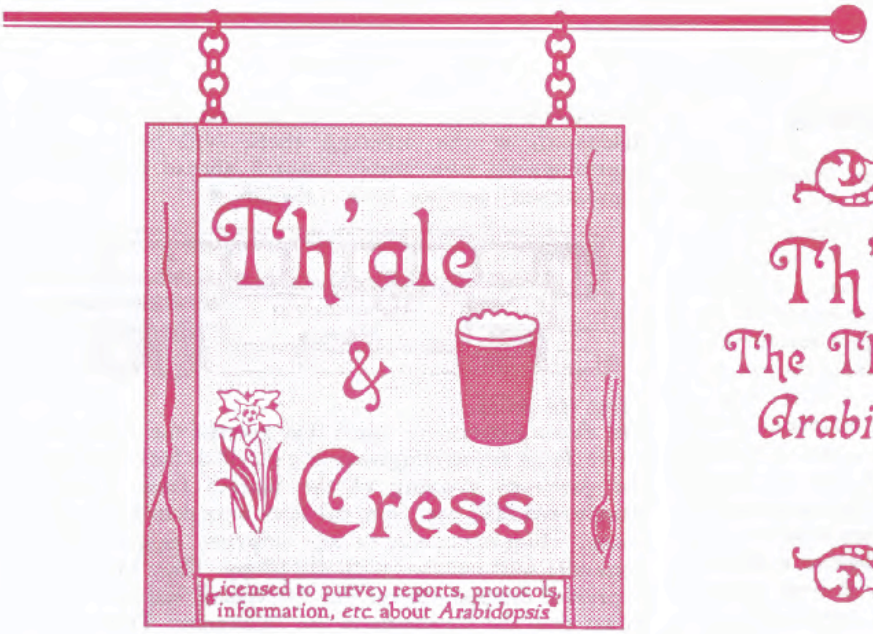


Th'ale & Cress

The Third AFRC PMB *Arabidopsis* Newsletter

July 1990

In This Issue:

Plus – attached

Late Final

Most of you will probably notice that this newsletter has been delayed in hitting the top shelf of W.H. Smith. This is largely because some of the project reports were extremely late in arriving and a few only appeared after considerable cajoulment. Indeed, the last to be included only arrived on 12th July. Not wishing to start *Th'ale & Cress* with a "Welcome to California, now go home" sort of introduction, the ACM, nevertheless, has to address a few stern words to the small minority of you who make his life somewhat miserable at newsletter production time. It may be a pain in the neck to have to write a paragraph, but it is only once every three months, so the ACM asks everyone to remember this obligation under the terms of their *Arabidopsis* grant (you all agreed to be co-ordinated) and get them in on time. On the other hand, thanks are due to the majority of you who have supplied reports in reasonable time, especially the (slowly) growing numbers who are submitting items by e-mail or on disc. (Re-typing a whole series of FAXes is no fun, particularly when the original was prepared on a word-processor.) The aim of the newsletter is to provide a forum for the mutual benefit of all the participants in the *Arabidopsis* Programme. To partake is therefore to benefit. And so please make a real effort to submit your next project report by **15th October**.

VIENNA CONFERENCE: REPORTS

From Caroline Dean...

THE Fourth International Conference on *Arabidopsis* research, held in Vienna between 2 and 5 June, showed how fast the *Arabidopsis* field is moving. Since the last meeting, which was held only seven months previously, there has been significant progress in many different laboratories. It is impossible to summarize briefly all the new and exciting developments so I highly recommend reading the Abstract book. *(If you genuinely cannot get hold of a copy from someone nearby, I will send a photocopy of the abstracts' book to worthy members of the AFRC progamme -- ACM.)* Gurd Jurgens (Munich) described the isolation of more than 200 putative embryonic pattern mutants (after screening 45,000 EMS-mutagenized lines. Caren Chang (Pasadena) described the isolation of a cosmid clone that conferred the *etr-1* (ethylene resistant) phenotype after transformation into wild-type plants. Interestingly, the cosmid contained two trancripts, one with significant homology to adenylate cyclase. Jerome Giraudat (Gif and Boston) also described the walk performed by his lab. to *abi-3,* and their analysis of the complementing cosmid.

Ken Feldman (Dupont) summarized the current status of the T-DNA insertional mutagenesis programme in which they now have 1800 kan resistant lines from the seed transformation protocol. He plans to continue screening for new insertions aiming for complete saturation of the genome. Two mutations isolated from this collection, *ag-1* and *gl-1* were described in detail by Meyerowitz (Pasadena) and Herman (Nebraska) respectively. Meyerowitz proposed a model for control of flower development and then presented convincing data to support this from the analysis of double and triple mutants.

Arabidopsis is now also being successfully used to analyze plant-pathogen interactions and pollen/stigma interactions.

A document entitled "A Longe-range Plan for the Multinational Coordinated *Arabidopsis thaliana* Genome Research Project" was distributed at the meeting. *(Again, if you **really** can't get hold of a copy, I will send you a photocopy -- ACM.)* This was put together by an interim steering committee (including Elliot Meyerowitz, Chris Somerville, Dick Flavell, Caroline Dean, Howard Goodman, Mark van Montagu, Jim Peacock and representatives from NSF and DOE) at two meetings, one in Bloomington last October and one in Denver over Easter. The document describes the co-ordinated plan for *Arabidopsis* genome research over the next 10 years with the ultimate goal being the complete sequencing of the genome. It also describes the establishment of resource centres, seed and clone, one of each in the US and Europe. Communication between labs and distribution of information will be achieved using an electronic bulletin board (PLEASE SIGN UP FOR IT - SEE THE ARTICLE, "ELECTRONIC *ARABIDOPSIS*" IN THIS NEWSLETTER). There will also be money for post-doctoral exchange programmes. The discussions we had about these last December in Norwich at the First Annual UK *Arabidopsis* meeting have come to fruition. The AFRC will soon be announcing the establishment of several fellowships for UK post-doctoral researchers. As discussed, at the meeting these will be used for approximately two years' research abroad followed by approximately one year back in the UK.

From the ACM...

THE shining executive coach that greeted the ACM as he drove down the buthingwhat of a shock, as his examination the previous evening of the viscera from a ritually slaughtered BSE cow from the farm next door had foretold doom. Indeed, much to his surprise, people seemed generally well satisfied with the jalopy. Only Mark Yong expressed disappointment that the vehicle had four wheels rather than the six shown in the illustration. This was not to prove an auspicious observation.

The ACM almost began to relax as the coach sped up the J.I. driveway with consummate ease. However, the feared for Nightmare on Colney Lane soon flickered onto the video screen at the front of the bus as the vehicle ground to a halt a mere 50 metres from the front gate. On the warmest day of the month, the air-conditioning had ceased to work. We soon re-started, but without a cooling breeze. Things went well until we were almost at Stratford tube-station, when the coach kangarooed to a halt. It then moved forward again in a series of lurches propelled solely by the starter motor. Memories of an old Marina came flooding back to the ACM -- the clutch must have gone. It had, it transpired, but fortunately only the hydraulic fluid had mysteriously disappeared; the clutch itself was, apparently, new. "Hmm, what sort of outfit doesn't bleed the clutch properly when fitting a new one?" thought the ACM with fingers crossed whilst nervously watching the requests at the front for a length of rope and the site of the nearest tree-filled park. One driver nipped down the road to Halfords whilst the other donned overalls -- the first time anyone there had seen someone wearing white shoes with such a garment. Less than an hour later we were moving again, even managing to arrive at the meeting point at the arranged time. The rest of the tour party soon appearred, with the noticeable exception of the Nott. Squad. "Fire on the Central Line," they said, by way of explanation. It was only several days later that alcohol forced them to confess that the fire had delayed them returning from the Tate Gallery, not.from the train from the grimy Midlands. Despite these delaying tactics, we arrived in plenty of time for the

appointed ferry and just about the whole party was soon standing in glorious sunshine to watch the white cliffs recede before heading to the Duty Free and bar. Apart from Andy Maule getting the driver to play a soft-porn video, just when everyone wanted to go to sleep, the highlight of the day was having to choose whether to dash across a French motorway, or to trek through the Ardennes and over a bridge to get to the Michelin version of South Mimms. Breakfast next morning was a much more pleasant affair, partaken in Austria, in a restaurant overlooking a lake which stood at the base of a very large rock. The variety of cakes on sale, even for breakfast, was only exceeded by the range of condoms in the machines in the toilets. "Must be for the German tourists," stated Bernie, somewhat Ridleyesquely. We arrived in Vienna at lunchtime, guided in by our local guide (see below), only an hour later than scheduled. And the rest is, inevitably history (see Caroline's article for the science bit in the middle), until the return journey....

The ACM got at least some revenge on Ladbrokes when a bet placed with them before departing from England that Tania Young and Helen North would be (i) late and (ii) the last to arrive, proved correct. Suffering from several days of concentrated science, plus (in some cases, so the ACM is led to understand) the odd excess of local beverage, together with last-minute sight-seeing and shopping, the overall energy level on the return trip was an order of magnitude lower than it had been on the way there. The deflatory experience of leaving was mirrored by a turn for the worse in the weather as we headed towards the German border. At some stage in the night, we stopped for a meal in Bavaria. Most ate; a few even woke for the occasion. Otherwise, sleeping was the order of the night for most. After a few tortuous hours, everyone managed to get up for the group photograph as we waited for the ferry in Calais. (Apologies for the poor quality, but it was taken on the ACM's Box Brownie. A deluxe version of the group photo, promised by Mark Yong, failed to arrive at the Newsletter's front office). Just as everyone was returning to sleep as we edged onto the ferry, a loud explosion suggested that the front tyre had blown out. Investigations revealed, much more seriously, a deflated gas-suspension system. The ACM then pointed out that he had been prescient with the newsletter illustration, as it only showed three wheels. Thus, just when it appeared that the trip was going to peter out to an anticlimax, a replacement coach had to be ordered. This eventually arrived at Dover Coach Terminal, but not before Keith Mitchelson had managed to fleece most of his fellow passengers of their remaining foreign cash at "Cheat". Surprisingly enough, the replacement coach made it both to Stratford, and to Norwich, without further incident. Any takers for a chartered 'plane for the next big *Arabidopsis* Conference?

The ACM thanks: AFRC for funding him and the coach; Ian Bancroft and Mary Holdsworth for being the "spy in the cab" and informing him and everyone else what was happening on the flight deck; Renate Schmidt for assisting with his (failed) "O-level" German; and, especially, Viennese native Trude Schwarzacher, who not only gave all those travelling from Norwich an excellent background introduction, but also navigated the neophyte bus drivers into and out of Vienna and acted as a constant fount of local knowledge and dispenser of assistance -- not least in persuading the Austrian Customs' Officials to let us into the country, when the papers provided by the Vice-Consul in North Walsham proved to have one eagle-stamp too few.

SEB CONFERENCE

It had been intended to advertise the SEB's Symposium on the Molecular Biology of Plant Development, to be held in Glasgow from Tuesday, 28th to Friday, 31st August 1990, which includes several *Arabidopsis* talks. A copy of the Registration form does come with this newsletter, but owing to the delay in its appearance (see remarks on the front page), the deadline for early registration has already passed. Apologies to Gareth Jenkins.

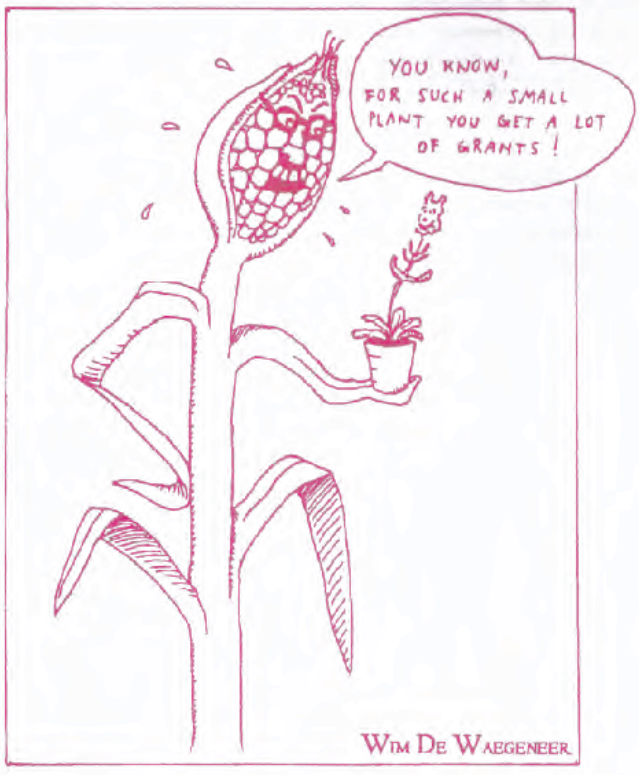
SPECIAL GUEST-STAR REPORT

It is a particular pleasure for the ACM to include in this edition of the Newsletter a progess report from a lab. not only outside the AFRC programme, but from outside the country. Furthermore, the report was recieved on time and *via* e-mail. If only....

From Roger Innes..

Roger Innes, Andrew Bent, and Brian Staskawicz, Department of Plant Pathology, University of California, Berkeley, CA 94720

Greetings from the U.S.! Brian brought us a copy of ARABIDIAN NOTES after his last visit to The John Innes labs (no relation). It so impressed us we decided to subscribe and make a contribution. We are using *Arabidopsis* as a model host to identify plant genes required for disease resistance. Resistance of a given crop cultivar to a specific pathogen is a heritable trait. In most cases, inheritance of resistance is controlled by a single plant



"Resistance gene" (R). Plant 'R' genes are required for recognition of specific components of pathogens. Production of these pathogen components is controlled by "avirulence genes" (A) of the pathogen. No R gene has been cloned from any plant, thus far, and it is not understood how the "recognition" event leads to disease resistance. Our project is similar in strategy to the project Mike Daniels described in the last issue of *Arabidian Notes*. The primary difference is that we are using the bacterial plant pathogen *Pseudomonas syringae* pv. tomato rather than *Xanthomonas campestris* pv. *campestris*. We have identified strains of P.s.t. that are virulent on ecotype Columbia (Col-0) and strains that are avirulent on Col-0. We now have four different cosmid clones (from two different avirulent P.s.t. strains) that convert virulent strains to avirulence on Col-0. We subcloned the "avirulence activity" from one cosmid and have identified a 1.4 kb fragment carrying an avirulence gene which we have designated avrRpt2. We have screened over 30 ecotypes of *Arabidopsis* for susceptibility to P.s.t. strains carrying the avrRpt2 clone. Ecotypes Po-1 and Hs-0 proved to be susceptible. These ecotypes were crossed to the resistant ecotype Col-0 and F_2 plants analyzed for segregation of resistance corresponding to avrRpt2. We have scored the F_2 from the Hs-0/Col-0 cross and find that resistance is dominant and is segregating as a single gene. The Po-1/Col-0 F_2's will be scored by early July. We are now gearing up to clone the disease resistance gene corresponding to avrRpt2 (Rpt2) in Col-0. Roger Innes has been developing a two element transposon tagging system based on *Ac* and *Ds*. So far we have observed a high rate of somatic excision (based on green sectors on leaves using a kan:*Ds* cassette) but no authentic germinal excision events. We are trying hooking up other promoters to the *Ac* transposase that specifically express in the flower meristem. We will also start the standard walking approach and are giving a shot at deletion mutagenesis followed by subtraction cloning.

LIAM1@VIOLET.BERKELEY.EDU

Also Starring...

Many thanks to Sue Albini for not only providing her project report, on time and by e-mail, but for also suppplying the following at the same time:

From Sue Albini...

The FEBS Winter School on Meiosis
Bundessportschule, Obertraun, Austria

In April this year I attended the FEBS lecture course on meiosis. We were promised an overview of recent knowledge of the biochemistry, genetics and ultrastructure of all aspects of meiosis, which was to lead to a synthetic concept of the meiotic process. The course was structured so that there were lectures in the morning and evening and sporting activities in the afternoons (more of this later). In addition to lectures, there were also general discussion sessions to which participants contributed short talks with slides. There were 18 lectures covering most of what is happening in the world in meiosis research. The yeast geneticists were there in force and they described and discussed the genes expressed during the initiation of meiosis, meiotic synapsis, recombination, segregation and late sporulation. Higher order chromosome structure, i.e., coiling and packing of the DNA and the structure of centromeres, were described in relation to function during cell division. Ultrastructural studies of the synaptonemal complex (SC) during prophase I of meiosis were discussed in a wide range of organisms from yeast and other fungi to higher plants, insects and mammmals. We heard how the techniques of molecular biology, immunology and biochemistry are being applied to the investigation of prophase I of meiosis. The components and sub-structure of the SC are being identified using antibody labelling and the interaction of DNA with the SC is being examined by *in situ* hybridisation of DNA probes to SC preparations. Associating gene conversion events with the early recombination nodules (ERNs) present in early prophase was presented as a model for the mechanism of homologous chromosome pairing. The link between reciprocal meiotic recombination and late RNs present in, e.g., pachytene nuclei, was reaffirmed. The potential role of the SC as a regulator of crossing over was also discussed. The more general subject of models of reciprocal meiotic recombination was addressed and the problems involved in relating these to homologous chromosome pairing and SC formation were also debated. Summaries of the topics and themes discussed will be published in FEBS letters.

The meeting was held in the Alps in Upper Austria and its main purpose was to enable post-grads and post-docs to grasp the basics of research into meiosis across a very wide range of organisms and methods of investigation. The relaxed and friendly atmosphere encouraged the exchange of ideas and even the most basic questions could be asked without embarrassment. As a scientific meeting it was a great success. Also successful were the sporting activities, the most glamorous of which was skiing. It was a great chance, for those of us who had never skiied before to have a go. There was free instruction for everyone, even absolute beginners, so with some trepidation I joined them. Skiing backwards and falling over were the skills I acquired on the first day, progressing to skiing backwards head first by day three. The flapping arms of the ski instructor three feet in front of me, as he shouted "SCNOPLOUGH SCHOO!" (snowplough Sue) are somthing I will always remember as is the black eye I suffered when hit by a madman's wayward ski pole. Despite my lack of aptitude for skis and slopes I did manage to ski down quite a big hill,without falling over, after only three afternoons. Participating in the sporting activities during the meeting engendered a sense of equality; the ski slope is a great leveller (in every sense of the word). Landing in an hysterical heap of limbs, skis and ski poles at the bottom of a hill with an eminent professor, is certainly an icebreaker.

My thanks to the AFRC PMB travel fund for the contribution which enabled my attendance at this meeting.

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.

Significant progress has been made since the last report in March. Electron microscope *preparations of* Arabidopsis thaliana SCs have been produced. So far, the yield is low but the preservation and identification of chromosome markers such as centromeres and nucleolus organiser regions means that a pachytene SC karyotype, which is analagous to a mitotic metaphase karyotype, will soon be produced. Buds in prophase I of meiosis are fairly difficult to find, and having to work with the small amount of material that can be found is a great hindrance. So far, it has been determined that the best plants to work with are the late flowering types with large inflorescences, as found growing around our greenhouses. The use of floral mutants such as the FLO10/AP2 double mutant (aka stud or big boy) produced by George Haughn, will be investigated. This mutant produces many anthers, but of course they may not all be fertile or develop synchronously. The prospect of using SC preparations as targets for *in situ* hybridisation of DNA probes is very bright. This is the next area the research will move o to. First the technique will be developed in another system more amenable to the study of prophase I of meiosis, and then transferred to *Arabidopsis*.

ALBINISM@UK.AC.BIRMINGHAM.COMPUTER-CENTRE.IBM3090

(See also Sue's report on the FEBS Winter School on Meiosis – ACM .)

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 of Science, Technology and Medicine, London SW7 2BB.

We have constructed a vector which allows origins of plant DNA replication to be cloned. First, we produced a hygromycin cassette consisting of (5'→3'): (1) the C-terminal two-thirds of the *E. coli* hygromycin phosphotransferase (HPT) gene; (2) a transcriptional termination sequence; (3) a region containing unique *Bam*HI, *Sma*I, *Hind*III, and *Kpn*I sites into which an origin of DNA replication can be cloned; (4) a CaMV promoter; (5) the N-terminal two-thirds of the HPT gene. This cassette was then inserted into the plant transformation vector Pbin19 and can thus be recovered *via* unique *Sac*I and *Xho*I sites. It is anticipated that when integrated into plant chromosomal DNA, intramolecular recombination will occur to generate a circular, replicating plasmid containing a functional hygromycin phosphotransferase gene.

We are at present testing our ORI vector with a known origin of DNA replication. Tomato golden mosaic virus DNA component A, from which the coat protein gene has been deleted, has been cloned into the *Hind*III site of the vector and tobacco leaf discs have been agroinoculated. We will know if the vector is functional in a few week's time.

From Jeremy Carmichael...

Molecular identification and analysis of genes involved in plant development and growth control.

Jeremy Carmichael and Jim Murray; Institute of Biotechnology, University of Cambridge.

The aim of the project is to isolate genes of *Arabidopsis* with functions relating to cell division and growth control. Our hope is to use complementation of yeast mutants to identify plant genes with conserved function. To this end I am hoping to make a cDNA library from cells grown in suspension cultures as an alternative to using buds or seeds which other groups are attempting. Suspension cultures have been established directly from leaf slices and indirectly using dispersed callus derived from leaf explants. I am trying to optimise the conditions for growth and subculture to obtain cultures with a high mitotic index before isolating RNA. I hope to construct the library in a vector or vectors suitable for expression in both budding and fission yeast.

Redundant PCR is an alternative approach to complementation. I have made some initial attempts at PCR using primers directed against the conserved cdc2 gene. Work continues.

JAHM@UK.AC.CAMBRIDGE.PHOENIX

From David Coates...

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

Drs D. Evans, D. Coates, B.S. Cox, with Drs J. Boyce, J. Coates and P. Askerlund; Plant Sciences Oxford and Pure and Applied Biology, Leeds.

The last three-month period has seen a lot of developments as the project has got fully underway. We have been joined by Dr Janice Coates (Leeds) as a part-time post-doc to work on *Arabidopsis*, and Dr Per Askelund (Oxford) to work on the biochemistry/reconstitution of the Ca pump in maize. Per comes from Lund and has considerable experience as a membrane biochemist. He is initially joining us as a Royal Society European Exchange Fellow. Janice has post-doctoral experience in protein biochemistry and seed-storage protein engineering.

Joy Boyce has identified a number of antibody-positive clones from a λgt11 Zea cDNA library. While the antibody is known to be specific to the plasma membrane calcium pump in plants, we still have some doubts as to whether these clones are it. Sequencing is in progress and we have found that most of our clones are identical; however, careful comparisons of cDNA homology between P-type ATPases suggests that homology at this level only occurs in small regions (hinge, ATP binding site, calmodulin-like region). We have also initiated alternative screening (oligos to known regions of peptide homology), as well as the use of PCR with oligos directed at these regions, and at redundant consensus regions derived from a sequence analysis of known, sequenced calcium ATPases.

Work on membrane biochemistry has involved investigation of the phospholipid of maize plasma membranes and of the lipid annulus of

the calcium pump (jointly with Dr David Cooke, Long Ashton). We have also undertaken a pilot study of the effect of the calcium-dependent neutral protease calpain on the system and Miss F.L. Theodoulou (a graduate student in the lab.) is continuing to prepare monoclonal antibodies which will be available for work on structue/function relationships. Dr Askerlund (who arrived with us last week) will shortly begin purifying plasma membrane samples before commencing work on reconstitution.
We are grateful to AFRC for supporting the link between Oxford and Leeds by funding Joy Boyce in a visit to Leeds to learn techniques and for providing the equipment needed for the work. One of us (DEE) is an invited speaker at the FESPP meeting at Umea in August.
GENOMIC LIBRARIES FROM ARABIDOPSIS. The shortage of a locally available *Arabidopsis* genomic library has prompted one of us (DC) to start constructing one, in collaboration with Dr Sarah Gurr (Biochemistry, Leeds). More details later to anyone interested, but it should be ready by late August.
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).

Isolation of the flowering-time gene fg

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

Despite several requests, nothing was received from George Coupland – ACM.

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. The second three months of the project has seen a consolidation of the effort to attain uniformity in inoculation conditions in which some problems have been encountered. We are trying to define the optimum conditions of inoculum content and concentration and abrasive application together with plant age and other factors to get reproducible infection characteristics. In addition to this, we have been preparing for later stages of the project by working up protoplast and transformation methodology. We will shortly be testing the interaction of different *Arabidopsis* ecotypes with variant strains of CaMV.
COVEY@UK.AC.AFRC.JII

From Caroline Dean...

(With typical delegatory talents, Caroline has let those concerned in her lab. write their own reports – ACM.)

John Chandler is investigating aspects of the physiology and vernalisation of late flowering mutants of *Arabidopsis thaliana*.... Physiological experiments have started, using Hussein's and Van der Veen's late flowering mutants, and concentrating particularly on the fca locus, in an attempt to determine the processes involved in late flowering. To do this, plants are being treated with various compounds known to be involved in floral induction and evocation, in whole plant tissue culture, and the vernalisation response of some of the mutants will be tested in a range of growth room conditions.
This work will also be complemented by producing double mutants containing a late flowering locus and one from a lipid, starch or ethene mutant. Plants are also being screened following EMS mutagenesis of the fca mutant, to find individuals no longer responsive to vernalisation.
Jonathon Clarke is mapping two major genes involved in the vernalisation requirement of the winter annual, Stockholm to Meyerowitz's RFLP map and Hussein's and Van der Veen's late flowering loci. He is also developing an *in vitro* culture system to facilitate quantitative genetic analysis of late flowering and the vernalisation requirement of Stockholm.
Tansposon tagging by Emily Lawson.... At the moment, I am concentrating on obtaining single copies of all my original insertion loci, by a programme of back-crossing wild-type Landsberg *erecta* to the transformants. This will allow me to examine the genetics of the autonomous *Ac* element in *Arabidopsis*, and also to examine the differences in excision rates between the wild type and the *Nae*I deleted elements. This deletion removes 537 base pairs of the untranslated leader of the transposase. Transformants carrying constructs where the deleted element is inverted into the streptomycin-resistance gene have given significantly higher levels of excision than those carrying the wild-type element. I am interested in investigating this phenomenon.
Clare Lister.... We are generating approximately 300 recombinant inbred (RI) lines from a cross between Landsberg *erecta* and Columbia. We intend to generate a large quantity of F_9 seed. The F_5 seed is presently being harvested. The F_9 seed will (hopefully!) be available early next year.
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 has commenced work on the cell cycle control genes in higher plants.

(What the ACM really dislikes about his job is having to edit down verbose reports.)

From Gary Foster...

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

Dr. Gary Foster, Dr. Rod Scott, Dr. John Draper, Mike Roberts and Rob Blundell; Botany Dept., University of Leicester, LE1 7RH. Tel: 0533-523393; FAX 0533-471001.

A number of pollen specific cDNAs from *Brassica napus* have now been sequenced and the homologous *Arabidopsis* genes pulled and characterised. Sequence analysis has revealed a family of related pollen-specific genes exhibiting a common repeated amino acid motif at the C-terminus. The promoter region from an *Arabidopsis* genomic clone, corresponding to a pollen-specific Brassica cDNA has been sequenced and is currently being analysed *via* reporter genes in transgenics.

This promoter, along with other known pollen-specific promoters, and CaMV, is being used to drive antisense transcripts antisense transcripts of all isolated pollen-specific cDNAs in in *Arabidopsis, B.napus,* and tobacco further to analyse pollen development. These promoters are currently being used to construct a pollen specific "helper transposase" which will, if according to plan, function in *Arabidopsis* prior to mitosis and possibly at an earlier stage of gametogenesis, and allow the generation of "germline"-only transposon-induced mutations that can be directly screened for in the F1 generation.

Finally, work is well underway to engineer YAC vector sequences into *Ds* elements with the intention of rescuing large portions of DNA flanking an insert.

The authors are also interested to hear from anyone who has knowledge of either proline and analine, or histidine rich, animal or plant proteins. Responses will be repaid *via* alcoholic beverages at the next conference.

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.

Karen Sweet and I have been screening M2 families from EMS-treated seeds for seedling lethal mutants that impair or alter the development of the shoot apex. The mutations are recovered from heterozygous sibs of the affected individuals. So far, we have screened approximately 400 families and have several promising mutants. The phenotypes of homozygotes of these lines range from essentially no apical development to massive over-proliferation of the apex. One of the latter class can be maintained as a hormone autonomous teratoma *in vitro*. Several pigment mutants have been recovered in the screen and these are being "bulked up" for use in the fate mapping project. (We intend to irradiate heterozygous plants and look for sectors.)

Paul Davison has been isolating RNA from meristems generated *in vitro* and from the apical teratoma line with the aim of producing an apex cDNA library. Ottoline Leyser and Nigel Kilby both presented abstracts and posters on their recent results at the Vienna Conference. Finally, Justin Ainscough is starting to introduce his attenuated 35s B-glucuronidase constructs into *Arabidopsis*.

From John Gray...

Trichome differentiation in *Arabidopsis*.

John Gray; Botany School, University of Cambridge.

Mandy Walker arrived in May and has initiated several lines of research on leaf cell differentiation. She is attempting to isolate the ttg locus (on chromosome 5) which results in glabrous leaves and seeds with a transparent testa. An interesting mutant! She is also screening Ian Furner's M2 families for mutants with altered stomatal density.

Ros Slatter, an SERC-supported postdoc, is planning to look at chromosomal scaffold attachment sites around selected genes in *Arabidopsis*, after developing methods with pea nuclear genes. However, the isolation of large numbers of intact nuclei from *Arabidopsis* is something of a problem. Does anyone have a method (and protocol) for the large-scale isolation of nuclei?

JCG2@UK.AC.CAMBRIDGE.PHOENIX

From Nic Harberd...

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

Mary Holdsworth and Nicholas Harberd; IPSR, Cambridge.

Further to our previous report, we understand that the *gal* locus has (probably) been isolated, by Tai-Ping Sun in Fred Ausubel's lab., using a subtractive hybridisation procedure similar to, although potentially of greater sensitivity than, the PERT method we have employed. The new method has great potential for the isolation of any gene for which a deletion mutant allele is available (see Stauss and Ausubel (1990) *Proc. Natl. Acad. Sci. USA* 87, 1889-1893). *(The Straus protocol comes with this newsletter. Things are not all gloom in Nic's lab. though. See the article on cDNA Libraries – ACM.)*

From Nick Harris...

Development of the silique of *Arabidopsis*.

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

Mrs. Jacqui Spence started as a research technician from 1.03.90.

The major morphological features of silique development in both the wild type and the meristic clv mutant have been described from sections of tissue taken at progressive stages of maturity. The clv mutant has a shorter, "club-like" fruit with an increase in carpel number from 2 to 3 or 4. As well as these differences there are also differences in carpel form, vasculature and fusion. Initial work has begun on characterisation of histo- and cytochemical changes associated with carpel differentiation and development. This has involved a range of enzyme amd immuno-cytochemical, and *in situ* hybridization techniques.

We have begun a screen of EMS treated seeds for silique mutants; we would be very pleased to hear from

anyone who spots and doesn't throw away any "odd" forms, in particular a non-dehiscent mutant.

From Pat Heslop-Harrison...

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

J.S. Heslop-Harrison and J. Maluszynska, Karyobiology Group, J.I. Centre for Plant Science Research.

Jola Maluszynska (from the Silesian University, Katowice, Poland) will join the project in July 1990 to work on the physical genome organization of *Arabidopsis*. Jola has already spent five weeks with us in Cambridge and has continued the research in Vienna in the meantime.

She has improved the method for making chromosome preparation of *Arabidopsis* root tips. Pretreatment with 0.02M 8-hydroxychinolin, fixation in methanol/acetic acid and enzymatic digestion before spreading gives several well-spread metaphases. The small chromosomes of *Arabidopsis* now look like real chromosomes with two distinct arms, centromeres and nucleolar organizer regions, and the five pairs can be easily identified. The preparations can be used for Giemsa C-banding and fluorescent staining, and will be essential for physical mapping of the genome by *in situ* hybridization.

From Eric Holub...

Identification and mapping of genes for resistance to fungal pathogens of *Arabidopsis*

Eric Holub and Ian Crute; IHR, East Malling, Maidstone, Kent ME19 6BJ.

Eric started work officially on 28 March, but since February he has been searching field populations of *Arabidopsis* for natural infestation with *Albugo candida* and/or *Peronospora parasitica*. Both pathogens have been found in several locations and are apparently endemic at East Malling. The spring season for collection has now ended, but we have established field sites for future observation of disease in Kent. *A. candida* has proved the easier pathogen to maintain and handle under growth chamber conditions, so we have begun to use a promisingly simple seedling assay to screen germplasm of the host for differential reaction to *A. candida*. So far, all genotypes tested have been susceptible. *P. parasitica* has thus far been more difficult to manage, but we have successfully inoculated seedlings of the host with this pathogen. Eric has also located a pycnidial fungus on *Arabidopsis* which he has been successful in isolating *in vitro*, reinoculating to plants and reisolating. The identity of the fungus (probably *Phoma*) and whether it is truly parasitic have yet to be established. Another major activity has been the collection of seed from local populations of *Arabidopsis*. This material will be used to characterize the genetic diversity of the species in Kent. We are also interested in screening germplasm from other sources, and would be delighted to receive seed collections of any geographic source for testing of response to these fungi.

HOLUBE@UK.AC.AFRC.EMRSA

From Gareth Jenkins...

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

Gareth I. Jenkins; Departments of Biochemistry and Botany, University of Glasgow.

The project is progressing steadily on several fronts. Our main objective is to produce transgenic *Arabidopsis* expressing various chimaeric constructs which we can subsequently use to screen for photoregulatory mutants. We achieved transformation early on and are now trying to glue together the various constructs that we need. At the same time we have been studying the *hy-4* mutant, which was reported to be deficient in the blue light control of hypocotyl extension. We have confirmed this finding and have been investigating other blue light mediated responses to see if they are also altered. It is rather too early to draw any firm conclusions, but the phenotype of this mutant seems to be quite complex. It is unlikely simply to be deficient in the blue light photoreceptor. Finally, we have been growing *Arabidopsis* in various different light environments in preparation for a screen for new mutants and we will shortly embark on the screening exercise.

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.

A fragment of the *hem*C gene, which encodes the porphobilinogen deaminase enzyme, has been amplified and isolated using the polymerase chain reaction from an *Arabidopsis* genomic library in λfix. This fragment, about 800bp in length, is currently being sequenced prior to using it as a probe for the library.

Similarly, PCR is being used to amplify the *hem*E gene, which encodes the uroporphyrinogen decarboxylase enzyme. The PCR products are in the process of being sequenced. Work on the *hem*B gene, which encodes 5-aminolaevulinic acid dehydratase is also underway.

A protocol has been devised to purify the 5-aminolaevulinic acid dehydratase and porphobilinogen deaminase enzymes from the *Arabidopsis* plant material. A batch of plants is being grown up for large scale isolation and it is hoped that sequence information about these two enzymes will be available shortly to complement the DNA data.

Both post-doctoral appointments have now been made.

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.

We have now accumulated a great deal of information about the growth of the first leaf of the Landsberg *erecta* wild type and the changes in chloroplast number with cell development (see Abstract p.124, Vienna meeting). The total cell

population in the developing leaf increases from c.12,000 seven days after sowing to c.130,000 after 22 days, when the leaf is mature. Within this population the proportion of mesophyll cells declines and the proportion of vascular cells increases such that in mature leaves the total cell population consists of 36% mesophyll, 36% epidermal and 28% vascular. We have described comprehensively the relationship between cell size and chloroplast number during development of these leaves and shown there to be a highly significant correlation ($n = 266$, $r^2 = 0.87$) between the two parameters throughout development. We now aim to look for mutants which deviate significantly from this relationship. Analysis of some available mutants, hy-1, hy-6, ch-1, ch-5, and lu, showed only hy-6 to have significantly fewer chloroplasts per unit cell plan area compared to wild type and we intend to look further at the development of hy-6. We have just sown our first trays of M2 seeds (from Lehle Seeds, Arizona) and are about to start screening.

From Keith Lindsey...

Insertional mutagenesis in *Arabidopsis thaliana*.

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

We're still bulking up *Arabidopsis* C24 transformants for analysis of GUS activity generated by native promoter-GUS fusions. Our tobacco work gives us much confidence: we've carried out a preliminary screen of 215 transformants and we've found GUS activity in 55-60%. The *Arabidopsis* work is being directed towards screening for GUS fusion gene activity in anthers, to identify tagged genes involved in microspore development. We have recently also received funding from the EEC BRIDGE initiative to expand the work to investigate genetic control of zygotic embryogenesis in *Arabidopsis*. We were also awarded an SERC CASE studentship to identify cell division genes (cdc homologues) in this species, with a view to studying expression in early stages of embryo development.

MEA@UK.AC.LEICESTER

From Andy Maule...

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

A.J. Maule, C.L. Harker and G. Boudazin; John Innes Institute, Norwich

Contrary to the view of one or two virologists at Vienna (there were only four), *Arabidopsis* definitely is a host for cauliflower mosaic virus, giving symptoms very similar to those in turnip. Virus-coded proteins can be detected, and at the ultrastructural level the same cytopathic changes take place. Most important for our work on factors controlling virus spread. The gross changes in plasmodesmal structure seen in turnip are also evident in *Arabidopsis*. We hope that in the near future we will be able to test plants transformed with the viral spread gene, for similar changes in plasmodesmata. From three transformed rape plants we have not been able to detect any protein expression even though the same binary constructs do give protein expression in tobacco. The constructions utilised the 35S promotor; I have been told told that others have found that this does not work well in rape? The problems associated with cloning multimeric CaMV constructs for "agro-infection" did not go away. This approach has now been shelved while we test direct CaMV DNA inoculation for the analysis of spread mutants.

From Mike McPherson...

Ammonium toxicity in *Arabidopsis*.

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

Most of our current efforts are directed towards the cloning of an *Arabidopsis* glutamate dehydrogenase (GDH) gene(s). In the last report we described the design of primers for PCR experiments. These primers are based on alignments of all known GDH sequences and are highly redundant. Previous work in my lab had shown that primers of greater than 8092-fold potential redundancy work efficiently. We have pushed these limits higher in this *Arabidopsis* project and are using primers of potential redundancies ranging from 2,048 to ...73,728! And they work.

Currently we have tested our "universal GDH primers" with a range of genomic DNA samples from bacteria, fungi and plants. The latter include potato, tomato, soy bean, wheat and *Arabidopsis*. By employing a nested primer approach to PCR we now have a number of uniquely amplified products (including one from *Arabidopsis*). Southern blotting and direct sequencing of the PCR products are now underway to confirm the identity of these products. Assuming everything checks out we will use the *Arabidopsis* GDH fragment as a hybridization probe to screen libraries.

We are growing plenty of *Arabidopsis* with the intention of constructing a root cDNA library if this proves necessary but we would like to screen any other cDNA libraries that might be available first. Can anyone help please ?

GEN6MJM@UK.AC.LEEDS.BIOVAX

From Keith Mitchelson...

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

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

The project is developing in two directions since it began in March. Firstly, the library of YAC clones (C. Somerville) is being prepared for identification of VNTR-containing sequences. Briefly, a multiplexing procedure is being used in the first round of screening to minimise the work-load. Pooled rows (12) and columns (8) of clones are prepared with equal representation of all member clones. Cells are immobilised in agarose and the YACs are fractionated from the host yeast-chromosomes by pulsed field electro-

-phoresis. DNA is transferred to nylon membranes prior to screening by Southern hybridisation. A YAC clone carrying a VNTR element will most likely be found at the clone common to both a positive row and a positive column. Individual YAC clones thought to possess the VNTR element will then be screened by the above procedure.
Secondly, DNA is being prepared from a variety of *Arabidopsis* ecotypes to allow identification of appropriate VNTR probes which display informative RFLP patterns. Work on both lines is continuing....

From Bernard Mulligan...

Genetic male sterility in *Arabidopsis*.

Zoe Wilson, Janet Fuller, Jane Russell & Bernard Mulligan; Nottingham University. MAC and MODEM available. Tel. 0602-484848 (ext 3467).

In the amazingly short time since our last report, we have continued with the allelism tests on our collection of male steriles. We have identified at least 3 separate *ms* loci, and have allelic sets of each mutant. Mapping of the *ms* genes is in progress. As mentioned in the first report, the male sterile mutants show a range of disturbances in pollen development: (i) empty anthers, (ii) obviously aberrent pollen, (iii) apparently normal pollen (based on malachite green/acid fuchsin staining). Of the latter group, some have pollen which takes up fluorescein diacetate and hydrolyses to fluoroscein, while in pollen of other mutants, no FDA conversion occurs. We are also setting up *in vitro* pollen germination assays. The method described by Monika Pickert (AIS 26, 39-42 (1988)) works very well.

From Denis Murphy...

Study of cytochrome b_5 as a component of fatty acid desaturation in *Arabidopsis*.

Steve Slocombe & Denis Murphy; John Innes Centre for Plant Science Research, Colney Lane, Norwich.

This project has concentrated on oleate desaturation during embryogenesis, using *Arabidopsis* as a convenient genetic model. Cytochrome b_5 is believed to operate in this system in plants and work is currently directed towards characterising the gene from *Arabidopsis*. The availability of a number of amino acid and DNA sequences of the mammalian cytochrome has enabled a PCR-based approach to be taken for cloning and analysing the gene. In addition, cDNAs of mammalian cytochrome b_5 kindly provided by Alan Steggles (of Northeastern Ohio Universities, College of Medicine), are being used as probes to screen *Arabidopsis* libraries and to monitor expression during embryogenesis in *Brassica napus*. It is thus hoped to differentiate between the role of cytochrome b_5 in the synthesis of membrane lipids and in the accumulation of oil in developing seeds. The possibility that there may be different sets of cytochrome b_5 (and other lipid-related) genes involved in storage lipid *versus* membrane lipid synthesis will be investigated

From Steven Neill...

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

Steven Neill; Bristol Polytechnic.

Ms Jackie Williams took up the position of research technician in January 1990 so the project is now six months old. So far we have determined the kinetics of stress-induced ABA accumulation, isolated poly(A) mRNA and begun to analyse the effects of stress by *in vitro* translation. We are now at the stage of attempting to construct a cDNA library from this mRNA using a λZap cloning system.

From Helen North...

Cell cycle control genes in *Arabidopsis*.

Jeremy Hyams and Helen North; Dept. of Biology, UCL.

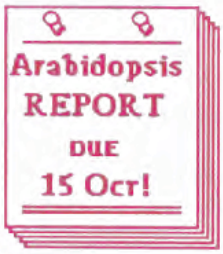
The project commenced April 1990. We are hoping to utilise *S. pombe* cell cycle mutants to identify functional higher plant cell cycle genes by complementation. After testing methods of transformation with *S.pombe* genomic libraries, the construction of a higher plant cDNA library and its subsequent transformation is planned.

From Jane Parker...

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

Jane Parker, Christine Barber and Michael Daniels; The Sainsbury Laboratory, John Innes Institute, Norwich.

Chris Barber and I have spent some time standardising the conditions under which our *Arabidopsis* plants are grown and inoculated to give reproducible responses to the various strains of *Xanthomonas campestris* pv. *campestris* (*X.c.c.*). We think we have got it right and we are now following up some interesting plant-pathogen combinations described by Mike Daniels in the last newsletter. We are concentrating mainly on the differential responses of *Arabidopsis thaliana* lines Columbia and Landsberg *erecta* to *X.c.c.* strains 8004 and 1067. Strain 8004 causes disease symptoms in both lines whereas 1067 infects only Landsberg *erecta*. Analysis of the segregation of resistance and susceptibility in the F_1 and F_2 plants of a cross between these two lines suggests the presence of a single dominant gene in Columbia governing resistance to 1067. We are now growing up the F_3 progeny of each individual F_2 plant to confirm the segregation and provide DNA for RFLP linkage analysis. We also want to undertake a large scale screening of EMS-treated Columbia plants for alterations in their response to the two bacterial strains.
In addition, we are starting to look for changes in expression of defence-related mRNAs and their protein products in Columbia plants inoculated with 8004 and 1067. We hope to characterize these responses in relation to resistance and susceptibility.



From Peter Quinn...

Thermal tolerance of fatty acid desaturase mutants of *Arabidopsis*

Peter Quinn; Biomedical Sciences, KQC.

After unavoidable delays, Mr Shrinivas Volety commenced tenure of the project as a graduate assistant on 21 May, 1990. He has initiated a review of data files generated in time-resolved X-ray diffraction studies of the phase behaviour of galactolipids prepared by semi-synthetic methods from chloroplasts. He will attend the 9th International Symposium on Plant Lipids during 8-13 July where collaborators on the project, Drs J. Browse and C. Somerville, will be presenting papers. This will provide an opportunity to discuss exchange of personnel between our laboratories.

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.

Not much news this time. Analysis of genomic clones is ongoing and sequencing of one is in progress. We have started to prepare a cDNA library but have had problems with the Pharmacia kit. Pharmacia have sent a new kit but this also gives us an artifact due to contamination. We battle on!

"[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 mutants which are defective in chloroplast protein transport functions. We have prepared M2 seed stocks from EMS-mutated *Arabidopsis* seeds and are currently using a Western-blotting approach to screen batches of M2 seedlings for the presence of accumulated precusor forms of chlorplast proteins.

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 two research workers on this project joined the lab in May. They are Dr. Huguette Albrecht from Strasbourg, who spent a year in John Bol's lab in Leiden, and Michael Witty from Christchurch, New Zealand. Having sorted out work permits, somewhere to live and getting over jet-lag, they have now embarked on characterising a clone from a λEMBL3 library of *Arabidopsis* DNA, which was isolated using a cDNA for HMBS from *Euglena*. In addition, oligonucleotide primers have been constructed from conserved regions of the published HMBS sequences (from *Euglena*, *E. coli* and human) and have been used to generate PCR fragments for use as probes to screen the library if the clone we have is not suitable for the promoter analysis we want to do.

TAS25@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.

After reminders, a FAXed copy of their Vienna abstract eventually arrived, perhaps appropriately, on Friday, 13th July. The abstract is on page 52 of the Vienna abstracts book, as the ACM did not have the time at such a late stage to re-type it (had it been sent by e-mail, however...)

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

How To Reach Us

Th'ale & Cress: the third AFRC PMB Arabidopsis Newsletter, July 1990.

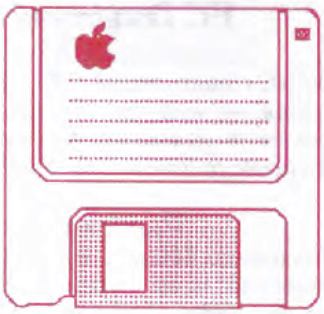
Assistant Circulation Manager, David Flanders, The Cambridge Laboratory, The John Innes Centre for Plant Science Research, Colney Lane, Norwich, NR4 7UJ, U.K.

Tel: 0603-52571

FAX: 0603-56844 or 0603-502270.

E-mail ARABIDOPSIS@UK.AC.AFRC.JII

Please send all contributions, wherever possible by e-mail, or failing that, on disk. Mac disks are ideal, but we can cope with MS-DOS (IBM) -- preferably a 3½" (high density, 1.4MB) disk with the file in word processor format *and* as a text-only (ASCII) file. 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. Further details about communicating via computer are given in the previous newsletter. Communication by modem is also available for the *cognoscenti*.



Acknowledgements

Special thanks to:

- Barrie Allen for offset-litho printing of both the masthead and the graphics.
- Andy Davies for printing the coach photo.
- Wim De Waegeneer for his cartoon.
- Laura Donohue for proof-reading.
- Terry Donohue and Richard Mitchell (The Underground Grammarian) for supplying some of the graphics.
- Anne Edwards for her cartoon.
- Black Rot for the crossword.

ARABIDOPSIS cDNA LIBRARIES

MANY of you have been requesting information about who has *Arabidopsis* cDNA libraries. Until recently, the ACM has been pointing everyone in the direction of Christine Raines. So many of you have approached her, however, that she appears to have gone into hiding. In order to spread the load around somewhat, could anyone that has, or is expecting to have, a cDNA library that they are willing to make available, please let the ACM know so he can then inform interested parties of the choice available. In order to start this, I'm reliably informed that Peter Jordan, Denis Murphy, Alison Smith, and Felicity Watts mentioned having or getting libraries at the Christmas meeting. Items in this and the previous two newsletters obviously show that things have changed somewhat, but the following have also mentioned libraries in their project reports: David Coates, John Draper and Rod Scott, Ian Furner, and Steven Neill. In addition, the ACM has some more details from Mary Holdsworth (as she's just down the corridor and so easy to interrogate) about a λFixII genomic library that she is currently making in Nic Harberd's lab. The library has been named Laer/gai/MLH and is from the gai dominant gibberellic acid insensitive dwarf mutant isogenic line. Watch this space for further details !

DELETION MUTANTS

A method has recently been developed by Donald Straus in Fred Ausubel's lab., for isolating the DNA that is absent in deletion mutants [Straus and Ausubel (1990) *Proc. Natl. Acad. Sci. USA* 87, 1889-1893]. This technique has been termed, genomic subtraction and has obvious implications for all you *Arabidopsis* gene-cloners. A copy of Staus's protocol comes free with this newsletter.

EC DIRECTIVES ON RELEASE OF GENETICALLY MODIFIED ORGANISMS

AFRC's International Office has supplied programme co-ordinators with the new EC directives on the use and release of genetically modified organisms. So, if you're desperate to have a peep, Caroline has a copy.

ELECTRONIC ARABIDOPSIS

REPRODUCED below is the text of a flyer put out by Chris Somerville at the Vienna Meeting. Basically, he is setting up an electronic bulletin board for the international *Arabidopsis* community. It's both easy and free to subscribe to this and it is strongly recommended that you do so. This is for two reasons: (i) philanthropic, as the more users Chris gets to sign up, the more facilities the bulletin board is allowed and (ii) selfish, as you may well miss out on some vital piece of information if you don't (sending stuff can also be beneficial, particularly for all you students and post-docs trying to make a name for yourselves so you might actually stand a chance of getting a jay-oh-bee). Judging by the few of you out there who communicate with the ACM by e-mail (many thanks to those of you who do), I don't really hold out much hope for Chris being swamped with users from the U.K. However, don't expect, the newsletter to duplicate everything on the bulletin board. If you miss out, you only have yourselves to blame. If you're at an AFRC Institute with a VAX running VMS, all you have to do to subscribe is :

$ mail <CR>
mail **send** <CR>
to: **cbs%earn-relay::pleiades.cps.msu.edu::request-athal** <CR>
subj: **Subscription request** <CR>
Your e-mail address <CR>
CTRL/Z

(For those of you using other mainframes or different operating systems, it should be just as easy.)

From Chris Somerville...

In order to facilitate communication among scientists working with *Arabidopsis*, the international steering committee for the *Arabidopsis* genome project endorsed the implementation of a "computer database" which would be readily and inexpensively accessible to all *Arabidopsis* workers. As a first step, we have implemented an "electronic news group". At present, the system comprises two electronic mail addresses which are housed on a IBM mainframe on the Michigan State University campus. One of the numbers is a mailing list which contains the e-mail addresses of everyone who asks to be on the list. The other number is simply an electronic re-mailer which sends everything it receives to everyone on the mailing list. Thus, for instance, if you have a news item or if you want to know if anyone in the community has something you need, you send an e-mail message to the re-mailer and it is instantly delivered by e-mail to everyone on the subscription list. Such a system is currently in use by participants in the human genome project and, in addition to items concerning methods etc., generates some discussion about other issues of common interest. Aside from the costs of having an account on your local mainframe, the system is entirely free.

In order to get the system upgraded to the status of a "bulletin board" with menus and slightly more selective options, we need to get 100 subscribers to the news group. *(At the time of going to press, there are 85 subscribers – ACM.)* Therefore, we would like to encourage as many subscribers as possible. Since our goal is to get all the seed stocks, and information about YACs, RFLPs, clones and sequence information on line, the ability to correspond with a large number of potential users will be useful in developing a database structure that is widely applicable. The numbers are accessible through the internet system.

We have been told that this system should be accessible without charge from all mainframes. However, it may be necessary to have your system manager add our mainframe to his/her list of "host files". The relevant information in this respect is:
The host name: pleiades.cps.msu.edu
The IP# (Internet Port): 35.8.12.140
- In order to subscribe to the news group, send an e-mail message to: request-athl@pleiades.cps.msu.edu
- To send a news item, commentary, request or whatever, send an e-mail to: info-athal@pleiades.cps.msu.edu

If you have problems with the system, please let me know and I will have our technical people contact your technical people. My e-mail (bitnet) address is 21847crs@msu

FUNDING SOURCES

Acciones Integradas Hispano-Britanicas '91/92

THIS progamme, which is jointly run and financed by the British Council and the Spanish Ministry of Education and Science, funds "pump-priming" (whatever that may mean) research links between labs in the two countries. As such, it provides grants for travel and subsistence, but not for research costs. Over 120 links were supported in 1990/91 and so free trips to spanish labs, with possible side tours to the Costa del Sol, are up for grabs. Further information and application forms are available from:
Director of Science and Exchanges, (Acciones Integrades), The British Council, Almagro 5, 28010 Madrid, Spain. Tel: 010-34-1-319 1250. FAX: 010-34-1-308 6375. Telex: 42769 INSBR E
The closing date for applications is 9 November 1990.

STANZAS FOR SCIENTISTS

As no-one has sent in any suggestions for this, it will have to be another of the ACM's choices. This piece is by Robert Frost, one of America's greatest poets this century. (For those without ready access to an Audubon guide, an oven bird is a kind of thrush, which builds a nest resembling an oven.)

The Oven Bird

There is a singer everyone has heard,
Loud, a mid-summer and a mid-wood bird,
Who makes the solid tree trunks sound again.
He says that leaves are old and that for flowers
Mid-summer is to spring as one to ten.
He says the early petal-fall is past
When pear and cherry bloom went down in showers
On sunny days a moment overcast;
And comes that other fall we name the fall.
He says the highway dust is over all.
The bird would cease and be as other birds
But that he knows in singing not to sing.
The question that he frames in all but words
Is what to make of a diminished thing.

T-SHIRT COMPETITION

ENTRIES are still welcome for the design of the motif for the second AFRC PMB *Arabidopsis* conference T-shirts. As with the Crossword, apathy reigns supreme. Only one entry has been received so far, although others have been promised. As an incentive, remember that the winner will be presented at the conference with a free T-shirt and a bottle of bubbly. So get designing (a rough idea will do) and send in your artwork.

THIS ISSUE'S QUOTE

THIS issue's quotes were all uttered by a certain person during a talk at the Vienna meeeting. A prize to the first person who can guess who said: "I don't want to precis the whole document and everything that's in it, as you've got it....We've inserted 'by any means' -- and you might want to put that into your copy."

THE NEXT ISSUE

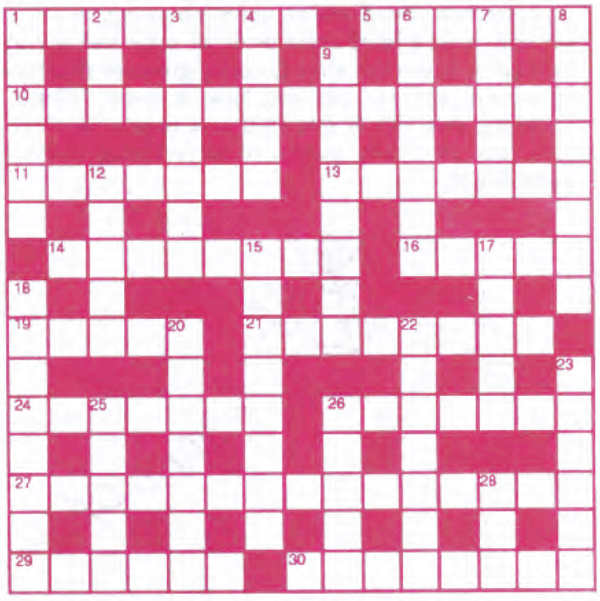
THE Fourth AFRC PMB *Arabidopsis* Newsletter should be appearing at the start of November. Highlights are scheduled to include: the latest RFLP map(s); a whole series of YAC-screening protocols from Renate Schmidt; your project summaries; and all the regular features. Contributions and ideas for items are welcome. In particular, it has been mooted that the newsletter run a Job Market Section, particularly for those situations where there is a few months' money left on a grant. There could well be someone out there with the right skills who is looking for six weeks' funding prior to taking up a post-doc in Antarctica. So, if you've got a job going, or are looking for one, send a brief description of what you are offering or what it is you are after (priority given to those arriving by e-mail) and we can see how it goes. Please note, however, that this is intended as a forum for those "odd" jobs, not as a free means of advertising conventional positions.

ARABIDOPSIS PRIZE CROSSWORD

THE response to the first crossword was underwhelming. Only one answer was received. Fortunately, it was virtually complete and so our congratulations go to Mr. Caxton of the Cambridge Laboratory, The John Innes Centre for Plant Science Research, who wins the £5 book token. Whether the lack of entrants is owing to the usual wall of apathy, or whether it reflects the long-held view in some circles that scientists are, in general, a bunch of literary Neanderthals, is difficult to determine at this stage. (It is perhaps worth noting that our winner is a photographer.) In order more thoroughly to examine this phenomenon of crossword blindness amongst workers on *Arabidopsis*, the ACM, at vast expense, has persuaded Black Rot to produce a somewhat easier, but still, hopefully, just as interesting a crossword for the current edition of the Newsletter. To make things even easier (for those who went on the recent junket to Vienna, at least); the majority of the answers have a decidedly Viennese flavour. Once again, there is a £5 book token for the first correct entry (assuming we get more than one) to be drawn out of the duty-free bag. So, have a go. Get your MaxPax, sit in the corner of the coffee room, pen in hand, and let your mind wander to Vienna as Radio One mistakenly plays *Strauss* in the background (that's one answer for a start).

WIENER SCHNITZEL

by BLACK ROT



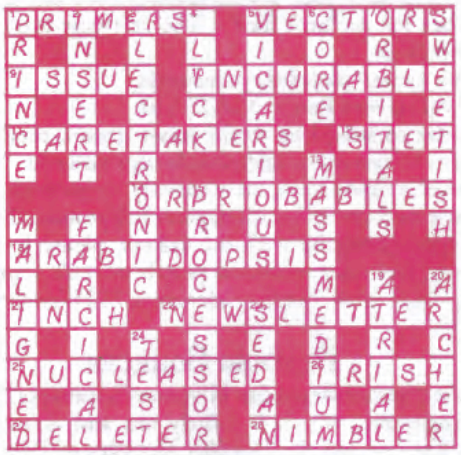
Clues Across

1. His eighth unfinished symphony ? (8)
5. Eine Kleine Komposer ? (6)
10. 29 youth singing group (6,4,5)
11. Looks heroic in frugal lantern light (7)
13. Made in USA, perhaps belonging to 5 (7)
14. Italian harp -- like broken chord (8)
16. Claud maybe of noble origin.... (5)
19. ...Or raised over East German home for 8 (5)
21. Assailant's confused on street (8)
24. One who fabricated tales from the Vienna Woods ? (7)
26. 29 dish provides lustred mixture (7)
27. 24's sad river dance (4,6,5)
29. Viennese of German origin (6)
30. Harry Lime in the field (5,3)

Clues Down

1. Uncivilised to put silver in broken vase (6)
2. Garden tool from Plymouth ? (3)
3. Ring time in first aid post not bar ? (7)
4. Automaton has personal problem in decay (5)
6. Home for Granny Smith ? (7)
7. Over and _____, in addition (5)
8. Old name for thrush (8)
9. Oscillators make me go starry (8)
12. Slowly does it for 1, 5, and 24 (5)
15. Giving a smooth appearance, like 23 (8)
17. Belief about long grass (5)
18. Tell's arm has angry bend (8)
20. Runt led astray in slow movement (7)
22. The incomplete oarsman could be 28 (7)
23. Glassy appearance of street directory in Scots valley (6)
25. Read, we hear, in French (5)
26. North Borneo (5)
28. Parisian's bad set up for run (3)

Just in case anyone is interested, here are the answers to the previous crossword:



DON'T FORGET!

Your next project summary is due by **15 October** 1990.

Rules of the *Arabidopsis* Prize Crossword:
This competition is open to all readers of this newsletter. In the unlikely event of a grant holder supplying the first correct entry, a project summary must have been received for them to be eligible for the grand prize. The answers as well as the winner's name will be in the next newsletter.