Greater London Plant Molecular Sciences Symposium – Agenda

20th September 2001, King's College London, Henriette Raphael Building, Guys Campus

9.00 am Opening Address – Paul Devlin

King's College London, Division of Life Sciences

9.10 am	Dr Shirley Coomber	*Overview and genes involved in root development in Arabidopsis.*
9.25 am	Dr Paul Devlin	*Genetic dissection of the Circadian Clock in* Arabidopsis

Royal Holloway, University of London, School of Biological Sciences

9.40 am	Prof. Peter Bramley	*Overview and Engineering of antioxidants in crops*
9.55 am	Dr Laurence Bindschedler	*The dynamic nature of the plant cell wall*
10.05 am	Dr Richard Anthony	*Growth signalling in Arabidopsis*
10.15 am	Dr Gary Warren	*Genes for low temperature tolerance in* Arabidopsis
10.25 am	Dr Enrique Lopez-Juez	*Tuning chloroplasts*

10.35 am **Coffee**

University College London, Department of Biology

10.55 am	Prof. Mike Evans	*Overview and Photosystem II and water oxidation*
11.07 am	Dr Saul Purton	*Molecular genetics of Chlamydomonas nuclear chloroplast interactions*
11.19 am	Dr Richard Strange	*Phytotoxins of Ascochyta rabiei and Fuarium oxysporum f. sp. ciceri affecting chickpea*
11.31 am	Dr Astrid Wingler	*Senescence and resource distribution*
11.43 am	Prof. Peter Rich	*Cytochrome bf complex and quinone binding and herbicides*

Imperial College London, Department of Biology and Wolfson Laboratories

11.55 am	Dr Pietro Spanu	*Fungi attacking plants*: *a quest to unravel biotrophy and pathogenicity*
12.10 pm	Prof. Jim Barber	*Rings and things*
12.25 pm	Mr John Tregoning	*Transgenic chloroplasts for the production of edible vaccines*
12.40 pm	Dr Richard Murphy	*Plant fibre materials: Ultrastructure, utilisation, durability and eco-profiles*

12.55 pm **Lunch**

Imperial College at Wye, Department of Biology

1.55 pm	Prof. John Mansfield	*Overview and Plant pathogen interactions.*
2.05 pm	Dr Murray Grant	*Plant disease resistance signalling*
2.15 pm	Dr Charles Ainsworth	*Sex determination in plants; Rumex as a model system*
2.25 pm	Dr Colin Turnbull	*Branching genes in plants*
2.35 pm	Dr John Rossiter	*Plant secondary metabolism; glucosinalates*
2.45 pm	Dr Alex Grabov	*Potassium transport and root development in Arabidopsis*
2.55 pm	Dr Glen Powell	*Aphids Phloem Interactions*

Royal Botanic Gardens, Kew, Jodrell Laboratory

3.05 pm	Prof Peter Crane (Director)	*Introduction and Overview*
3.20 pm	Dr Vincent Savolainen	*Phylogenetic data, molecular evolution and biodiversity*
3.35 pm	Dr Ilia Leitch	*Loss and recovery of Arabidopsis-type telomere sequences in the evolution of a major radiation of flowering plants*
3.50 pm	Prof Monique Simmonds	*Natural product research at the Royal Botanic Gardens, Kew*

4.05 pm **Tea**

Queen Mary, University of London School of Biological Sciences

4.25pm	Prof. Peter Heathcote	*Overview plus TBA*
4.35 pm	Prof. Conrad P. Lichtenstein	*Teaching mother nature to suck eggs: natural GM during plant evolution*
4.45 pm	Dr Andrew Leitch	*Sequence homogenization in allopolyploid plants*
4.55 pm	Dr Yoong Lim	*Molecular cytogenetic analysis in plants*

Horticultural Research International, East Malling

5.10 pm	Dr Yiguo Hong	*Overview and Plant virus-encoded pathogenicity determinant suppresses post-transcriptional gene silencing*
5.25 pm	Prof. David James	*Regulation and targeting of transgene expression in fruit crops*

5.40pm Closing address – Enrique Lopez-Juez followed by a **Wine reception**

Kings College London, Division of Life Sciences

Light input to the circadian clock in Arabidopsis

Paul Devlin
King's College, London, Division of Life Sciences

All organisms properly tested show a rhythm of metabolism, of physiological processes or even of behaviour in tune with the day night cycle of the earth. These phenomena are not purely responses to the external environment but are controlled by an endogenous oscillator known as the circadian clock. To be useful in allowing anticipation of dawn and dusk, an organism's circadian clock must be correctly entrained to the daily cycle of day and night by light signals at dawn and dusk. Both the red light-absorbing phytochrome and blue light-absorbing cryptochrome families of photoreceptors are involved. Plants show a plasticity in recruitment of different photoreceptors in different environmental conditions in gathering information about the light environment in order to set the clock. Analysis of double and triple mutants under a range of conditions demonstrates both additivity and redundancy between the various photoreceptors involved. Significantly, *Arabidopsis cry1 cry2* double mutants still show robust rhythmicity under all conditions indicating that cry does not form a part of the central circadian oscillator in plants as it does in mammals.

Royal Holloway, University of London, School of Biological Sciences

Overview

Peter M Bramley
Royal Holloway, University of London
School of Biological Sciences, Centre for Plant Molecular Sciences

The Centre comprises 10 members of academic staff. Research focuses on the biochemistry and molecular genetics of plant metabolism, differentiation and environmental responses in three key areas:

Gene discovery
Genes conferring freezing tolerance in Arabidopsis and functional analysis of gene products
Novel genes and transcription factors for isoprenoid biosynthesis
Identification of gene promoters using novel pattern recognition techniques
Profiling of genes involved in floral quality and longevity

Metabolic engineering
Enhanced levels of lipophilic and hydrophilic antioxidants in food crops
Integration of cellular function
Elucidation of mechanisms that regulate lignin and polysaccharide biosynthesis
Discovery of mechanisms involved in the generation of active oxygen species and the role of protein kinases in plant defence responses
Elucidation of metabolic networks regulating antioxidant levels in tomato
Molecular dissection of MAPK signalling pathways and their role in regulating cell growth and division
Molecular basis of light quantity acclimatory responses which influence leaf cell development and chloroplast biogenesis.
Molecular dissection of the mechanisms through which light and plastid signals regulate photosynthetic genes

Engineering Antioxidants in Crops
The focus of this work is to enhance the quality and levels of lipophilic (carotenoids, vitamin E) and hydrophilic (flavonoids, polyphenols) antioxidants in crop plants, particularly the tomato. In addition, the cross talk between carotenoid and polyphenol formation is under investigation. A range of genes under the control of constitutive and fruit-specific promoters has been used, and selected examples will be presented.

THE DYNAMIC NATURE OF THE PLANT CELL WALL
A MODEL SYTEM FOR SECONDARY WALL SYNTHESIS.

L. Bindschedler, E. Gay, ER Wheatley, GP. Bolwell
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A cell suspension culture of tobacco transformed with the *Agrobacterium Tcyt* gene, which leads to high endogenous levels of cytokinin has been established. *Tcyt* tobacco culture shows increased cell aggregation, elongated cells and a five-fold increase in wall thickness, correlating with enhanced production levels of vascular nodule containing fibre cells and tracheids, in addition to cells that remain meristematic. Increased amounts of xylan, cellulose and phenolic components reflect the enhanced activity of enzymes involved in xylan biosynthesis during secondary wall synthesis and tracheid differentiation.

Work with xylogenesis in French bean indicated that UDPglucose dehydrogenase and to some extent UDPglucuronate decarboxylase control flux into hemicellulose. A dehydrogenase and a xylan synthase were first purified from French bean. The three enzymes involved in the synthesis of xylan: the dehydrogenase, the decarboxylase and the xylan synthase are being purified from from xylogenic *Tcyt* tobacco cultures. Like in bean, the soluble dehydrogenase showed homology to ADH and had dual specificity for UDPglucose and ethanol in contrast to the mammalian-like dehydrogenase. A 40 and 87 kDa soluble decarboxylases have been purified and trypsin digest has been subjected to MALDI TOF analysis. The 40 kDa enzyme shows homology to other dcarboxylases, but not the 87 kDa enzyme, despite activity. Xylan synthase activity was present in microsomal preparations and was maximum for 6 day old *Tcyt* cultures, and coincided with a broad peak of cinnamyl alcohol dehydrogenase..

Blee KA, Wheatley, ER, Bonham, VA, Mitchell, GP, Robertson, D, Slabas AR, Burrell
Robertson D, Smith C, Bolwell GP (1996) Biochem J. 313: 311-317.
Rodgers, MW, Bolwell GP (1992) Biochem J. 288: 817-822.

Growth signaling in *Arabidopsis*

Richard G. Anthony and Laszlo Bögre

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Mammalian 3-phosphoinositide-dependent kinase-1 (PDK-1) phosphorylates and activates a group of the AGC subfamily of protein kinases, including PKB isoform, p70 S6K, PKC isoforms and SGK. The AGC group of kinases has emerged as a key mediator of the phosphoinositide 3-kinase (PI3K) signaling pathway. PDK-1 thus stands at a pivotal point in signaling and consequently there has been considerable research activity, in animal systems, to understand how PDK-1 function is regulated. An *Arabidopsis* PDK1 homologue has now been partially characterised. Like the mammalian protein, the *Arabidopsis* PDK-1 possesses a kinase domain at the N-terminal and a pleckstrin holomolgy domain at the C-terminus. As a first step to understanding the role of PDK-1 in plants we have identified several interacting partners, including two Serine/threonine kinases and also a novel kinase, which shares some homology with human GAK (cyclin G-associated kinase). We are currently investigating both putative upstream activators and downstream targets in this key growth- signaling pathway.

Implication of Frost-Sensitive Mutants

Gary Warren, Glenn Thorlby, and Irene Bramke
Royal Holloway

Mutations affecting cold hardiness, if their effects are specific, identify proteins that normally protect against chilling or freezing. This offers an approach complementary to the study of cold-induced genes and the cold signal transduction pathway.

The *sfr* (sensitive to freezing) mutations of *Arabidopsis* display distinguishable behaviours after freezing, which may reflect deficiencies in different protective mechanisms. The *sfr2* mutant, with its unusual latency period for injury, may be affected in protection against a slow-acting lesion.

The strong phenotypes of the *sfr2* and *cls8* mutations, and the absence of segregating modifier genes, have allowed precise mapping for gene identification. On the other hand, the *sfr6* mutation, although dramatically affecting cold-inducible gene expression, is more difficult to map by freezing phenotype. We are introducing a reporter gene into the *sfr6* line so that complementation can be detected in a rapid, non-destructive assay that is unambiguous in individual plants.

Indirect effects on freezing tolerance - eg. via reduced vigour - seemed unlikely to be informative, and we therefore gave low priority to mutations that had profound pleiotropic effects. The *sfr6* and *cls8* mutations were in this category but have turned out to affect transduction of the cold signal.

Tuning chloroplasts

Enrique López-Juez
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The light environment of a plant can be highly variable. As light harvesting organelles, chloroplasts need to be finely attuned to their light environment. We are trying to understand these tuning mechanisms, in the model *Arabidopsis*, at two stages:
During first dark-to-light transition, nuclear genes for photosynthetic products are expressed under light (phytochrome) control. Evidence from us and others indicates that (1) a phytochrome signal transduction pathway exists which is specific for photosynthetic gene expression, and closely related to autoregulatory "plastid signals" by which plastids control nuclear gene expression. (2) At least one kind of such plastid signals involves tetrapyrrole metabolites. (3) Plastid signals are ultimately of a positive nature, as shown by the examination of nuclear gene expression in the absence of chloroplasts. We are currently trying to identify novel genes specific for phytochrome control of photosynthetic gene expression.
Changes in light quantity also alter leaf and chloroplast development. Evidence from us and others indicates that (1) *Arabidopsis* is highly sensitive to changes in light quantity. (2) At least two separate mechanisms sense high light. (3) One mechanism is primarily sensitive to blue light, controls palisade cell development and the expression of genes involved in light utilisation. (4) Another mechanism senses changes in the redox state of chloroplast components, controls genes for light harvesting, and probably for photosynthetic reaction centres. We are establishing genetic screens to identify components in both regulatory pathways.

University College London, Department of Biology

The biogenesis of the photosynthetic complexes of eukaryotes.

Saul Purton

Department of Biology, University College London

The photosynthetic complexes embedded in the thylakoid membrane of chloroplasts are exquisitely elaborate structures. They are composed of numerous protein subunits together with the various redox centres and other co-factors required for the light-driven electrochemistry. Each complex is a genetic chimera where most of the core subunits are encoded by chloroplast genes, and the remaining subunits encoded by the nuclear genome. The biogenesis of each complex therefore requires a sophisticated genetic circuitry to ensure the co-ordinated synthesis of all of the subunits and co-factors, and their assembly into a functional complex. We are taking a molecular-genetic approach to investigate the role of nuclear genes in the biogenesis of the photosynthetic apparatus. Our analysis of nuclear mutants of the green alga *Chlamydomonas* that fail to accumulate one of the complexes, has led to the identification of novel genes encoding factors required for the expression of individual chloroplast genes or the attachment of specific co-factors. More recently, we have started a new project to investigate the role of the nucleus in the biogenesis of the respiratory complexes in the *Chlamydomonas* mitochondrion. We have characterised a novel component of cytochrome *c* oxidase and identified a nuclear gene required for the expression of the mitochondrial *COXI* gene.

Metabolic Regulation of Leaf Senescence and Resource Allocation

Astrid Wingler, University College London (a.wingler@ucl.ac.uk)

Metabolites are important regulators of plant metabolism, development and resource allocation. I am mainly interested in two aspects of metabolic regulation: (i) The role of sugar signalling in the regulation of leaf senescence and (ii) the nitrogen-dependent regulation of biomass allocation in clonal plants.
(i) Sugar accumulation can result in the down-regulation of photosynthesis, in accelerated leaf senescence and, as a consequence, in the remobilisation of nitrogen. We have shown that accumulation of sugars in senescing leaves can override the senescence-delaying effect of cytokinins. To analyse the sugar signalling pathways during leaf senescence in more detail, we are planning to isolate Arabidopsis mutants that are affected in the sugar-mediated regulation of senescence.
(ii) A high supply of nitrogen usually results in a decreased allocation of biomass to the roots. In clonal plants, this effect can, however, be reversed when ramets growing at high nitrogen supply are connected to ramets that are growing at low nitrogen supply. This plasticity of clonal plants can be exploited to analyse the molecular regulation of biomass allocation.

Assessment of the pathways of dark reduction and oxidation of the plastoquinone pool in thylakoid membranes of higher plants and green algae

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Several possible 'chlororespiratory' pathways have been proposed for both reduction and oxidation of the plastoquinone pool in the dark in cells of both higher plants and green algae. However, bioinformatic analysis of the recently published genome of *Arabidopsis thaliana* sets limits to the types of respiratory-related components that could be present in higher plant thylakoids: for example, there are no nuclear-encoded homologues of subunits I, II and III of cytochrome *c* oxidase, succinate dehydrogenase or thylakoid-targetted homologues of the NADH-reactive fragment of mitochondrial complex I (the 75kDa, 51 kDa and 30 kDa subunits) that might provide an NADH dehydrogenase fragment for the other complex I homologues that are encoded in the chloroplast genome. In parallel to these considerations, direct assay of pathways of dark reduction and oxidation of plastoquinone has been made in intact leaves, in cells of *Chlamydomonas reinhardtii* and in isolated thylakoid membranes. Combination of these measurements with effects of a range of specific inhibitors of various quinone-reactive sites has allowed assessment of number, types and extents of pathways for dark redox change of plastoquinone.

Imperial College, Department of Biological Sciences

Fungi attacking plants: a quest to unravel biotrophy and pathogenicity

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Department of Biological Sciences, Imperial College

Our aim is to elucidate mechanisms that are fundamental in the establishment and maintenance of fungal infection in plants. We currently work with two pathosystems: barley powdery mildew (*Blumeria graminis* f sp *hordei*) and tomato leaf mould (*Cladosporium fulvum*). *B. graminis* is an obligate biotrophic pathogen and, although this poses limits to the type of investigations that can be carried out, it probably represents some of the most "compatible" interactions between plant and pathogens. We have sequenced a number of ESTs isolated from fungus growing on the host. These, together with ESTs identified and characterised in other laboratories, will be the basis for the construction of micro-arrays for the gene expression profiling during development and infection. In *C. fulvum* we are analysing the function of a class of cell surface proteins called hydrophobins (*C. fulvum* has at least six) by targeted gene deletion. We have found that the major hydrophobin is probably involved in facilitating dispersal. Finally, we test the hypothesis that in incompatible interactions tomatoes kill *C. fulvum* by producing toxic levels of reactive oxygen. We are producing mutants with heightened resistance to reactive oxygen *in vitro*. We will then observe whether disease resistance is altered.

Plant fibre materials: Ultrastructure, utilisation, durability and eco-profiles.

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ABSTRACT

The current research profile of our group (2 academics, 2 Post-docs, 6 postgraduates) evolved from a focus on the microbial degradation of wood-based materials. Fundamental knowledge on the cell wall structure and microbial colonisation /breakdown of wood is deployed to generate new strategies for the protection of wood against fungal decay mechanisms and in the design and targeting of biocidal treatments against such deterioration. Specific aims of our present research are to understand the bio-transformation of fungicides by bacterial and fungal colonists of wood and to characterise the role of extra cellular mucilage in basidiomycete wood decomposition.

The cell wall structure/ultrastructure of several plant fibres (bamboos, flax, jute, kenaf) is being investigated by FE-SEM, TEM, laser confocal and light microscopies. Characteristics such as microfibrillar orientation (including the role of cortical microtubules) and the extent of cell wall lamellation are being studied in order to interpret the mechanical and other properties of plant fibre products. The increased interest in renewable resources based on plant fibre materials has prompted us to develop research to understand their environmental profiles in comparison with 'synthetic' materials using a whole life cycle assessment (LCA) approach.

Keywords: Plant fibre materials, cell walls, environmental profile, LCA, biodeterioration, microorganisms

Imperial College at Wye

Signalling pathways in plant pathogen interactions.
M. Grant, M. de Torres, A. Al- Daoude, K. Jolive, W. Trueman, W. Bryne.

(i) Signalling pathways underlying the hypersensitive response.
We are dissecting signalling mechanisms underlying the HR using a model pathosystem comprising the bacterial biotroph *Pseudomonas syringae* pv. *tomato* strain DC3000 carrying the *avrRPM1* gene and *Arabidopsis thaliana* carrying the *RPM1* gene. We employ a combination of transcriptomics, proteomics and bioluminescence approaches to identify candidate genes implicated in hypersensitive cell death.
(ii) Expression of innate immunity:
Innate immunity is an ancient defence system utilised by mammals, plants and insects and represents the first mechanism of active defence initiated against an invading parasite. We have shown pathogen challenge results in a rapid and significant alteration of the expression patterns of the host's genome. We are using transcriptional profiling and 2D SDS-PAGE to reveal
(i) host responses to microbial occupation of the apoplast
(ii) establishment of a susceptible interaction eliciting disease responses.
We anticipate this approach will generate an overview of activated and repressed disease pathways as well as other events associated with establishment of an immune response.

Dr Charles Ainsworth

Sex determination in plants; Rumex as a model system

The dioecious plant Rumex acetosa (Polygonaceae) is being used as a model system in which to study sex determination. R. acetosa has a sex determining system similar to Drosophila/C. elegans where the sex of the individual is controlled by the ratio of the number of X chromosomes to the number of autosome sets: males have 12+XYY, females have 12+XX. The inappropriate organs are initiated in both male and female flowers but arrest at an early stage. TEM studies show clear differences in the morphology of cells in the arrested organs relative to neighbouring cells in organs which continue develop normally; the arrested cells are more vacuolated (both more and larger vacuoles) and both mitochondrial and and plastids have altered morphologies. The molecular basis of organ arrest, and whether or not it involves an apoptosis-like mechanism is currently unknown. The C function gene, however, may play a role since expression of this gene ceases in the arresting organs. The main focus of current research is to identify genes expressed early during inflorescence development which may have roles in sex determination, by cDNA AFLP display. --

Branching genes and novel long-distance signals in plants

Colin Turnbull, Department of Agricultural Sciences, Imperial College at Wye, Wye, Kent TN25 5AH

Our research concerns genes and signals that regulate shoot branching. We have developed a model of branching control in pea that incorporates five *Rms* (*Ramosus*) genes and two novel signals that appear to be transported in opposite directions along the plant axis. Using a series of *rms* mutants and a range of grafting techniques, we have shown that shoot branching of intact plants can be controlled by signals from the roots. Although the two hormones classically implicated in branching control, auxin and cytokinin, are often altered in level and/or response in these mutants, it is highly unlikely that they are the signals directly regulated by the *Rms* genes. Typically xylem cytokinin content is low in branching *rms* plants, contrary to the notion of cytokinin as a branching promoter. Likewise, auxin (the classic inhibitor) is never depleted in *rms* mutants, nor is auxin transport impaired. A collaborative program with University of Queensland, INRA, and University of York is now attempting to identify and allocate functions to the *Rms* genes. In parallel, we have recently developed grafting procedures to test the model with the *silvescens* (*sil*) mutant of *Antirrhinum* and *max1* (*more axillaries*) in *Arabidopsis*. Results indicate that graft-transmissible inhibition of branching also operates in these species. The ability to graft *Arabidopsis* seedlings has considerable potential for elucidating many other areas of plant signalling systems, including flowering time control, systemic resistance and abiotic stress responses.

POTASSIUM TRANSPORT AND ROOT DEVELOPMENT IN *ARABIDOPSIS*

Alexander Grabov and Francisco Vicente-Agullo

Department of Agricultural Sciences, Imperial College at Wye

Root hairs are known to be an ideal model system for studying ion transport and developmental events on cellular level. A number of genes including *AXR2, TRH1* and *RHD2* block root hair growth in *Arabidopsis* at the transition between the stages of initiation and elongation. ***t***iny ***r***oot ***h***air ***1*** mutant impaired in root hair elongation was recently isolated from a population of T-DNA transformed lines. The TRH1 gene was cloned by plasmid rescue and turned out to encode a polypeptide belonging to the family of KUP/HAK transporters. K^+ transport capacity of TRH1 was accessed in ^{86}Rb uptake experiment. We found that *trh1* is by 40% less efficient than wild type plants in ^{86}Rb accumulation. Although percentage of TRH1 contribution in the total K^+ accumulation was higher at low external $[K^+]$, the transporter was capable to mediate a low affinity transport as well. A role of TRH1 and other K^+ transporters in plant development and mineral nutrition will be discussed.

aphid-phloem interactions

Glen Powell

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Aphids are able to locate phloem sieve elements within plants and extract sap in a way that avoids the normal responses to wounding. Incorporation of insect and plant in an electrical circuit allows monitoring of stylet activities during these processes. Aphids may show two consecutive behaviours following stylet puncture of a phloem sieve element. The first activity represents injection of saliva into the sieve tube, whereas a second activity is associated with uptake of phloem sap. Components of aphid saliva injected immediately after phloem puncture probably inhibit the normal sieve tube responses to damage (callose deposition and P-protein gellation) and enable sap uptake without phloem sealing. Comparisons of insect responses to nearly isogenic resistant and susceptible plants suggest that resistance factors are expressed in the phloem and may involve modified sealing mechanisms. While most studies of aphid-plant interactions have focused on aphid responses to plants, molecular approaches are starting to reveal how plants respond to aphids. Recent studies with Arabidopsis have demonstrated that aphid feeding induces multiple plant response pathways.

Royal Botanic Gardens, Kew, Jodrell Laboratory

Phylogenetic data, molecular evolution and biodiversity

Vincent Savolainen

Molecular phylogenetics has had a major impact on understanding plant evolution. A new familial classification of angiosperms was published in 1998 by the Angiosperm Phylogeny Group, based on analyses of many genes over the last decade; in particular rbcL has now been sequenced for over 8,000 taxa, thereby allowing studies of the molecular evolution of photosynthesis. Complete species-level phylogenetic trees for key genera have also been produced to identify causes of diversification (e.g. pollinators and evolutionary development of floral shape). Time scales in phylogenetic trees can also be depicted from molecular clocks, and we have applied these techniques to study speciation (e.g. sympatric palms with delay in flowering time on oceanic islands) and the origin of major plant groups. In general, dating with molecules provides older ages in comparison with the fossil record, indicating that rates of molecular change might have globally decreased towards the present, but the cause of this phenomenon is unknown. Correlates of evolutionary rates have also been studied, and we found that rates of molecular evolution co-vary with the number of species in each family; in particular, the rate at which species have formed in plant families is linked to the rate of neutral mutations in DNA. The causes for an increased rate of mutations, such as ultraviolet or infrared radiation, have also been investigated at a global scale in species-rich families; the efficiency of DNA repair systems is now being studied in taxa exhibiting higher mutation rates.

Loss of 'typical' telomere repeat sequences from a major radiation of monocots.

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FISH and Southern blotting were used to demonstrate that the otherwise ubiquitous telomere repeat sequence 5'-$(TTTAGGG)_n$-3' (the 'typical' telomere), isolated from *Arabidopsis thaliana,* was absent from chromosomes of the plant genus *Aloe* (Adams *et al*. 2000. *Chromosoma* 109: 201-205), a distant relative of *Allium* which also lacks this sequence (Pich *et al*. 1998. *Plant Systematics and Evolution* 202: 255-264). The phylogenetic tree developed by the Angiosperm Phylogeny Group (Fay *et al.* 2000. In: Monocots: Systematics and Evolution. Eds. KL Wilson, Morrison DA. (CSIRO: Melbourne) pp 360) was then used in a predictive manner to select 27 species from 16 families of the monocot group Asparagales for further investigation. The survey showed that 16 species in 12 families lacked 'typical' telomere sequences. Phylogenetically these taxa clustered in a derived clade within the Asparagales, estimated to contain c. 6,300 species (Adams *et al. Proceedings of the Royal Society*, in press). Based on these observations we predict that the 'typical' telomere was lost, probably as a single evolutionary event following divergence of Doryanthaceae, perhaps 80 million years ago. One genus, *Ornithogalum* (Hyacinthaceae), gave a surprising result in that it had the 'typical' telomere suggesting the sequence can be recovered.

Natural Product Chemistry at the Royal Botanic Gardens, Kew: Past and future

M.S.J. Simmonds, R.J. Grayer, G.C. Kite, N.C. Veitch, P.C. Stevenson, T. Kokubun, E.A. Porter, P.W.C. Green, M. Yule and A. Scott Brown

Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB

Different strategies have been used at Kew to identify plants for biological activity studies. The emphasis has been on the use of taxonomic and ethnobotanical data to identify plants that contain biologically active molecules; for example, plants that contain compounds with medicinal properties or those that can influence the feeding behaviour of insects. These latter compounds are used to further our understanding of the role of plant chemistry in the evolution of insect-plant interactions.

Alkaloids

Current areas of interest include the glucosidase inhibiting polyhydroxyalkaloids and the anti-malarial bisbenzylisoquinoline and indole alkaloids from South American Apocynaceae and Menispermaceae. The insecticidal and pharmaceutical activity of a range of alkaloids, including the amaryllidaceae alkaloids, from the monocotyledons are also being studied.

Flavonoids

Widely studied for their biological activity and value in chemosystematics our focus lies in the families Graminae, Leguminosae and Lamiaceae. Our research includes, investigating the cancer protective properties of tricin, the role flavonoids play in legume-pest interactions and the role of flavonoids as characters to assist authenticate extracts from plants.

Terpenoids

Our emphasis has been on the insect antifeedant properties of neo-clerodanes diterpenoids and limonoids from Lamiaceae and Meliaceae.

The phytochemical knowledge and instrument-based chromatographic techniques available to the group at Kew, including GC-MS, LC-MS and nuclear magnetic resonance spectroscopy, provides the group with the expertise and tools to address a range challenges resulting from the advances in genomics.

Queen Mary, University of London, School of Biological Sciences

Teaching mother nature to suck eggs: natural GM during plant evolution

Lena Murad[1], Joseph Bielawski[2] Yoong Lim[1], Roman Matyasek[3], Ales Kovarik[3], Andrew Leitch[1], & **Conrad Lichtenstein**[1], [1]School of Biological Sciences, Queen Mary, University of London, London E1 4NS, England UK. Tel: +44 120 7882 3049 Fax: +44 120 8983 0973 email: c.p.lichtenstein@qmw.ac.uk. [2]Dept. of Biology, UCL, London NW1 2HE. [3]Institute of Biophysics, Czech Academy of Sciences, Brno, Moravia, Czech Republic;

Geminiviruses (GV) are small circular single-stranded (ss) plant DNA viruses that replicate in the nucleus by rolling circle replication. The viral Rep protein, generates the ss viral DNA circular monomers from a *cis*-essential origin mapping next to the *rep* gene. While expressing antisense *rep* transgenes in tobacco to engineer resistance [1,2], we serendipitously discovered that uninfected wild-type tobacco carries multiple direct repeats of a geminiviral origin sequence adjacent to a truncated *rep* gene [3]. We also found multiple copies of these elements only in the genomes of three related *Nicotiana* species [4,5]. Phylogenetic analysis of these geminiviral related DNAs [GRDs] together with geminiviral *rep* suggest a unique integration event in a single cell of an unknown ancient GV about 25MYA. GRD became amplified, diversified and methylated. During evolution and speciation of this plant section another GRD family arose in some species, possibly by transposition, in a different single locus on another chromosome. GRD resembles, both structurally and functionally the helitrons –a newly discovered family of transposable elements [6].

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Sequence homogenisation in allopolyploid plants is associated with interphase nuclear activity.

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Recently we discovered in tobacco (*Nicotiana tabacum*), an allotetraploid between progenitors of *N. sylvestris* (maternal genome donor) and *N. tomentosiformis* (paternal genome donor) that gene conversion has homogenised most, but not all 18-26S rDNA. Only about 8% of the *N. sylvestris* origin rDNA units remain. In contrast 5S rDNA does not show inter-locus homegenisation. It is likely that 18-26S sequences are vulnerable to gene conversion at interphase of the cell cycle since it is here that they are decondensed and active within a nucleolus. We discuss sequence homogenisation in allopolyploids in the context of interphase nuclear organisation and cytosine methylation patterns

Evolution in *Nicotiana* section *Tomentosae*

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We present perhaps the first molecular cytogenetic phylogeny that has been constructed from an analysis of the distribution of repetitive DNA mapped by fluorescent *in situ* hybridisation. The phylogeny demonstrates species relationships within the plant genus *Nicotiana* section *Tomentosae*. We also show karyotype divergence during speciation of the allopolyploid *N. tabacum* (tobacco), and observe intergenomic translocations between chromosomes of the parental species. During the course of section Tomentosae evolution there is sequence dispersal, locus gain and loss, sequence amplification and reduction, all within the regular framework of the basic genome structure. We demonstrate that one repetitive sequence, called GRD of geminivirus origin, can be used to trace the ancestry of tobacco. Reasons for the different behaviour of repeats during evolution of the section are discussed.

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Plant virus-encoded pathogenicity determinant suppresses post-transcriptional gene silencing

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The monopartite geminivirus *Tomato yellow leaf curl China virus* (TYLCCV) is a member of the genus *Begomovirus* in the family *Geminiviridae*. Expression of TYLCCV C2 protein and green fluorescent (GFP) fused to C2 protein (C2-GFP) in *Nicotiana benthamiana* from a potato virus X (PVX) vector induced necrotic ringspots on inoculated leaves, and necrotic vein banding and severe necrosis on systemically infected leaves. The localization of GFP fluorescence in plant cells infected with PVX/C2-GFP and in insect cells transfected with baculovirus expressing C2-GFP indicates that TYLCCV C2 protein is capable of shuttling GFP into plant and insect cell nuclei. Our data demonstrate that TYLCCV C2 protein may contribute to viral pathogenicity *in planta* and is nuclear localised.

The TYLCCV C2 protein domains that may play a role in viral pathogenicity have been further investigated. Alignment of the TYLCCV C2 protein with 67 homologues from monopartite and bipartite begomoviruses revealed that a putative zinc-finger motif C_{36}-X1-C_{38}-X7-C_{46}-X6-H_{53}-X4-$H_{58}C_{59}$ and four potential phosphorylation sites (T_{52}, S_{61}, Y_{68} and S_{74}) are highly conserved. When expressed from a potato virus X (PVX) vector, TYLCCV C2 protein mutants C2-T52M, C2-H58S, C2-C59S, C2-S61R and C2-S74D, like the wild-type C2 protein, induced local necrotic ringspots and systemic necrosis in *N. benthamiana* plants. Mutants C2-H53P and C2-Y68D produced irregular necrotic lesions on inoculated leaves that were distinct from the wild-type phenotype. In contrast, zinc-finger mutants C2-C36R, C2-C38N and C2-C46I induced chlorosis and mosaic symptoms rather than necrosis. These results suggest that amino acids C_{36}-X1-C_{38}-X7-C_{46}-X6-H_{53} are functional in the zinc-finger motif. Of these, the three-cysteine residues are essential for C2 protein-mediated pathogenesis and amino acids H_{53} and Y_{68} may also participate in this process.

An easy and fast system has been developed to assay TYLCCV C2-mediated suppression of post-transcriptional gene silencing (PTGS). Our data demonstrate that the TYLCCV C2, like African cassava mosaic virus AC2, is a suppressor of PTGS. The mutations in the putative zinc-binding motif described above abolished the ability of C2 protein to suppress PTGS although these zinc-finger mutants are still nuclear localised.

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Regulation and Targeting of Transgenes in Fruit Crops

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Abstract:
It is desirable that the expression of transgenes in genetically improved crops is restricted to the tissues requiring the encoded activity. Our group has a long-standing interest in targeting gene expression to a variety of tissues in the fruit plants apple and strawberry. We are examining the expression patterns of several heterologous and cloned homologous promoters to drive expression of the β-glucuronidase (*gusA*) marker gene in different tissues of transgenic fruit plants. In apple we are interested in fruit specific promoters that control expression of genes involved in ethylene biosynthesis to improve shelf life, whilst in strawberry we have cloned promoters that will be of use in conferring pest and disease resistance. A floral organ-specific promoter has been isolated to target gene expression to petals and stamens of the plant for *Botrytis* resistance and a root-specific promoter has been isolated to target expression to roots to confer resistance to vine weevil larvae, a leading European pest of modern strawberry production systems.
In plastid transformation technology the plastid genome (plastome) is targeted for genetic modification as opposed to the more traditionally targeted nuclear genome. Although difficult to achieve there are several attractive aspects of this technology and current progress, using strawberry as the target crop, will be reviewed.