulate in systemically-infected *Arabidopsis* leaves. Plant differences in response to CaMV infection are reflected in alterations to the pattern of viral products. The next stage will be a comparison of the effects of genetic variants of CaMV on *Arabidopsis* ecotypes.

COVEY@UK.AC.AFRC.JII

From Ian Crute...

Identification of genes for resistance to fungal pathogens.

Ian Crute; IHR, East Malling.

Dr Eric Holub has recently been appointed to work on this programme at IHR East Malling and will start as soon as a work permit can be arranged. Eric is a plant pathologist from the University of Wisconsin, Madison and has spent some time in Paul William's laboratory working with "Fast Plants" (rapid cycling brassicas). Eric is already in Kent; his English wife is a post-doc at the University of Kent in Canterbury. During his first visit to East Malling, Eric and I "rediscovered" white blister disease (*Albugo candida*), well established in our local *Arabidopsis* population.

Two new walk-in growth chambers will soon be completed for controlled inoculation studies, so we are "champing at the bit".

CRUTE@UK.AC.AFRC.EMRSA

From Andy Cuming...

Ammonium toxicity in *Arabidopsis*

Andy Cuming[1], Mike McPherson[2] and Kerrie Jones[2]; [1]Genetics Dept. & [2]Biotecnology Unit, Leeds University.

We have grown our first *Arabidopsis* plants in the Genetics department growth room. As it is small, green and insignificant, it is virtually indistinguishable from the other inhabitants of the room (*Physcomitrella patens* -- I promised my head of department a plug).

Our initial aim is to isolate the glutamate dehydrogenase gene, and to this end we have generated an alignment of all the GDH amino acid sequences currently available. Only one of these is of plant origin, from *Chlorella*, and interestingly, this is more highly homologous with the bacterial sequences than with vertebrate sequences. It isn't clear, however, whether this is a cytosolic or a plastid enzyme, although the gene itself is nuclear in location. Based on this alignment, in which the individual amino acids have been attractively colour-coded (no extra charge), we have synthesised four oligonucleotides for use as PCR primers. Two of these correspond to the catalytic domain, one to the co-enzyme binding site, and the fourth to a position between these two regions of the sequence.

We are now preparing root mRNA, in which the level of GDH has been enhanced by ammonium ions, for cDNA synthesis. We intend to amplify a short cDNA fragment and use this as a homologous probe for full-length cDNA and genomic clones. More news, we hope, in our next exciting installment. *(Hopefully, on disk and on time! -- ACM)*

From Mike Daniels...

Infection of *Arabidopsis thaliana* with *Xanthomonas campestris* pathovar *campestris*: a model system for molecular genetic studies of plant-pathogen interactions.

Michael J. Daniels; The Sainsbury Laboratory, John Innes Institute, Norwich.

Xanthomonas campestris pv. *campestris* is the most serious disease of brassica crops worldwide. We have studied the genetics of pathogenicity of *X.c. campestris* for some years and have identified several classes of gene required for invasion and symptom production, and in parallel experiments the response of plants to infection in terms of gene expression has also been documented. We have recently begun to use a genetic approach to understand the plant side of the interaction, taking advantage of the fact that the host range of *X.c. campestris* includes essentially all known crucifer crops. Under suitable conditions the bacteria can be inoculated into *A. thaliana* leaves where they multiply and after a few days produce disease symptoms resembling those incited in *Brassica* leaves under the

same conditions. The disease is not produced by *X. campestris* pathovars which cannot normally infect crucifers, or by *X.c. campestris* mutants of reduced pathogenicity to brassicas. We have screened numerous ecotypes of *A. thaliana* and independently isolated wild strains of *X.c. campestris* to find natural variation in the interaction phenotype. While most combinations gave the same type of compatible interaction, i.e., the plants were susceptible to infection, we have identified certain combinations worthy of further study. Using two tester strains of *X.c. campestris*, one being the wild type used routinely in the laboratory from which all mutants are derived, we have found that ecotytpe Columbia is susceptible to one but resistant to the other. The resistance is dominant and appears to be determined by a single gene. An avirulence gene has been cloned from the avirulent *X.c. campestris* strain which interacts with the Columbia resistance gene in the typical gene-for-gene manner. Ecotype Oy-0 is resistant to both *X.c. campestris* strains, but the resistance seems to be determined by different systems; resistance to one strain is dominant, but recessive to the other. We have also begun screening M2 mutagenised *A. thaliana* plants for variation in symptom type or resistance, and two mutants of interest have been found. Our experiments have demonstrated the usefulness of the *A. thaliana*-*X.c. campestris* system, and by exploiting the molecular genetic resources and technology available for *A. thaliana* it should be possible to analyse the biology of the interaction in great detail.

Much of the work required to develop the system was carried out by Fan Mi-jiao, a visiting scientist from Nanning, China, supported by a Royal Society fellowship, together with Christine Barber and Belinda Clarke of the Sainsbury Laboratory. Jane Parker took up her appointment on March 1st to continue the project, supported by an AFRC grant.

From Caroline Dean...

This report summarises all the work going on in Caroline Dean's lab. Some of this is not funded by the PMB programme, but has been included as it may be of interest to other *Arabidopsis* researchers.

Construction of an overlapping library of the Columbia ecotype.

This project is an international collaboration with Chris Somerville (Michigan), Howard Goodman (Boston) and Pablo Scolnik (DuPont). Dr Renate Schmidt and Gerda Cnops, two EC funded fellows, have made great progress in this project. The two PMB SO posts have yet to be appointed, but interviews recently took place. At Norwich, we will be linking up the YAC clones that cover the top halves of chromosomes 4 and 5. The 2300 YAC clones from Erwin Grill and Chris Somerville have been plated out at high density using a 96-prong replicator that transfers clones from 96-well microtitre plates to petri-dishes (using 8 offsets). The library has been probed with all the RFLP markers from Meyerowitz and Goodman that lie on the top of chromosomes 4 and 5 and at this present time 50% show at least one positive signal. Dr Ian Bancroft has optimised the IPCR technique to generate end-probes from the YAC inserts. In the first test, an IPCR fragment generated from the right end of the insert of YAC clone 17A2 was used to probe the YAC library. It hybridised to the YAC clone it was amplified from and to one other. We will screen more YAC clones so that we can analyse at least two positively hybridising YACs for each probe. This will ensure that a particular YAC clone that shows hybridisation to an RFLP marker or IPCR fragment is not composed of non-contiguous genomic regions. We have replicated the YAC library and have thus far distributed it to three labs in Europe and one in the UK.

Transposon tagging in Arabidopsis.

The HSO position on this grant has yet to be filled but we (C. Dean, Emily Lawson and Clare Lister) have continued screening the Landsberg *erecta* transformants carrying various derivatives of the maize transposable element *Ac* in a streptomycin resistance marker. Excision of the element from the streptomycin marker results in green sectors on white cotyledons in seedlings germinated on streptomycin media. The variegation on the seedlings is absolutely lovely and has made the front cover of the IPSR Annual Report 1990 so watch out for your copy! Fully green individuals also arise due to germinal excision and their progeny can be screened for mutations caused by insertion of the element at new locations in the *Arabidopsis* genome. Southern and PCR data have confirmed that the element excises and re-integrates into the genome. We intend to collect 500 fully green seedlings before performing a mutagenesis screen. A deletion created in the 5' untranslated leader region of the *Ac* transcript significantly increased the level of transposition and we are currently testing whether this is equally effective at transactivating a *Ds* element (Dr Joanne Burn, short term visitor). In order to map the genomic positions for both T-DNA insertions and new *Ac* positions we are using IPCR to amplify the flanking plant DNA. The IPCR fragments will then be used to probe RFLP blots. We have sequenced two IPCR fragments generated from two independent T-DNA insertions in order to ensure the correct DNA was amplified. The fragments showed the expected sequence to the T-DNA RB and then unknown sequence, which we assume to be the flanking plant DNA.

Molecular genetic analysis of the vernalization requirement of *Arabidopsis thaliana*.

There are three aspects to this project. The first is to clone the *fca* locus (which confers a late flowering phenotype to the plant that can be corrected by vernalization). Dr Ian Bancroft is currently mapping the *fca* mutation onto the RFLP map. He will walk to the *fca* locus using the YAC clones from the Columbia ecotype. He is also constructing Landsberg *erecta* YAC libraries in order to isolate the corresponding YAC clone from Landsberg *erecta* which will then be used in the complementation studies. The second is to perform an EMS mutagenesis experiment on the *fca* mutant to find a plant that no longer responds to vernalization. This will be done by Dr John Chandler who will take

up the HSO position for this project (funded by PMB) on 1 May, having just completed his PhD with Prof. Dale in Edinburgh. John will also perform some physiological experiments on the late flowering mutants to try to determine which processes are involved. The third aspect is being studied by Jonathan Clarke who is mapping the major genes involved in the vernalization requirement of the winter line, Stockholm, in order to see if they are allelic with any of the late flowering loci mapped in Landsberg *erecta*.
ARABIDOPSIS@UK.AC.AFRC.JII

From John Doonan...

Identification and analysis of genes regulating the cell division cycle in plants.

John Doonan; Dept. Cell Biology, John Innes Institute.
Dr Hanma Zhang starts on 1 April.

From John Draper...

Regulation of Ds transposition in higher plants and evaluation of rapid techniques for the cloning of flanking DNA.

Dr John Draper and Dr Rod Scott; Botany Dept., University of Leeds.
PROJECT SYNOPSIS. The proposed work aims to improve tansposon insertion mutagenesis as a tool to isolate plant genes, and to evaluate "transposon hopping" methods for chromosome fine mapping and for the creation of overlapping genomic libraries. We intend to develop a pollen-specific *Ds* "helper transposase" construct which will function in *Arabidopsis* prior to meiosis and possibly at later stages of gametogenesis, and allow the generation of "germline"-only transposon-induced mutations that can be directly screened for in the F1 generation. At a later stage of the project, it is intended to engineer YAC vector sequences and dominant marker genes into *Ds* elements to allow rapid rescue of large portions of DNA flanking an insert.
PROGRESS. This project has been underway since December 1989 with the appointment of a post-doctoral worker (Gary Foster) and a research technician (Rob Blundell) and has continuing input from a Ph.D. student (Mike Roberts) The project has developed by virtue of the fact that we have been working on flower development in *Brassica napus* for the past 2.5 years. During this time, we have isolated several pollen-specific cDNAs which exhibit very tight temporal regulation in brassicas. In the past few months, we have constructed *Arabidopsis* genomic libraries and pulled out several genomic clones homologous to the brassica cDNAs. Several of these genomics are currently being characterised with a view to making promoter fusions with an *Ac* transposae cDNA and repoter genes (e.g., GUS) for analysis in transgenic *Arabidopsis* and *B.napus*. Transformation attempts with *Arabidopsis* and *B.napus* have proved successful, but in the latter species some improvement in efficiency is still desirable.

From Ian Furner...

Towards a molecular genetics of apical development in *Arabidopsis thaliana*.

Ian Furner; Dept. of Genetics, University of Cambridge, Downing Street, Cambridge, Tel. 0223-333959.
The main focus of research in my laboratory is plant development. My proposal in this initiative was directed at trying to gain some insight into apical development in *Arabidopsis*, using a combination of molecular and genetic approaches. The two new researchers hired on the initiative have been here less than two months and are, therefore, just starting up. They are Karen Sweet and Paul Davidson. Karen and I are taking the genetic approach, looking for mutants affecting the apex, fate mapping, etc. Paul is taking a more direct biochemical approach, looking for cDNAs which are highly expressed in the apex.
Three other researchers in the laboratory work on *Arabidopsis* and though they are not formally part of this initiative, their projects are relevant and are mentioned here. Ottoline Leyser is a fourth year post-graduate student and her work has centred on fasciated mutants of *Arabidopsis* (fasciation is a term used to describe a variety of physiological and genetic disturbances to apical meristem development). Ottoline's research has involved a considerable amount of EMS mutagenesis and her protocol comes with this newsletter. Nigel Kilby is a post-doctoral researcher and his work concerns the potential of T-DNA tagging as an insertional mutagen in *Arabidopsis*. He has been using the Valvekens transformation largely as outlined in the last *Arabidopsis* newsletter. In the course of the work he ran into some technical problems and solved them. These problems and solutions are attached to this newsletter.

The final member of the *Arabidopsis* group is a first-year post-graduate student, Justin Ainscough. He is looking for [illegible]an and tissue-specific enhancers using an attenuated 35S b-glucuronidase gene on a T-DNA vector.
We will be continuing a variety of M2 screens of EMS treated Landsberg *erecta* and if any of you have a particular, and fairly obvious, visible phenotype you are interested in, please contact me If I come across it I will save the relevant seed and send it to you.

From John Gray...

Trichome differentiation in *Arabidopsis*.

John Gray; Botany School, University of Cambridge.
The project will finally get started in mid-March when Mandy Walker, the post-doc appointed on the grant, arrives fro[illegible]iz Dennis's lab at CSIRO, Canberra, Australia. It looks as though a large amount of the original project proposal will soon be completed in the US (see the papers from David Marks's lab[1]). I have been growing a range of *Arabidopsis* isolates, from as far afield as Kashmir, Finland, Cape Verde Islands and Morocco (thanks to George Coupland), to look for natural variation in leaf epidermal morphology, including stomatal density.

[1] *The Plant Cell* (1989) 1 1043-1050
1051-1055.

From Nic Harberd...

An attempt to clone the gal locus by phenol-enhanced DNA re-association.

Mary Holdsworth and Nicholas Harberd; IPSR, Cambridge.

Intragenic recombination analysis of mutant alleles at the gal locus suggests that the irradiation-induced gal-31.89 allele may be a deletion mutation, covering approximately half the length of the genetic fine structure map of this locus (1). We are attempting to isolate DNA from this locus by use of a method that allows cloning of DNA fragments absent in a deletion mutant strain. We are using the phenol-enhanced re-association technique (PERT, 2). This method uses phenol-accelerated competitive DNA re-association. DNA from plants homozygous for gal-31.89 was sheared and combined in 1000-fold excess with Mbo leaved DNA, isolated from the Landsberg *erecta* progenitor strain. The DNA was melted by boiling, and the mixture then subjected to phenol enhanced re-association. Three types of termini are expected following re-association. Most will have sheared DNA ends, a few will be hybrid molecules with one Mbo 1 and one sheared end, and the least frequent will be molecules that have re-associated perfectly and have complete Mbo I ends. These are selected from the re-associated mixture because they alone can be ligated into the BamH1 site of pUC19. The resulting plasmid library should be enriched for DNA deleted in the gal-31.89 strain. We are testing clones from this library in an attempt to tify inserts which hybridise with DNA from L. *erecta*, but not with DNA from gal-31.89. Such inserts may contain DNA from the gal locus. If we are successful in identifying such clones. we will use them to isolate larger genomic DNA clones containing the entire gene. If this method proves to be successful it may be generally applicable to the isolation of genes for which a potential deletion mutant allele is available.

References: (1) Koorneef, M. *et al.* (1983) Genetical Research **41**, 57-68. (2) Kunkel, L.M. *et al.* PNAS (USA) 82, 4778-4782.

From Nick Harris...

Development of the silique of *Arabidopsis.*

Nick Harris and Phil Gates; Dept. of Biological Sciences, University of Durham.

The silique is the fruit: a pod-like structure, but with a central septum. It is a determinate organ whose development is initiated, but temporarily arrested until restarted by fertilisation of the enclosed ovules. This project will determine the major histological features of differentiation and the development of both wild type and mutant siliques (including clv1; clv2) both prior to and after fertilisation. Using hybridisation, immuno- and enzyme histochemistry, we will identify the temporal and spatial patterns of differentiation of the significant tissue types. Screening will be undertaken to isolate a range of other silique mutants, including a non-dehiscent form.

From Pat Heslop-Harrison...

The chromosome structure and genome organisation of *Arabidopsis* in reconstruction of nuclei.

J.S. Heslop-Harrison; IPSR Cambridge.

Preliminary results from *in situ* hybridisation of λ-clones to cell spreads of *Arabidopsis* are showing that hybridisation signal can be detected at specific points on the chromatids at the electron microscope level. If confirmed, this would be the first step towards physical *in situ* mapping of clones.

From Jeremy Hyams...

Cell cycle control genes in *Arabidopsis.*

Jeremy Hyams; UCL.

Person to start soon.

From Gareth Jenkins...

Isolation and characterisation of photoregulatory signal transduction mutants in *Arabidopsis.*

Gareth Jenkins; Dept. of Biochemistry, University of Glasgow.

Readers will be pleased to know that *Arabidopsis* thrives in the moist Glasgow climate (actually in petri dishes). What's more, the wee beastie has been transformed -- is this the first transgenic weed in Scotland? Constructions containing luciferase coding sequences are now being introduced.

From Peter Jordan...

The genes encoding the early enzymes of the chlorphyll biosynthesis pathway in *Arabidopsis thaliana* and their regulation.

Prof. P.M. Jordan; Biochemistry and Molecular Biology Laboratory, Queen Mary & Westfield College, University of London, Mile End Road, E1 4NS.

The initial aim of the programme is to isolate genes encoding the early enzymes of the chlorophyll biosynthesis pathway using information from direct protein sequencing of enzymes isolated from *Arabidopsis thaliana* and/or using information from related cDNA or gene derived protein sequences from other organisms. As the generation of *Arabidopsis* leaf material is still in the process of being scaled up, the latter approach has been used. Accordingly, we have synthesised several appropriate DNA oligonucleotide sequences and have used these in the polymerase chain reaction (PCR) to amplify the DNA, encoding two segments of the *hemC* gene from an *Arabidopsis* gene library. The *hemC* gene encodes the enzyme porphobilinogen deaminase which catlyses the first step in the formation of the tetrapyrrole ring. (The library is in λ-fix and was kindly sent from Washington, USA, by Dr. Raineri.)

Two 300-350kb segments of DNA have now been obtained which will be used to probe the gene library as a prelude to the determination of the complete *hemC* gene nucleotide sequence. PCR primers have also been prepared for the investigation of genes encoding three other early enzymes of the chlorophyll pathway and a similar strategy will be adopted.

The appointed research staff will be joining the group at Easter.

From Rachel Leech...

An analysis of leaf development and chloroplast division in *Arabidopsis thaliana.*

Rachel Leech (PI) and Kevin Pyke (RA); Dept. Biology, University of York.

Cellular development in *Arabidopsis*

thaliana var. Landsberg *erecta* first-leaves has been analysed in resin-embedded sections using image analysis. Plants were grown in constant environment cabinets at 20°C, 16 hour light period and 70% RH. Under these conditions the first leaf becomes visible 7 days after sowing and is highly meristematic until day 13 after which leaf cell number remains constant. Differentiation of palisade mesophyll cells is first noticeable after 7 days.

Subsequent pairs of leaves appear at about 3 day intervals. In young leaves there is a gradient of expansion, with cells nearer the leaf base and the petiole remaining meristematic for up to 4 days longer than cells nearer the leaf tip. By volume, fully expanded *Arabidopsis* leaves are composed of 59% mesophyll, 27% airspace, 13% epidermis and 1% bundle cells. Chloroplast number per mesophyll cell increases linearly with cell size during cell expansion. In mature cells, the chloroplast number is *circa* 100. For those who thought *Arabidopsis* cells might be especially small, leaf mesophyll cells are similar in size (*circa* 4500um^2) to hexaploid wheat mesophyll cells, which contain about 70 times more nuclear DNA! *(Perhaps even more? — ACM)* The leaf development in chloroplast number mutants is currently being examined.

From Keith Lindsey...

Insertional mutagenesis in *Arabidopsis thaliana*.

Keith Lindsey; Mike Clarke, Jennifer Topping and Wenbin Wei; Leicester Biocentre, University of Leicester.

This project forms part of our interest in using transformation techniques to identify native genes and regulatory elements that are important in controlling plant development. The aim of this particular project is to investigate the use of insertional mutagenesis as a means of: (*i*) inactivating; (*ii*) studying the regulation of the expression of; and (*iii*) tagging native genes that are expressed during development in *Arabidopsis*. The novel approach we are adopting is to introduce a promoterless *gus* reporter gene into a population of plants, to generate native promoter-*gus* fusions, and to screen for both developmentally regulated *gus* expression and for phenotypic mutants.

This technique has advantages over chemical/physical mutagenesis and standard T-DNA insertional mutagenesis techniques. Not only are mutagenised genes tagged by physical linkage to *gus* sequences, but by measuring GUS activity by quantitative (fluorimetric) and qualitative (histochemical) analysis, it is possible to study promoter function *in vivo* before gene isolation. Furthermore, this functional assay provides a means of isolating genes by insertional mutagenesis in the absence of a visually obvious phenotypic change to the plant.

We have already made the required gene constructs, and are directing current efforts primarily towards improving the efficiency of *Arabidopsis* transformation. We have had some success already, producing kanamycin-resistant plants of ecotype C24 using the root transformation procedure of Valvekens *et al.* Putative transformants are currently being analysed. We are also trying out the seed transformation method of Feldmann's laboratory, but it is too early to quantify its efficiency in our hands. We are confident of our general experimental approach: we have transformed about 150 tobacco SR1 plants with the promoterless *gus* constructs, and between 10-30% exhibit GUS activity differentially in different organs.

From Andy Maule...

Identification and expoitation of the interaction between a protein and host factors which control virus spread.

John Innes Institute, Norwich.

On 1st February, Carole Harker started her post-doc position to examine the molecular interaction between cauliflower mosaic virus (CaMV) and cuciferous hosts which controls the ability of the virus to move from cell to cell. She was joined at the same time by Dr. Genevieve Boudazin from INRA, Versailles who will be visiting for one year. The initial phase of the work will be to establish a procedure for assessing the behaviour of CaMV "spread gene" mutants after infection of wild type rape or *Arabidopsis*. Complementation of the mutants in transgenic plants expressing the spread gene will verify that these mutants are not defective in virus replication. We are currently checking some transgenic rape for protein expression and Caroline Dean has kindly offered to put our CaMV constructs into *Arabidopsis*. To obtain a measurable infection of spread mutants in plants, we are planning to use the technique of "agroinfection" of plant viruses. This is achieved by using *Agrobacterium* to insert a multimeric construct of CaMV into the host, from whence an infectious unit can be released by either recombination or replication, or both. I am not a popular person for suggesting this as we now find that even CaMV monomers cloned at particular restriction sites have an amazing capacity for recombination in *E.coli*. If anyone has a particular interest in unwanted plasmid DNA re-arrangements, or preferably, if anyone has *E.coli* strains completely defective in plasmid recombination then please let me know.

From Keith Mitchelson...

Identification and cloning of hypervariable loci from *Arabidopsis thaliana*.

Keith Mitchelson; Dept. of Molecular and Cell Biology, University of Aberdeen

Activity is about to commence in Aberdeen with the imminent arrival of Dr. Deborah Silcock to undertake the research post.

From Bernard Mulligan...

Genetic Male Sterility In *Arabidopsis*.

Bernard Mulligan and Greg Briarty; Nottingham University. MAC and MODEM available. Tel. 0602-484848 (ext 3467).

The team is now complete. Janet Fuller, Jane Russell and Dr Zoe Wilson are currently building up a collection of recessive male sterile mutants by screening our home-made EMS and X-ray M2 populations of Landsberg *erecta*. Well over 20 lines which fail to set seed unless fertilized with wild type pollen have been isolated so far. Examination of the stained anthers of these sterile plants shows a broad spectrum of aberrations in

pollen develoment. We have been using a staining cocktail containing malachite green and acid fuchsin for this analysis. In some mutants, pollen grains appear normal (stain red-purple), but the anthers do not dehisce. In others, the anthers contain little or no stainable pollen grains. Yet others contain pollen possessing little cytoplasm (stain blue/green) and are of abnormal shape. Anthers of the Wageningen mutant ms1 contain a purple staining material, but no identifiable pollen grains. We are currently carrying out a more detailed EM and light microscopical analysis of some of these mutants. Crosses to test for allelism between the new male sterile mutants are currently being carried out. When this work is complete, we plan to map any new ms loci we discover using a fertile derivative of Maarten Koornneef's multiple marker line W100. Since in o[illegible] species, certain male sterility genes control aspects of meiosis in the anther, a visitor to Nottingham, Dr Maria Vieira from the University of Sao Paulo, Brazil, is working out a minimally frustrating method for reproducible analysis of chromosomes in meiosis. This noble task is nearly done.

From Denis Murphy...

Purification of enzymes and cloning of genes involved in the regulation of storage and membrane lipid biosynthesis in *Arabidopsis*.

Denis J. Murphy, Steve Slocombe (post-doc), Ceri Batchelder (Ph.D. student), Ian Cummins (RA); Dept. of Biological Sciences, University of Durham.

We have started by trying to purify the microsomal cytochrome b5 involved in 18:1 and 19:2 desaturation. This is a relatively abundant and easily assayed enzyme. We are simultaneously using heterologous DNA probes (based on sequence data from other organisms) in an attempt to clone directly the cyt b5 gene.

In another project, we have purified an abundant seed protein (oleosin) involved in storage oil body formation. The genomic gene(s) for this protein is being cloned using a cDNA probe from rape seed. The structure of the flanking elements of this gene will be compared with those of other genes involved in the formation of seed storage products during embryogenesis in *Arabidopsis*. Conserved regions within the oleosin ORF are now being used to make anti-sense constructs in order to study the effects of down regulation of the oleosin on storage oil accumulation in the seed.

From Jim Murray...

Identification and analysis of genes involved in plant development and growth control.

Jim Murray; Institute of Biotechnology, University of Cambridge.

This project, which should get under way in May 1990, aims to clone plant homologues of genes already known to be involved in fundamental cellular processes in other organisms. We intend to use two approaches: (i) complementation of equivalent mutations in yeast with *Arabidopsis* genes; or (ii) redundant PCR using oligos made from the protein sequence of domains conserved between several species.

JAHM@UK.AC.CAMBRIDGE.PHOENIX

From Steven Neill...

Identification of water stresses and ABA regulated genes using wilty mutants of *Arabidopsis thaliana*.

Steven Neill, Bristol Polytechnic.

The aims of this project are to identify genes encoding products induced by abscisic acid (ABA) or by water-stress, including those coding for the enzymes of ABA biosynthesis. cDNA libraries will be prepared from water-stressed leaf tissue and subjected to differential screening with RNA isolated from stressed wild type and well-watered ABA-deficient tissue.

From Peter Quinn...

Thermal tolerance of fatty acid desaturase mutants of *Arabidopsis*.

Peter Quinn; Biomedical Sciences, KQC.

Work on my project has not commenced as I am waiting for my assistant to take up his post.

From Chris Raines...

Genetic analysis of regulatory factors determining the development of the photosynthetic apparatus of plants.

C.A. Raines, N.R. Baker and M. O'Farrell; Dept. of Biology, University of Essex.

At long last we are in possession of a genomic library which we prepared using the variety Columbia and the vector λ fiXII. The primary library has 140,000 recombinants. Screening of this library is underway and we will have some results early in March. We intend to amplify this library when we complete our work with it and will make this available to anyone wishing to screen it.

"[2573,2573]"@UK.AC.ESSEX

From Colin Robinson...

Isolation and analysis of *Arabidopsis* chloroplast biogenesis mutants.

Colin Robinson; Biological Sciences, University of Warwick.

The aim of this project is to isolate *Arabidopsis* mutants that are deficient in one of the various components of the chloroplast import machinery. In the screening procedure, mutant plants will be identified by the presence of precursor proteins (detected by Western blotting) and subcellular fractionation techniques will be used to determine whether the defect lies in the chlorplast import machinery or the stromal processing peptidase. Chloroplasts from mutant plants will be used in *in vitro* import assays to probe the mechanism and specificity of the import/maturation system affected.

From Alison Smith...

Investigation of the gene for hydroxymethylbilane synthase from *Arabidopsis* in transgenic tobacco plants.

Alison Smith; Dept. of Botany, University of Cambridge.

The aim of the project is to investigate the regulation of expression of the gene for the porphyrin synthesis enzyme hydroxymethylbilane synthase (HMBS; also known as porphobilinogen deaminase or PBGD). The approach will be to construct chimeric genes with the upstream regions of the HMBS gene from *Arabidopsis* with the reporter gene GUS, and to

monitor GUS expression in transgenic plants in different tissues and under different environmental conditions. Although HMBS is a housekeeping gene, and therefore must be expressed constituitively in all tissues, the enzyme is needed in much higher amounts in photosynthetic tissue to make chlorophyll. It is hoped that there will be someone in post to do this work within the next two months.
AS25@UK.AC.CAMBRIDGE.BIOLOGY

From Felicity Watts...

Generalised technique for cloning *Arabidopsis* genes involved in complex biochemical pathways.

Felicity Watts, Tony Moore and Julian Burke; University of Sussex.
We have appointed two people for our AFRC-funded posts: Dr. Neil Butt (RA1A), and Mrs. Anna Clarke (Technician Gr5). We also have a self-funded D.Phil student, Phil Layfield. Current work involves the screening of cDNA and genomic *Arabidopsis* libraries with yeast cell-cycle genes, and PCR using oligonucleotides corresponding to conserved sequences of RAS and cell cycle genes.

Please send your next summary to the newsletter by Friday, 15th June, at the very latest.

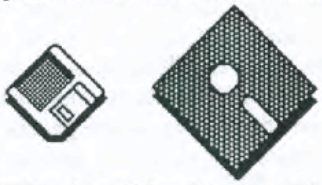
How To Reach Us

In brief, please send any contribution to the newsletter in one (or more) of the folowing ways (in roughly descending order of preference):
1. to ARABIDOPSIS@UK.AC.AFRC.JII
2. on a Macintosh disk (1.44Mb, 800kb or even 400kb)
3. via modem (for the ambitious)
4. on a 3½" (high density, 1.4MB) IBM disk in wordprocessor and text only (ASCII) format
5. as 4, but on a 5¼" (double density, 720kb) disk
6. as 4, but on a 5¼" (high density, 1.2Mb) disk.

A longer explanation of the above:
ARABIDOPSIS@UK.AC.AFRC.JII
Many thanks to those of you who sent your contributions, protocols, junk mail, etc., by e-mail to the above address using a mainframe computer (usually a VAX). This is a very efficient method for getting reports directly into the newsletter. The text can be cut and pasted on screen from the Arabidopsis VAX account directly into the newsletter's Macintosh word-processing programme and so one doesn't have to post disks back and forth. For those already sending stuff by e-mail, please carry on, and for those who haven't tried it yet, please have a go. (There's usually somebody in the lab or lurking down the corridor that has to get a daily fix on a terminal.) For anyone at an AFRC institute, just go and see your friendly local VAX manager. It doesn't take long to get an account and to learn how to log on and send mail. And once you've discovered the joys of e-mail, it has many uses; such as mailing Saturday's football result to your colleague currently in the US, etc.
Instead of e-mail, posting a Macintosh disk with a WriteNow/MacWrite file would be excellent (for the few Mac users amongst you, don't forget that graphics are welcome too). For the majority of you though, the newsletter can, albeit sometimes a little indirectly, cope with IBM outpourings. We can read Word Perfect 5.0 files directly, providing they are on 3½" disk. For any other programme, for the present (although the ACM is currently playing with file translators and so the situation may improve), please send your output in your standard word-processor format *and* as a text-only (ASCII) file. This is because the newsletter should be able to read this standard text format if we cannot manage to decipher the wordprocessed file. All IBM-type word-processing programmes can save as ASCII files. In WordPerfect, for example, one merely has to:
- open the document
- Crtl/F5
- option 1 (DOS - text)
- option 1 (Save)
- give filename to save as.

Please send your wordprocessor file together with its corresponding ASCII file on a 3½" (high density) disk, as this can then be read directly in the Macintosh's drive.

If you really are unable to save to a 3½" disk, the newsletter can, with some difficulty, cope with 5¼" disks (double density, if posssible), but sending 3½" ones makes life much easier at this end. As a last resort, we can (just) cope with BBC, Decmate and Amstrad disks, providing the files, as above, are also in ASCII format. (All of these systems can save files in this format).



Whatever the disk, please enclose a printed copy and *ensure* that the disk and originating machine are virus-free. Disks will, of course, be returned.
For the more adventurous, the newsletter's Mac also has a modem, and so if any of you also have a modem-equipped computer, perhaps we can try some direct file transfer via the telephone (from any computer). Give the ACM a ring you are game or if you are having trouble following any of the above directions.

Arabidian Notes: the second AFRC PMB Arabidopsis Newsletter, March 1990.
Assistant Circulation Manager, David Flanders, John Innes Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UH. Tel: 0603-52571; FAX: 0603-56844.
Special thanks to:
- Barrie Allen (Principal photographer, Cambridge Laboratory) for offset-litho printing of both the masthead and the graphics in the summary section.
- Mike Daniels for being the guinea-pig for WordPerfect PC to Mac file translation.
- Andy Davies for the conference photo.
- Laura Donohue for proof-reading.
- Terry Donohue and Richard Mitchell (The Underground Grammarian) for supplying the typefaces and some of the graphics.
- Anne Edwards for the cartoon.
- Bob Findlay for his BBC-disk reading talents.
- Paul Fretter for PC advice.
- Jonathan Jones for use of the Sainsbury lab's Laser Printer and PC disk drive.
- Black Rot for the crossword.

Arabidopsis cDNA Library

Following on from the item in the first newsletter, Christine Raines reports that her lab have made polyA RNA from both Columbia and Landsberg *erecta* ecotypes. From Northern blot analysis, the RNA looks good. Christine will now borrow a cDNA synthesis kit, which if not completely successful, will at least tell her if the RNA is indeed of good quality. Depending on the results of this test, she may have a λZAP expression library commercially made.

Tips & Questions

From Denis J. Murphy...
We would like to have expression libraries from developing seeds and (possibly) germinating seeds. If anybody else is interested, contact DJM. We will need lots of embryos for this!
- Is anybody interested in using microspore-derived embryo culture?
- We have (rapidly diminishing) EMBL and GEM-11 genomic libraries.

From Keith Mitchelson...
Does anyone (else) find that *Arabidopsis* seedlings are extremely subject to collapse within the first two weeks of germination? Any solutions, or is it due to too much solutions??

From Phil Gates and Nick Harris...
'Gardening Note': fluid drilling of seeds.
Easy way to sow large numbers of *Arabidopsis* seeds over the surface of a seed tray: suspend seeds in a sloppy agar and suck suspension into 10ml syringe; agitate on "whirlimixer" for circa 30 seconds to produce uniform suspension; squirt out agar drops with seeds onto moist seed compost or other growing medium. Sowing density can be varied by adjusting number seeds/volume of agar. Fungicides etc. can be incorporated into sloppy agar if required.

Location Changes

- As of late March 1990, the Durham Oilseeds Reseach Group will re-locate to IPSR, Norwich, where Denis J. Murphy will be Head of the Brassica Research Dept.

- Peter M. Jordan has moved from Southampton University to Queen Mary & Westfield College, London. He reports that his new laboratory is almost fully functional for molecular biology and will soon be at full strength.

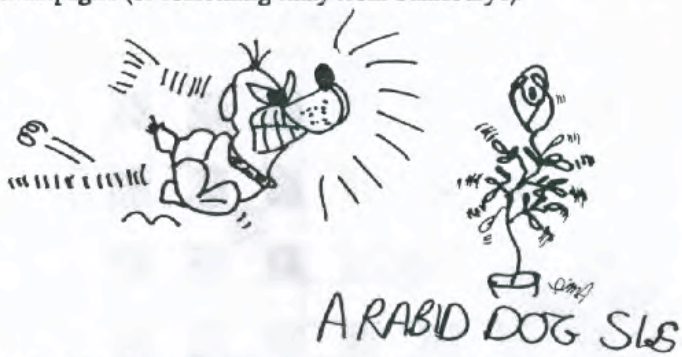
Announcements

- Bryan C. Clarke, Editor of *Proceedings of the Royal Society, Series B*; is canvassing for high quality papers for this soon to be re-born journal. It will have a new cover and format and will be a general biological journal publishing short papers (up to 4000 words) very quickly. For those seeking to put *Arabidopsis* into this renascence, contact, Prof. Bryan Clarke, F.R. S., Professor of Genetics, School of Biological Sciences, Queens Medical Centre, Nottingham NG7 2UH.

- The Third Afrc Pmb Arabidopsis Newsletter will be appearing at the end of June. Please have your contributions submitted by 15th June at the latest.

Win a Bottle of Bubbly!

Did anyone spot the glaring omission from the First Annual *Arabidopsis* conference? Well, it obviously was not a conference photograph, as can be seen on page 2. The answer?... conference T-shirts! The next meeting will have its own designer T-shirt. And the designer? the high priest of cruciferous couture -- none other than (as they say on the Good Old Days) ... yourselves.
Please send your designs to the ACM. The winning entry, chosen by a totally biased and bribable committee, will be used as the motif for the second *Arabidopsis* conference T-shirts. In addition to this considerable kudos, the winner also will be presented at the conference with a free T-shirt and a bottle of champagne (or something fizzy from Sainsbury's).



This edition's cartoon comes from the pen of Anne Edwards of the John Innes School of Fine Art. Other budding artists are encouraged to submit *apropos* items. Please send your masterpieces to the ACM.

Letters

In addition to project progress reports, protocols, and sundry other items, letters to the newsletter are welcomed. Please note that the *Arabidopsis* Newsletter reserves the right to edit letters and all other contributions.

From Dave Wilson...
With reference to your pamphlet "The first AFRC PMB *Arabidopsis* Newsletter". I note that you describe a "sieve", made from a 100ml beaker, giving detailed manufacturing instructions. I have to say that I find myself rather puzzled by your omission of any credit to myself for the invention of this item. I have been making this item for the last twelve years at least here in Nottingham University, and for the past eighteen months as a commercial enterprise, trading as WILSON SIEVES.
I have in fact supplied your institute with sieves on two occasions.
If you could give my name and address to the readers of your newsletter, I am sure that they would be interested in my very reasonably priced product.
Wilson Sieves are at, 2 Long Acre, Common Lane, Hucknall, Nottingham, NG15 6QD. Tel. 0602-630164 (6-11p.m.), FAX 0602-455388 (f.a.o. "Wilson Sieves").

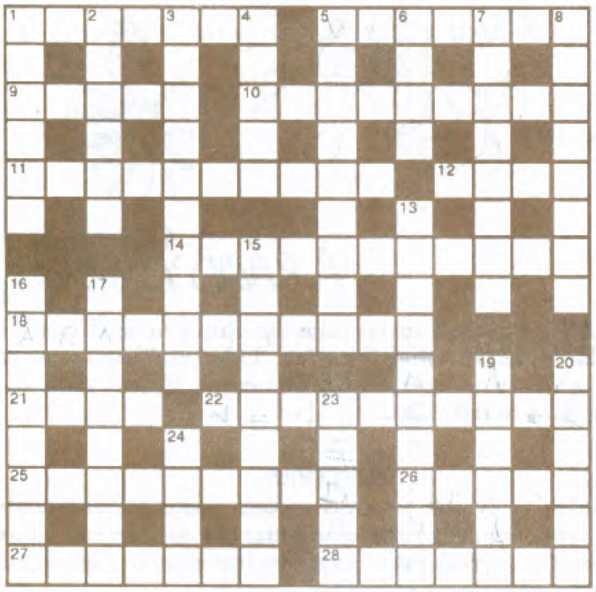
This Issue's Quote

"It's a scientific fact that if you stay in California you lose one point of your IQ every year." --Truman Capote. (This one is for Caroline. Suggestions for any others to the ACM.)

Arabidopsis PRIZE Crossword

The good news is that a £5 book-token will wend its way to the first correct entry drawn out of the bench-top centrifuge. The bad news: it's a cryptic crossword set by a (molecular biologist) regional finalist in last year's Times Crossword Competition. Once you get into it, it's good fun though, and most of the answers have a distinct biological (even Arabidian flavour). To help you on your way and to get you in the mood: in 5 across, Oberon's helper in Shakespeare's *A Midsummer Night's Dream* was Robin Goodfellow, a type of sprite called a **Puck** and also known by that name. A typical himalayan herbivore is a **Yak**. PUCs and YACs (*heard* to be) are both ....

by BLACK ROT



Across

1. ABC's for PCR's ? (7)
5. Oberon's helper and himalayan herbivore heard to be in this group (7)
9. Child's journal (5)
10. Nail Bruce over obstinate plasmid (9)
11. Custodians have to sit again in vehicles (10)
12. Let crazy test stand (4)
14. In English trial, a win for possibles __ _____ ? (2,9)
18,22 After tirade relents, I paw boss possibly for weedy paper (11,10)
21. Measure small island (4)
22. See 18
25. Restricted with old but endless power source initially (9)
26. Language of love of Blarney ? (5)
27. Engineer discovered in the Tyrol, where they yodel eternally (7)
28. Broken limb set in Newcastle last year made me more lithe (7)

Down

1. Ivy league college requires weight loss for Andrew (7)
2. DNA fragment has sulphur in an unreactive form (6)
3,7. spd vote Reagan in change over M25's construction (10,8)
4. Professional pollution (5)
5. Clergymen appear to suffer when about debt (9)
6. Nucleus is initially composed of reticular elements (
7. See 3
8. To find protein like thaumatin in dessert is hard (8)
13. Boston psychic on TV or radio (4,6)
15. Anagramatist, word has it (9)
16. Libelled scholar Nigel is confused over notice (8)
17. Ridiculous to place Channel Islands in distant California (8)
19. A test of the heart chamber (6)
20. Tell Sagittarius ? (6)
23. The chair for mad Scandinavians (5)
24. Experiment to note on way (4)

Rules of the *Arabidopsis* prize crossword:
This competition is open to all who bother to read this newsletter, except for Black Rot, the ACM and anyone who has access to their desks. The final catch is that in order to be eligible for the grand prize, the entrant must have sent in their next project summary, if one is due! The answers as well as the winner's name will be in the next newsletter.

STANZAS FOR SCIENTISTS

This is inspired by the "Physics for Poets" course that the ACM noted on a visit to Stanford University several years ago (he got on the wrong tour bus). Any suggestions for *apropos* poems are welcome. To get things rolling, we have a vaguely botanical piece by the late Philip Larkin from the collection *High Windows* published by Faber and Faber:

Cut Grass

Cut grass lies frail:
Brief is the breath
Mown stalks exhale.
Long, long the death

It dies in the white hours
Of young-leafed June
With chestnut flowers
With hedges snowlike strewn,

White lilac bowed,
Lost lanes of Queen Anne's lace,
And that high-builded cloud
Moving at summer's pace.

DON'T FORGET!

1. Vienna Coach.
2. The next summary of the progress on your project is due by **15 June** 1990.