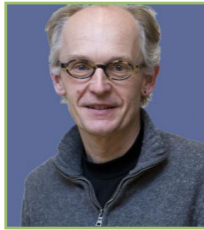


GARNet
2020

Five More
Years...

Welcome to the July 2015 Issue of GARNish

David Salt, GARNet Chair



Since its genesis 15 years ago, GARNet has been led by three exceptional UK plant scientists, Professors Ottoline Leyser, Andrew Millar and Jim Beynon, and has been continually funded by BBSRC through a series of competitive grant applications. In 2015 the GARNet leadership moved into the capable hands of Prof Jim Murray (University of Cardiff) with the funding of a fourth BBSRC responsive mode GARNet grant. Over the coming five years GARNet will continue its tradition of assisting the wider plant research community to take full advantage of public resources and services, and opportunities for funding, training and knowledge transfer. However, to equip plant scientists for the high density data-driven science of the future, GARNet will also provide access and training to allow plant scientists to effectively analyse, store, share and re-use large datasets generated across multiple scales. GARNet will also work to ensure plant scientists are equipped with appropriate resources and information to apply their research in the agricultural, bioenergy and industrial biotechnology sectors, and also to emerging areas such as synthetic biology.

As well as it being an exciting time due to the funding extension, it is also a time of renewal at GARNet. Over the past few years Charis Cook and Lisa Martin have been the driving forces behind GARNet, including organisation of the very well received GARNet2014 meeting. However, Charis and Lisa have now moved on to pastures new. The GARNet Advisory Committee would like to offer

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Thanks to: Ryan Joynson, Kathleen Greenham, Anthony Hall, Scott Poethig, Claire Stoker, Mimi Tanimoto, Katherine Denby and the plant science researchers at the University of Warwick.

their warmest thanks to Charis and Lisa for all their excellent work, and wish them all the best in their future endeavours. No doubt our paths will cross again in the future.

The loss of trusted team members opens the way for new talent, and we are pleased to announce that Dr Geraint Parry has taken over as full-time GARNet Coordinator, where he will benefit from continued help and advice from Dr Ruth Bastow. Geraint has transitioned to this role after time as a lecturer and group leader at the University of Liverpool, which followed PhD studies with Professor Malcolm Bennett and postdoctoral work in the USA. Geraint has long-held experience

The GARNet Committee

David Salt

University of Aberdeen
GARNet Chair November 2014–August 2016

Jim Murray

University of Cardiff
GARNet PI (from February 2015)

Katherine Denby

University of Warwick
Committee member November 2014–August 2017

Antony Dodd

University of Bristol
Committee member January 2013–August 2016

John Doonan

University of Aberystwyth
Committee member January 2012–August 2015

Anthony Hall

University of Liverpool
Committee member January 2012–August 2015

Nicholas Harberd

University of Oxford
Committee member January 2013–August 2016

Ian Henderson

University of Cambridge
Committee member November 2014–August 2017

Sabina Leonelli

University of Exeter
Ex-officio member

Sean May

Nottingham Arabidopsis Stock Centre
Ex-officio member

Zoe Wilson

University of Nottingham
Committee member November 2014–August 2017

Cyril Zipfel

The Sainsbury Laboratory, Norwich
Committee member January 2012–August 2015

within the worldwide Arabidopsis community, which should prove invaluable as GARNet takes up the new activities and opportunities offered through our new funding.

As ever, there are plenty of exciting GARNet events in the coming months. Most immediate is the CRISPR workshop, co-organised with OpenPlant, which is taking place in Norwich in early September. At the time of publication there is still time to register, so please do so if you are interested in learning about this potentially transformative technology. In 2016, and in collaboration with the University of Exeter,

GARNet will be organising a meeting on the topical issue of 'Reuse of Big Data'. Later in 2016 there are two meetings planned on 'Technological Developments in Plant Science', and 'Natural Genetic Variation as a Tool for Gene Discovery and Crop Improvement'. Watch all the usual spaces for details...



Follow @GARNetweets on Twitter. Also don't forget the **Weeding the Gems blog** at <http://blog.garnetcommunity.org.uk>. Please contact Geraint at geraint@garnetcommunity.org.uk if you would like to write a guest post on any topic.



Lisa Martin, who previously worked with GARNet, joined the GPC in February as the new Outreach & Communications Manager. As well as supporting Ruth Bastow in the day-to-day running of the GPC, Lisa is also curating information for *Plantae.org*, currently being developed by the American Society for Plant Biologists.

Providing a one-stop online destination for researchers, students, industry professionals and educators, *Plantae* is designed to be the leading plant science resource hub as well as a community gathering place, with expanded capabilities for connecting, learning and sharing.

The GPC has also recently recruited two New Media Fellows. Sarah Jose from the University of Bristol is helping to drive the GPC's marketing activities, while Amelia Frizell-Armitage from the John Innes Centre is managing the GPC blog.

A GPC initiative called DivSeek was officially launched in January. DivSeek aims to characterise crop diversity, provide easy access to genotypic and phenotypic data associated with genebank germplasm via an online information management platform, and help to develop standards, workflows and protocols to allow effective data exchange, integration and interoperability.

The GPC will be holding a Stress Resilience Research Symposium and Discussion Forum in Brazil in October. This meeting, in collaboration with the Society for Experimental Biology, will bring together experts working on the development of stress-tolerant plants to discuss the challenges and opportunities for joined-up global research in this area.

Please visit the GPC's website, www.globalplantcouncil.org; blog, www.blog.globalplantcouncil.org; follow on Twitter @GlobalPlantGPC (or in Spanish at @GPC_EnEspañol!), or contact Lisa at lisa@globalplantcouncil.org.



You can read more about this year's PlantSci 2015 conference at Harper Adams University on pages 18–21.

UKPSF provided national coordination for over 30 UK Fascination of Plants Day events. More than 60 organisations were involved in this global plant science outreach initiative, which included hands-on activities, interactive exhibits, guided walks, talks and debates.

As agreed at last year's AGM, the UKPSF's priority objective is to convene key stakeholders from across the plant science sector to develop a Roadmap for UK Plant Science for the next 10 to 25 years. An application has been submitted for funding to support this project and, if this is successful, workshops to outline the Roadmap will be held during the autumn.

Also this autumn are UKPSF elections to appoint four new UKPSF Executive members. Nominations will take place in October, during which each member organisation will be asked to nominate one or more Advisory Group members to stand for election. The UKPSF is drafting an Access and Benefit Sharing briefing paper to raise stakeholder awareness around compliance measures for users of genetic resources in accordance with the new EU Regulation (511/2014) and UK implementation of the Nagoya Protocol. If you are interested in

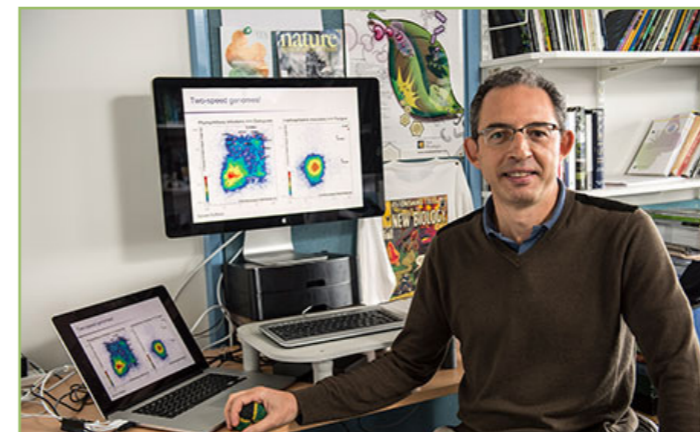
contributing to the briefing, or if you would like further information about the new regulations, please contact UKPSF Executive Officer Mimi Tanimoto: mimi@societyofbiology.org.uk.

Finally, the UKPSF Executive has submitted several funding applications to support the Federation's continued work. We await the outcome of these applications and hope to share positive news soon. Nevertheless, establishing a secure financial future remains challenging and we continue to seek new funding sources to support our core running costs and specific projects.

The date for the 2015 UKPSF AGM on Monday 30th November in London. If you or your organisation would like to contribute to the continued success of the UKPSF, please do get in touch with Mimi.



Congratulations to The Sainsbury Laboratory's Sophien Kamoun, who was recently elected to receive EMBO membership. Sophien is acknowledged for his long-standing contribution to plant science, particularly for his work in the area of plant interactions with the filamentous oomycete pathogen *Phytophthora*. Fewer than 100 scientists per year receive this



Professor Sophien Kamoun.
Photo: The Sainsbury Laboratory

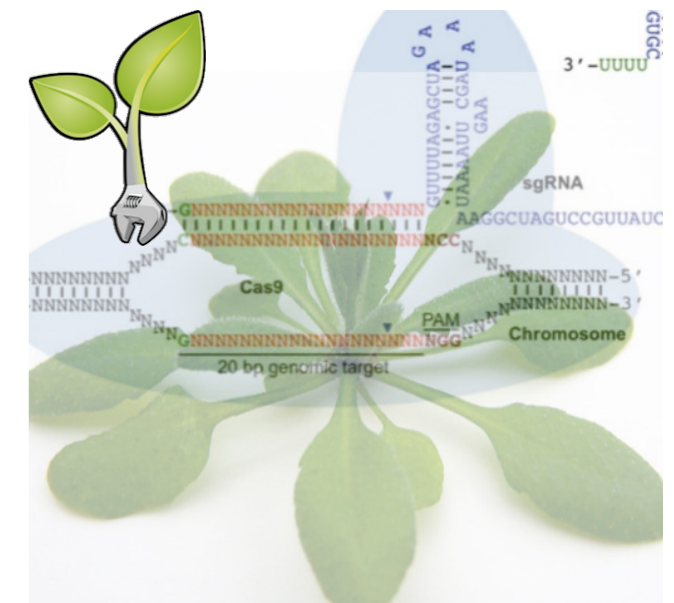
honour.



In collaboration with OpenPlant, GARNet will be running a workshop about CRISPR at the John Innes Centre in Norwich on 7–8th September 2015. The first day of the workshop is open to all interested researchers and will be composed of presentations from scientists using the CRISPR-Cas genome editing technology in their plant science research.

The second day is a practical, hands-on genome-editing workshop, lead by Dr Nicola Patron, Head of Synthetic Biology at The Sainsbury Laboratory. Day 2 is limited to 22 attendees; priority will be given to PhD students or early career plant scientists and is by application only.

For more details see <http://www.garnetcommunity.org.uk/node/643>.



iPlant UK: Democratising Access to High Performance Computing and Enabling Computational Analysis Across the UK Life Science Community

Ryan Joynson and Anthony Hall, Institute of Integrative Biology, University of Liverpool

Based in the USA, the iPlant Collaborative is an initiative funded by the National Science Foundation. It was established to put in place a cyberinfrastructure that gives life scientists direct access to some of the world's best and biggest supercomputers. iPlant users can store and analyse their data using web-based environments designed to take the sting out of bioinformatics analysis. Best of all, you don't need to pay a penny!

After the success of the US iPlant Collaborative project, the BBSRC awarded a grant to The Genome Analysis Centre (TGAC) and the Universities of Liverpool, Warwick and Nottingham to extend the existing iPlant cyberinfrastructure across the Atlantic and into the UK.

At the University of Liverpool, we are working to create next generation sequencing (NGS)-based bioinformatics workflows for iPlant UK, which will help life scientists across the country to analyse "big data". Critically, minimal bioinformatics training or experience will be required.

The first sets of workflows will be made to facilitate the analysis of data produced by some of the most common NGS-based applications, with workflow development for RNA-seq analysis and genome assembly already underway. These workflows will be made available in a graphical web-based user interface on iPlant called the 'Discovery Environment', which enables wet lab scientists with little or no bioinformatics training to analyse their data by removing the stumbling block that is the Command Line, providing pre-installed programs in the form of "apps", and a place to store and share data safely.

One of the first workflows created for iPlant UK is an RNA-seq analysis workflow based around the use of Tophat and Cufflinks (the so-called 'Tuxedo Suite' in iPlant US-speak). The workflow will

enable users to map splice junctions, assemble transcripts, detect differential expression and create comparative plots for multiple conditions, tissues and time points with multiple biological

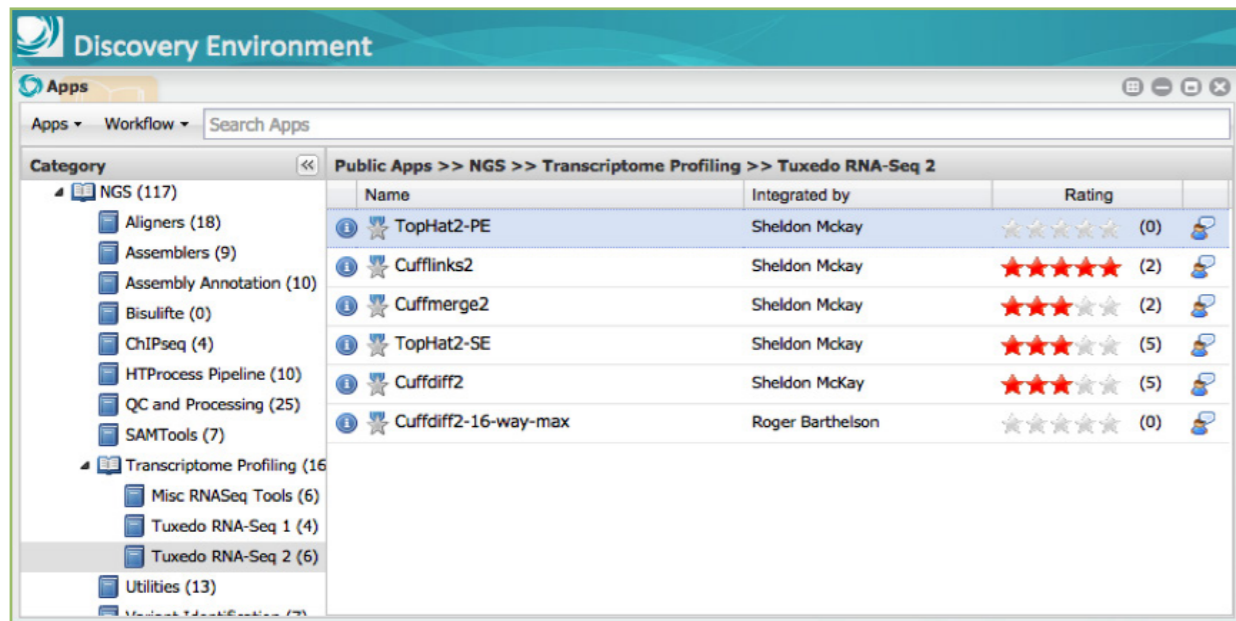


Figure 1. The iPlant Discovery Environment interface: an intuitive link between bioscientists and high performance computing.

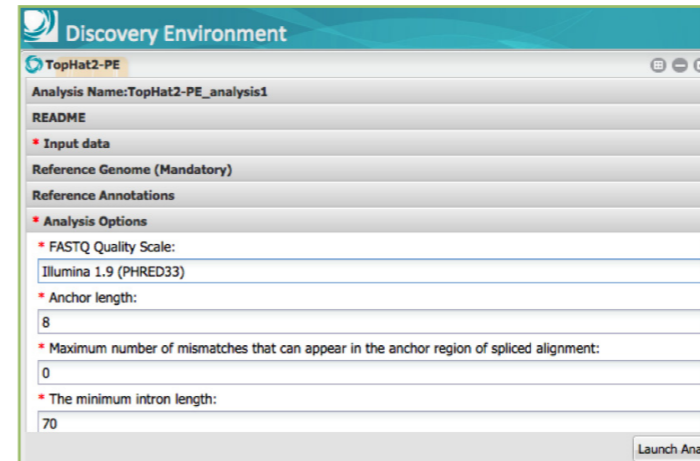


Figure 2. Launching analyses in iPlant - simple selection of data and analysis options.

replicates – all within a few clicks using computing power based at TGAC.

The workflow will save the outputs from each stage of the Tuxedo Suite pipeline to the user's personal data store, as well as creating some common differential expression analysis, publication-ready, plots using the *R* package CummeRbund. To use the workflow, users will simply need to upload and select the sequence data to be analysed, set options for the Tophat2 and Cufflinks programs using drop-down menus (if different from the default), and click submit.

iPlant will then run the analysis and even notify you when the job is done. This will allow users to comparatively analyse multiple sets of RNA-seq

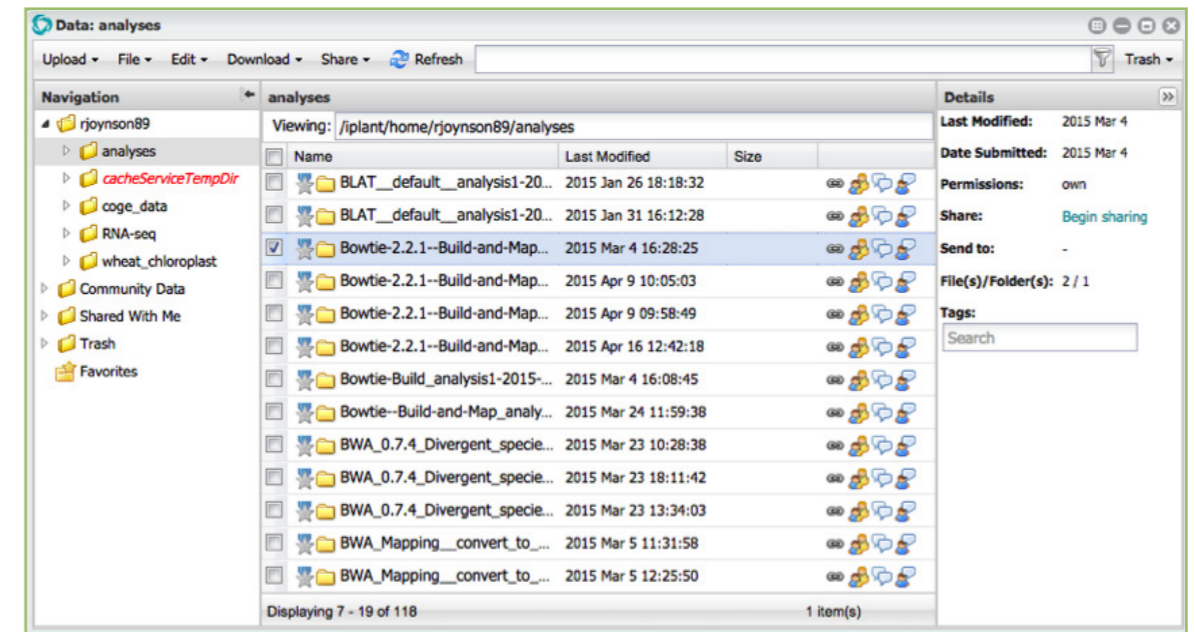


Figure 3. The iPlant Data Store, where users can store up to 1Tb of data and analysis output.

data simultaneously in a "fire-and-forget" manner with no user input required between stages of analysis – all on-demand and free of charge.

A beta version of the workflow will be made available on iPlant in the coming months. The development of iPlant UK workflows will be community-driven, so we want to encourage users to leave comments or indicate to us what they would like to see from future versions. This will enable us to tailor workflows in iPlant to the needs of the UK plant science community.

To help users get the most out of iPlant, multiple tutorials have been created to help analyse RNA-seq data, including differential expression analysis using the Tuxedo Suite of bioinformatics tools, along with a tutorial demonstrating how to assemble transcripts when no reference is available.

These tutorials can be found at: <http://www.iplantcollaborative.org/learning-center/all-tutorials>.

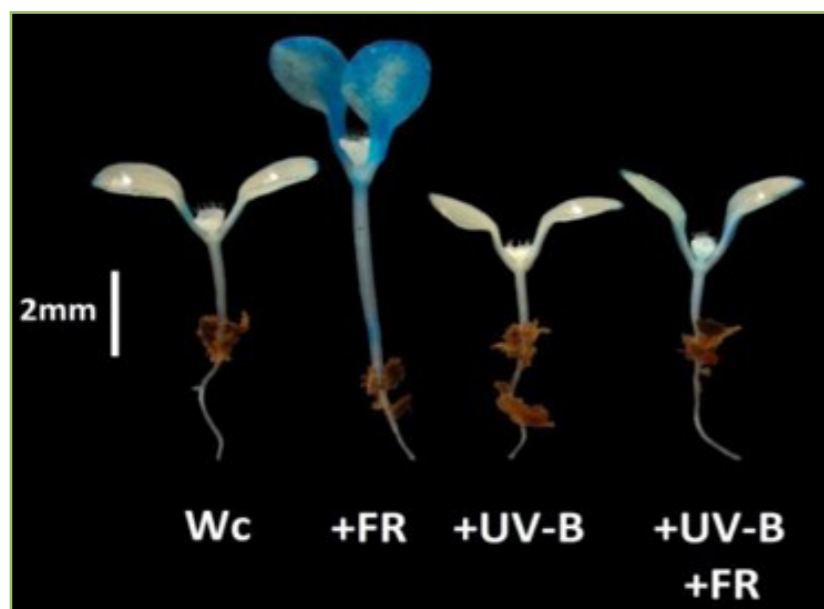
New Arabidopsis Grants

Arabidopsis researchers continue to be very successful in BBSRC responsive mode funding rounds. Here's a round-up of grants awarded to members of our community in the BBSRC Responsive Mode 2014 Round 2. Congratulations to the PIs and also the researchers in post working on these exciting projects!

Integrating UV-B signalling into plant photomorphogenesis networks

Kerry Franklin, University of Bristol
Gareth Jenkins, University of Glasgow

Plants detect reflected far-red light signals from neighbouring vegetation using phytochrome photoreceptors. In dense stands, this triggers rapid stem elongation to escape encroaching neighbour shade. Shade avoidance can reduce plant stability



Kerry Franklin: UV-B inhibits auxin activity and hypocotyl elongation in shade avoidance. DR5:GUS expression in seedlings grown for 3 days in white light, followed by 4 days of white light (Wc), +Far-Red light (FR), R:FR= 0.05, +narrowband UV-B (1 $\mu\text{molm}^{-2}\text{s}^{-1}$) or +FR and +UV-B treatments combined. Blue colouration represents auxin activity. Reproduced from Hayes et al. (2014) PNAS.

and yield and is therefore a major determinant in crop planting density.

Simultaneous exposure to both sun (UV-B) and threat of shade (low red to far-red ratio) signals will, however, occur prior to canopy closure and through canopy gaps. The mechanisms through which plants integrate these two conflicting signals are only starting to emerge.

In a recent collaboration between the Franklin and Jenkins laboratories, we showed that UV-B perceived via the UVR8 photoreceptor strongly inhibits shade avoidance responses in *Arabidopsis thaliana* (Hayes et al. 2014, PNAS 111, 11894–11899). This process involves a dual regulatory mechanism, which converges to inhibit the abundance and activity of PHYTOCHROME INTERACTING FACTOR (PIF) transcription factors, biosynthesis of the growth hormone auxin and plant elongation. In this way, UV-B perception by UVR8 provides plants with an unambiguous signal of sunlight, triggering a dual growth restraint mechanism to prevent the unnecessary allocation of resources towards neighbour competition. The initiation of this pathway by UV-B in non-shaded conditions results in plants with a dwarfed stature and reduced biomass.

Our previous work directly linked UV-B perception to plant hormone signalling, providing a long-awaited molecular explanation for the effects of UV-B on plant architecture. However, considerable work is still required to fully understand this important signalling network. In this BBSRC-funded project, we hope to provide deeper mechanistic insight in to how UV-B signals are integrated with other photoreceptor pathways to control plant architecture in sunlight. More specifically, we aim to elucidate how UV-B

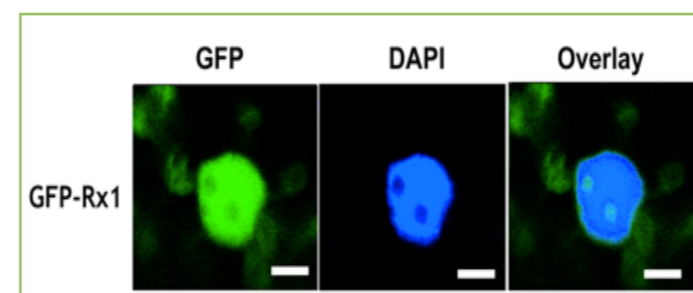
signals control PIF abundance and activity, how phytochrome, cryptochrome and UVR8 signalling pathways converge to regulate plant architecture in dense canopies, and how altered levels of photosynthetically active radiation affect UV-B responses in glasshouses.

The mechanistic basis of plant NLR signalling in effector triggered immunity

Martin Cann, Gary Sharples and Lars-Olof Palsson, Durham University

Plant NLR proteins trigger disease resistance in response to pathogen effectors. Several NLRs act in the nucleus, yet conserved nuclear targets that support their role in immunity are unknown.

In this work the group will investigate how a model NLR protein functions in the plant nucleus. The group will use the Rx1 NLR of potato to study the molecular basis of nuclear-localised immune signalling. Potato is the fifth most important global crop after wheat, corn, rice, and sugar cane, with a UK annual production of 6 million tonnes.



Martin Cann: Nuclear localization of the Rx1 protein.

In work leading up to the proposal the group has developed a methodology for the production of a recombinant Rx1 protein. Rx1 is therefore useful to study because it we can use biochemical tools developed by the group, and molecular and genetic tools developed by others. The group has used a combination of techniques to demonstrate

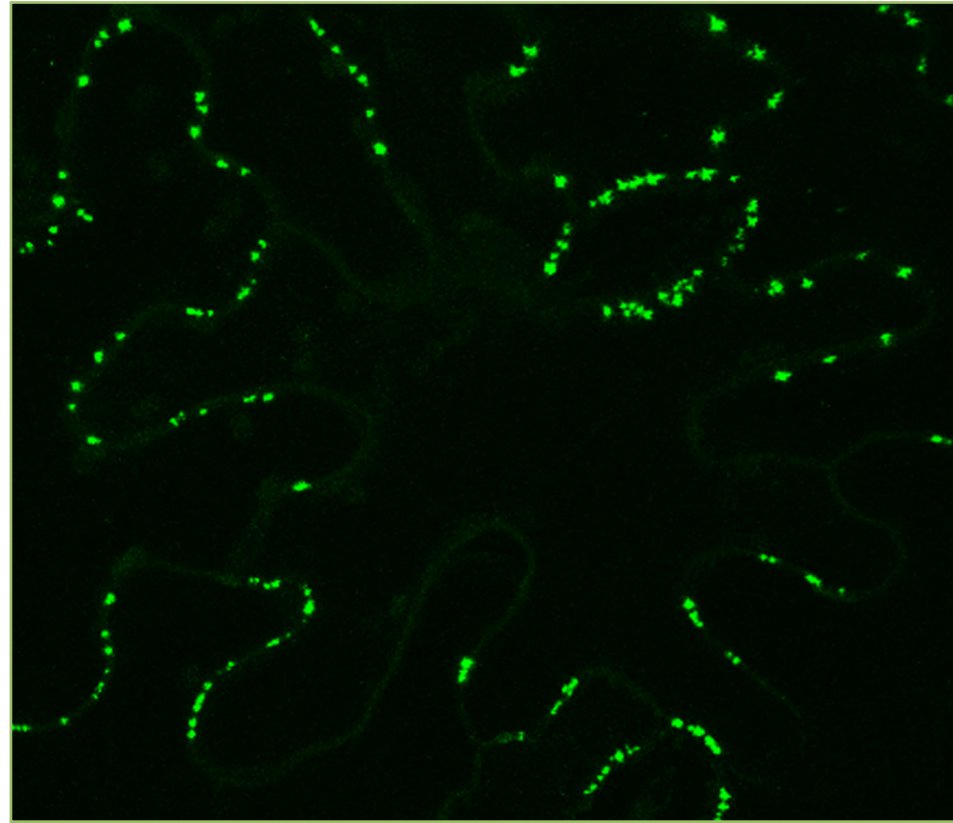
that the Rx1 protein interacts with nucleic acids *in vitro* and in the plant cell, and further demonstrated that Rx1 forms part of a signalling complex active at chromatin. It is therefore hypothesised that Rx1 can function as bridging molecules that respond to pathogen effectors by distorting DNA and activating a protective transcriptional response.

The group proposes to use *in vitro* analysis, with supporting experiments in plants, to develop a model for Rx1 DNA binding in immune signalling. First, fluorescence lifetime techniques will be used to examine how DNA binding is altered in Rx1 proteins with altered spatial localisation, inter- and intramolecular contacts, and immune signalling function. Second, mass spectrometric and ChIP techniques will be used to identify Rx1 interacting proteins that target Rx1 to DNA and provide a mechanistic basis for Rx1 in transcription initiation. Third, biochemical assays will identify how an interacting transcription factor targets Rx1 to specific DNA sequences to permit subsequent chromatin remodelling. The eventual aim of these experimental approaches will be to provide a link between immune activation and processes required for transcriptional activation.

Push on through to the other side : the molecular basis of viral cell-to-cell movement in plants

Jens Tilsner, University of St Andrews and the James Hutton Institute Dundee.

Plant viruses are major crop pathogens that cause significant economic damage and pose a threat to global food security. After a virus has been introduced to its host plant by mechanical damage or a vector organism, it initiates replication in a usually very small number of recipient cells. In order to continue the infection and spread



Jens Tilser: The fluorescently labelled movement protein of a plant virus localises to the plasmodesmata lining the lateral walls of epidermal cells on a *Nicotiana benthamiana* leaf.

throughout the plant, the virus then needs to transport its genome into adjacent, as-yet uninfected cells.

Because of the presence of the cell wall, the only way for plant viruses to move between host cells is through plasmodesmata, membrane-lined channels with a complex internal structure that leaves only a few nanometers of free space for the virus to pass through. Even at the viral scale, these dimensions are so narrow that the virus quite literally has to “push” its infectious genome through the channels.

This form of intercellular transport is unique to plant viruses and constitutes a severe genetic bottleneck and a critical infection step: once the infected cell has recognised the intruder, its defence mechanisms become activated.

The main antiviral immunity in plants is provided by the RNA silencing machinery, which utilises sequence-specific RNA degradation by ribonucleases programmed with virus-derived small RNAs. These small RNAs are themselves mobile through plasmodesmata and prepare naïve recipient cells to effectively stop the incoming virus. Viruses are thus in a “race” with the host plant’s defence responses, and the speed with which the virus is able to move through plasmodesmata is a major factor influencing the ultimate success or failure of the infection. Virus movement is therefore a promising target for new antiviral crop protection strategies.

However, the transport mechanism is not currently understood at a molecular level. It has been known for several decades that plant viruses encode so-called ‘movement proteins’, whose functions are to target plasmodesmata, dilate the channels and transport the viral cargo across the cell wall. But whilst the transport of movement proteins and viral genomes to the plasmodesmata has been extensively studied, no clear mechanism has emerged for exactly how movement proteins mediate the RNA transfer through the channels.

This project will address this fundamental question using *in vitro* experiments simulating the transport process. Purified movement proteins will be used to reconstitute membrane-associated ribonucleoprotein complexes and analyse their biochemical and biophysical properties. The project will test a specific hypothesis that posits

common features shared by the movement mechanisms of various disparate viruses. If proven correct, this will open up possibilities for new antiviral strategies that will potentially be applicable against a broad variety of crop pathogens.

Functions of the WHIRLY1 protein in chloroplast-nucleus crosstalk

Christine Foyer, University of Leeds

The WHIRLY family of ssDNA-binding proteins have a quaternary structure with a ‘whirligig’ appearance, but they form much larger oligomeric structures in chloroplasts and mitochondria. WHIRLY1 is targeted to chloroplasts but is also found in the nucleus. It was first characterised as a binding subunit of the nuclear transcriptional activator, PBF2, and is involved in plant defence gene expression. WHIRLY1 is required for the expression of chloroplast genes, senescence-associated genes and salicylic acid-regulated genes.

In this project, we will test the hypothesis that WHIRLY1 is involved in chloroplast-to-nucleus signalling. Specifically, we will determine whether the localisation and functions of WHIRLY1 are subject to redox regulation in the chloroplasts of *Arabidopsis thaliana* and barley. We will also characterise the WHIRLY1 interactome and determine whether WHIRLY1 is involved in the epigenetic control of stress tolerance.

The vision of these studies is to deliver an improved understanding of the regulation and functions of WHIRLY1 in chloroplast-to-nucleus signalling, together with a deeper knowledge of how this information might be used to maximise the productivity of crop plants such as barley under stress conditions.



Rupert Fray: An example of a *Arabidopsis* plant with low levels of m⁶A methylation (left) compared to an equivalent wildtype (right).

Defining the plant epitranscriptome

Rupert Fray, University of Nottingham
Gordon Simpson, Geoff Barton, University of Dundee

N⁶-methyladenosine (m⁶A) is a ubiquitous modification present in the messenger RNA of most eukaryotes. Global levels of m⁶A can be measured relatively easily, and when this is done, methylation in *Arabidopsis* is found to vary according to developmental stage, organ type and environmental conditions.

Typically more than half of all transcripts could be methylated in some tissues, but – until recently – it was not possible to map the modification in specific transcripts. This changed with the development of Me-RIP-seq, a procedure in which RNA is fragmented and m⁶A-containing polynucleotides isolated using an anti-m⁶A antibody. Methylation is then mapped following sequencing of the input and immune precipitated libraries.

The Arabidopsis enzyme MTA is required for mRNA methylation. Null mutant alleles are embryo lethal, indicating that mRNA m⁶A is essential for plant survival but, by partial complementation of such mutants, plants can be made with less than 10% normal mRNA methylation levels. These plants show increasingly severe developmental defects as m⁶A levels are reduced. MTA has several partner proteins that are required for its activity and it appears that the same partners are conserved in different eukaryotes.

For example, FIP37 was shown to functionally interact with MTA in Arabidopsis, and the mammalian homologue of this, WTAP (Wilm's Tumor Associated Protein), was subsequently shown to be an essential component of the methylation complex in humans. However, the exact composition of the 'writer' complexes, their regulation and the degree of conservation remains to be determined. It is clear that not all mRNAs are methylated and not all potential consensus target sites are methylated either. However, the mechanistic or regulatory basis of m⁶A selectivity is unknown.

Methylation of transcripts appears to either promote translation or target transcripts to P-bodies, depending upon the identity of the

proteins that interact with the modification. m⁶A can be "read" directly by YTH domain-containing proteins that specifically bind m⁶A. There are 13 Arabidopsis genes predicted to encode YTH domain-containing proteins, but their functions are almost wholly uncharacterised. In addition, m⁶A can directly influence the stability or conformation of RNA in the absence of RNA binding proteins.

The aims of this new project are to define the Arabidopsis epitranscriptome, determine how it is regulated, and assess the impact on gene expression of disrupting individual writer-complex components. The conservation of methylated transcripts between Arabidopsis and various crop species will also be assessed and the function of the different m⁶A "readers" will be addressed.

This collaboration combines expertise in RNA methylation (Fray), the molecular and proteomic analysis of RNA processing (Simpson) and quantitative analysis of high throughput sequencing data (Barton).

CDKG, a Ph1 related kinase involved in meiotic chromosome pairing

John Doonan, Aberystwyth University
Graham Moore, John Innes Centre

Reversible protein phosphorylation plays a central role in regulating most cellular processes in eukaryotes. Protein kinases modulate the activity of specific groups of substrate proteins by adding negatively charged phosphate groups, while protein phosphatases can remove these groups, providing an energy-efficient, highly specific and rapid means of modulating protein activity.

Cyclin-dependent protein kinases comprise a

family of multi-subunit kinases, whose members have diverse functions (Doonan & Kitsios, Mol Biotechnol, 2009 42:14–29).

The prototypical CDKA group (cdc2/CDC28 in yeast, CDK1/2 in mammals) was originally implicated in mitotic progression and the role of other groups is gradually being revealed. Ph1 defines an orphan group of cyclin-dependent protein kinases (CDKs) restricted to monocots that is related, both structurally and functionally, to the phylogenetically conserved plant CDKG/ mammalian CDK11 class of kinases.

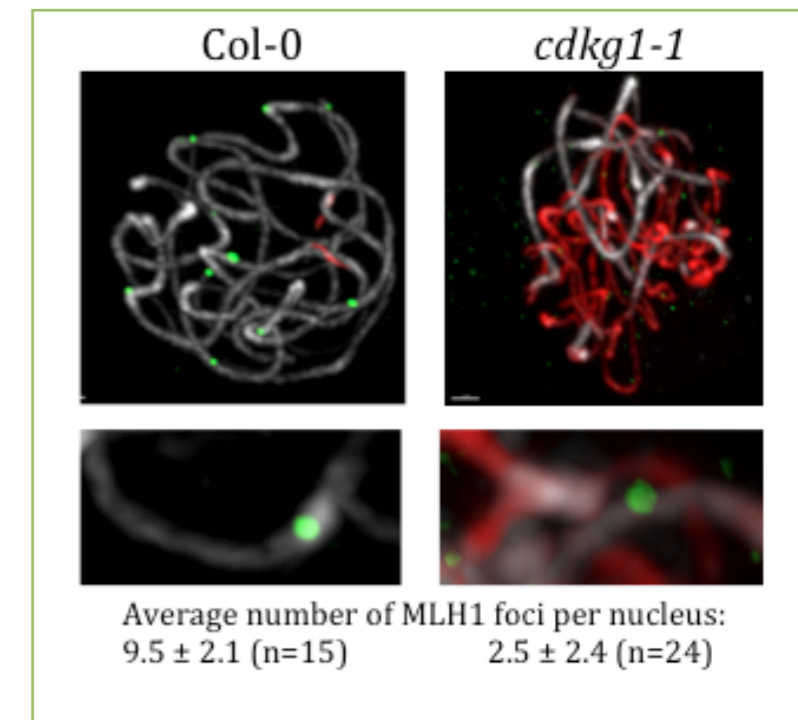
Ph1, the major chromosome pairing locus in wheat, has a profound effect on recombination between related chromosomes or homeologues (Griffiths *et al.* Nature, 2006 439:749–52), and is required for genome stability in hexaploid wheat. Deletion of the Ph1 locus allows introgression of foreign DNA from wheat relatives, but has the disadvantage of promoting mis-segregation events and is therefore difficult to exploit in a routine breeding context.

To evaluate the function of these Ph1-related kinases, we exploited the genetic versatility of the Arabidopsis model system and used high-resolution immuno-imaging approaches that retain the 3D conformational layout of meiotic chromosomes. T-DNA insertional mutants in the CDKG1 are temperature-sensitive male sterile because of a failure of chromosomal pairing at high ambient temperature (Zheng *et al.* PNAS, 2014 111:2182–7).

Recombination, as assessed by MLH3 nodules, is also reduced in the mutant at high temperature (see figure, right). Mutations in the cognate cyclin (CYCLIN L) behave as genetic enhancers (double mutants are sterile at all temperatures). These phenotypes are consistent with the previously

reported cytological effects of the Ph1 deletion. The BBSRC-funded project aims to test the hypothesis that CDKG /Ph1 group kinase activity influences the stringency of chromosome pairing. By transiently modulating CDKG and Ph1 kinase activity, genetically and/or chemically, it will test how these kinases affect homologous or homeologous chromosome pairing and recombination in Arabidopsis and wheat.

We also aim to identify substrates and define the signal transduction pathway that modulates pairing behaviour. Understanding how these kinases function should allow us to design methods to transiently manipulate their activity, and thereby exploit them in dissecting the molecular basis of homologous chromosome pairing in both model and crop species.



John Doonan: Recombination is reduced in the *cdkg1-1* mutant at high temperature. Meiotic nuclei immunolabeled with anti-ASY1 (red), anti-ZYP (grey) and anti-MLH1 (green) that marks late recombination nodules.

Genome editing: is it genetic modification?

Claire Stoker,
University of Warwick
clainestoker@warwick.ac.uk



United Nations experts have estimated that food production will need to double by 2050 in order to feed the rising population, which is predicted to reach over 9 billion within 35 years. Clearly this task will not be easy; the amount of global arable land is not increasing so we need to grow more from less. Furthermore, climate change is bringing increasing temperatures and more erratic weather patterns, all of which makes crop production challenging.

New techniques in plant science such as genome editing may help researchers feed our rising population. Genome editing allows crop researchers to manipulate the plant genome in a highly specific and targeted manner to introduce mutations that will help plants adapt to new, potentially hostile, environments. Unlike genetic modification (GM) or traditional plant breeding methods, which often lead to random modifications

to the plant genome, genetic engineering allows changes on a nucleotide-specific basis.

Genome editing is a relatively new technology. To address the questions and concerns that members of the public might have about it, *Sense about Science* hosted a Q&A session (<http://www.senseaboutscience.org/pages/gene-editing.html>) allowing the public to ask expert plant researchers for their opinions and information. The panel of experts comprised of Professor Robbie Waugh and Dr Glenn Bryan from the James Hutton Institute in Dundee, Dr Huw Tyson from BBSRC and Professors Jonathan Jones and Ottoline Leyser from The Sainsbury Labs in Norwich and Cambridge, respectively.

The panelists answered questions centered on five common themes: What is genetic engineering? How long does genome editing take/how does this time frame compare to current practices? Is it safe? Why is there a call for genome editing/what benefits could it bring? Should it be classed as GM and why does this matter in terms of current regulations?

Jonathan Jones answered the question: *"Please define 'standard genetic engineering'. Is it a bit like*



'standard civil engineering'?" He explained that standard genetic engineering is usually carried out in one of two ways, either using *Agrobacterium* or particle bombardment to insert DNA into plant cells. He also explained the use of marker genes to detect for mutated plants. Dr Jones then outlined the potential drawbacks of these techniques including the risk of multiple, uncontrollable and essentially random gene insertions and the need to screen hundreds of plants to find the desired single copy, correct location insertion.

Another group of questions revolved around the timescale for genome editing and/or GM, including, *"Is it faster to add new traits to plants via genome editing or conventional breeding?"* Genome editing is potentially much faster than the conventional selective breeding methods currently used in agriculture, which can take many years. Genome editing could be achieved within as little as three generations (a minimum of 3 years for most food crops), whereas selective breeding is much more time intensive.

In addition, many questions asked how the timescale for generated genome edited crops differed to that for GM crops? Panellists explained this largely depends on the regulations surrounding genome editing; the reason GM takes so long and is so costly to get to the field is because of strict regulations. If genome editing falls under the same regulatory framework as GM, then genome editing will also take an extremely long time, and a lot of money, to get to the field stages.

The need for genome was highlighted in answers to questions such as *"The UN says small farmers are already providing food security. So more GMOs are not needed then?"* It was explained that the food supply chain is complex and faces many challenges if it is to address future food security issues, and that genome editing and GM



Image from www.systembio.com

are both attractive options for crop improvement, hence increased food security as they can improve pre- and post-harvest performance. Importantly, it is critical to note that neither technology is a panacea that will immediately solve food insecurity, rather both can be part of the solution.

The main body of questions were related to the benefits that could genome editing could bring to food production. These included, *"Will these new techniques be used for more than increasing yield or reducing pesticide use?"* and *"What kind of benefits could genome edited crops offer us?"* Answers to these questions really highlighted the great array of benefits that genome editing could bring to the world of crop production.

For example, it was explained that genome editing is able to confer vital traits into crop plants such as increased abiotic and biotic stress tolerance (which would in turn decrease pesticide usage). Moreover, plants could be modified to have a higher nutritional value to humans, for example when many vegetables (e.g. potatoes) are fried they

produce carcinogenic acrylamide – by using genome editing, the levels of this could be greatly reduced.

Understandably, many members of the public were concerned about the safety of this new technique. The experts seemed to agree that genome editing is no more or less safe than marker-assisted breeding or current GM practices. The disadvantages of the imprecision of marker-assisted breeding were emphasised, when it was revealed that non-desirable as well as desirable traits are selected for because of 'linkage drag'. The experts confirmed that genome editing and GM have already been well tested and that the techniques are well understood. Furthermore, because of the low cost of sequencing, any non-target mutations could be easily identified if a crop were to enter the food supply chain.

Many of the answers to questions such as, "By asserting that genome engineering is distinct from GM, are scientists at risk of being seen to imply that GM is dangerous?" referred to the regulations on genome editing or/and GM and how pivotal these are to determining the ease and success of these techniques. With regard to scientists being seen to imply that GM is dangerous, many panellists agreed that this is unlikely to be the case as most scientists openly advocate the use of GM. They try to make it clear that the main reason to distinguish between the two approaches is related to the amount of regulation that would be necessary in each case.

Many experts agreed that genetic modification and genome editing are different techniques that can be used to achieve the same results. Furthermore, they agreed it was better to characterise any plant by specific trait changes due to the genetic alterations, rather than the technique used to make the genetic change *per se*. If genome editing was to be considered a GM technique it would

be much more expensive to introduce to food supply chains due to the strict regulations. This means many useful non-profit alterations to crop genomes may well be made in research institutes but will never see the light of day.

Perhaps the most fundamentally important question of the session was: "What do the panelists think: should genome editing techniques be considered GM?" This question raised many complex issues and concerns. The general consensus among the experts was that a plant altered via genome editing is not GM as there is no remaining foreign DNA in the edited plants. Genome editing focuses more on introducing mutations that may have occurred naturally in plants through practices such as selective breeding.

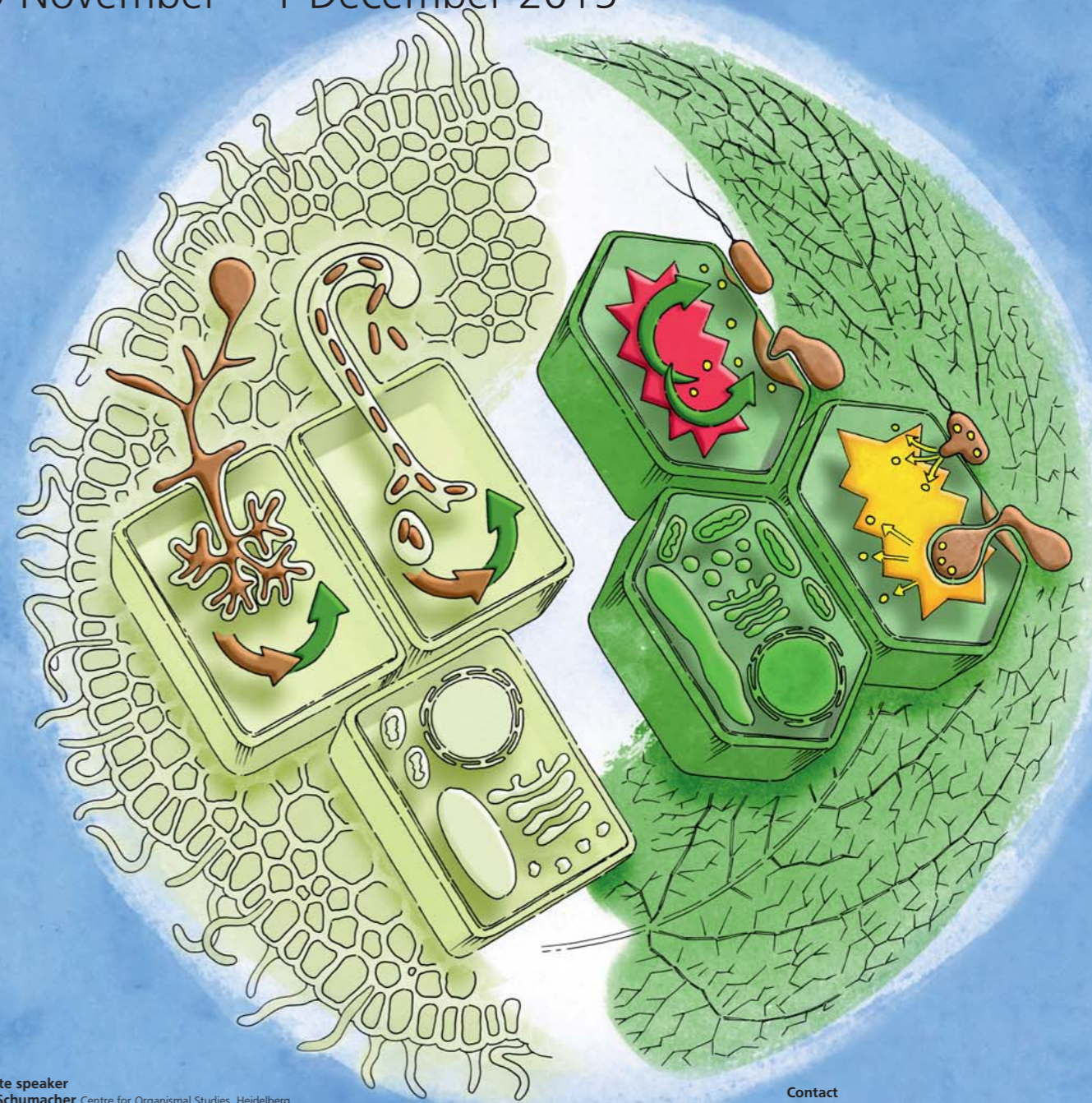
The public asked some very insightful questions and raised many real concerns regarding genome editing. I think by hosting this Q&A the *Sense about Science* panel managed to communicate complex ideas and concepts to the public in a very concise and understandable way, which reassured the public of the safety and usefulness of this new, exciting technique. If any members of the research community are interested in using one form of genome editing, CRISPR-Cas technology in their research then please consider registering for GARNet-OpenPlant workshop in early September.

Details can be found here:
<http://www.garnetcommunity.org.uk/node/643>.



Cell biology at the plant-microbe interface

Eden Hotel Wolff, Munich, Germany
29 November – 1 December 2015



Keynote speaker
Karin Schumacher Centre for Organismal Studies, Heidelberg University, Germany

Speakers
Ton Bisseling Wageningen University, the Netherlands
Paola Bonfante University of Torino, Italy
Peter Dodds CSIRO, Canberra, ACT, Australia
Gunther Döhlemann MPI for Terrestrial Microbiology, Marburg, Germany
Christine Faulkner John Innes Centre, Norwich, UK
Maria Harrison Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY, USA
Noriko Inada Nara Institute of Science and Technology, Ikoma, Nara, Japan
Regine Kahmann MPI for Terrestrial Microbiology, Marburg, Germany
Sophien Kamoun The Sainsbury Laboratory, Norwich UK
Richard O'Connell INRA Versailles-Grignon, France
Thomas Ott University of Munich (LMU), Germany
Jane Parker MPI for Plant Breeding Research, Cologne, Germany
Martin Parniske University of Munich (LMU), Germany
Silke Robatzek The Sainsbury Laboratory, Norwich, UK

Nick Talbot University of Exeter, UK
Hans Thordal-Christensen University of Copenhagen, Denmark
William Underwood University of California, Berkeley, CA, USA
Barbara Valent Kansas State University, KS, USA
Claire Veneault-Fourrey INRA, Nancy, France
Shunyuan Xiao University of Maryland Biotechnology Institute, MD, USA

Organisation
Silke Robatzek The Sainsbury Laboratory, Norwich, UK
Peter Dodds CSIRO, Canberra, ACT, Australia
Maria Harrison Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY, USA

Contact
New Phytologist Trust
Helen Pinfield-Wells
np-symposia@lancaster.ac.uk

New Phytologist Central Office, Bailrigg House, Lancaster University, Lancaster, LA1 4YE, UK.

Twitter: @NewPhyt.

Facebook: fb.com/NewPhytologist

The New Phytologist Trust is a non-profit making organisation dedicated to the promotion of plant science.

Complete details and registration at

www.newphytologist.org





UK PlantSci 2015:

Lisa Martin
GARNet

lisa@garnetcommunity.org.uk



This year's UKPSF PlantSci 2015 conference was held on the 14th and 15th April at Harper Adams University in Shropshire, set amid some glorious English countryside with rather fitting yellow fields of oilseed rape. The two days of scientific sessions included talks by over 30 speakers covering a broad range of topics. Though overall the meeting certainly had a more "applied science" feel to it than in previous years, the whole gamut of plant science research was represented, from model to crop, with a few interesting curve-balls thrown in for good measure.

Harpers' Vice-Chancellor Peter Mills formally opened the meeting, apologising for the smell of farm animals drifting in through the air conditioning unit! The lecture theatre was opposite a pig research area – he promised us we'd get used to it!

After a welcome from the UKPSF Executive Officer Mimi Tanimoto, the first keynote lecture of the day was from Guy Smith, Vice-President of the National Farmers' Union and head of an Essex farm officially recognised by the Guinness Book of World Records as the driest in the UK! Guy discussed the fact that unpredictable weather conditions, a decline in crop protection products available in Europe, and a challenging regulatory framework means that Britain's food supply systems is simultaneously becoming more insecure and less competitive. With this in mind,

he stressed the need for effective translation and knowledge exchange along the pipeline from bench biologists to practitioners out in the field, to improve agricultural productivity and sustainability whilst minimising impact on the environment and on biodiversity.

The first scientific session of the day, entitled '*Plants and Agriculture: Breeding the Next Green Revolution*',

was chaired by Professor Vicky Buchanan-Wollaston from the University of Warwick. In this session, Chris Burt from RAGT Seeds discussed how new genetic technologies are impacting on the science of wheat breeding, while Jim Monaghan from the host university explored how changing the growing environment of leafy salad crops, rather than the genetics, could confer gains in yield and produce quality. Mike Gooding explained ongoing research at IBERS to breed high-sugar perennial forage grass species optimised for livestock digestion, resource use efficiency and stress tolerance. Finally, Warwick's Robin Allaby – an evolutionary geneticist – gave a fascinating presentation about how ancient DNA is providing clues about the origins and evolution of agriculture.

The next session was all about trees and included talks by Kate Hutchinson from Forest Research, Sheffield's David Beerling, Dan Bebber from the University of Exeter, Richard Buggs from Queen Mary's University of London, and Newcastle University's Samuel Logan.

Before a fantastic poster session, drinks reception and BBQ dinner, all of which provided the opportunity for plenty of quality networking, the final session of Day 1 was a panel discussion chaired by West Midlands MEP Anthea McIntyre, who also sits on the European Parliament's Agriculture Committee. Angela lent her support



MEP Anthea MacIntyre chaired a lively panel discussion on 'Building a roadmap for UK plant science'. Photo: Lisa Martin

to the UKPSF's continuing mission to create a roadmap for UK plant science, which is currently in development.

The other panel members represented each of the four UKPSF working groups, established last year to drive forwards the recommendations made in the UKPSF's report, "UK Plant Science: Current Status and Future Challenges". Rick Mumford from Fera represented the 'Translation' working group, which is looking at ways to improve the pipeline from fundamental research to in-field applications and innovations. Rothamsted's Huw Jones represented the 'Regulation' working group, which is exploring the policies and frameworks governing plant science, in an effort to remove regulatory barriers and ensure evidence-based policy-making.

Filling in for an absent Elizabeth Warham from UK Trade and Investment, Stefan Kepinski from



NFU Vice-President Guy Smith gave the opening keynote lecture at UK PlantSci 2015.

Photo: Lisa Martin

Leeds represented the 'Funding' working group, and Simon Leather from Harper Adams University represented 'Training & Skills'. Professor Leather commented that he would like to see "every young person engaging in plant science at each stage of education". MEP McIntyre supported this by stressing the need to convey to biology students, "you don't have to be a medical doctor to save the planet."

Day 2 of the conference kicked off with a keynote lecture from Professor Caroline Dean at the John Innes Centre, who gave a fascinating overview of her decades of research into the epigenetic mechanisms regulating flowering.

Then we looked at cells. Jill Harrison from Cambridge explained how computational modelling is yielding new insights into branching

pattern in mosses; Birmingham's George Bassel revealed how he uses the same computer modelling software used to create Pixar movies to explore plant cell growth dynamics; and Heather Whitney from Bristol gave a beautifully illustrated and very interesting talk about iridescence in flowers.

In the Roots and Soil session, chaired by Bill Davies from Lancaster University, Malcolm Bennett from Nottingham enlightened us about adaptive responses of the roots to water stress, while Miriam Gifford from Warwick explained her research into using cell-specific genomics to understand the scale of symbiosis. Emily Schofield, an undergraduate student at Kew Gardens, gave a confident talk about her student project to understand fungal symbionts and their role in the germination and seedling development of British orchids – what a fantastic opportunity for an undergraduate!

Other root researchers who gave talks in this session were Carly Stevens from Lancaster, Yoselin Benitez-Alfonso from Leeds, and Beth Penrose from the NERC Centre for Ecology and Hydrology.

As usual, a highlight of the meeting was the 'Future Generations'



Former GARNet committee member Malcolm Bennett was at UK PlantSci 2015 to talk about root phenotyping work at the University of Nottingham's Centre for Plant Integrative Biology.

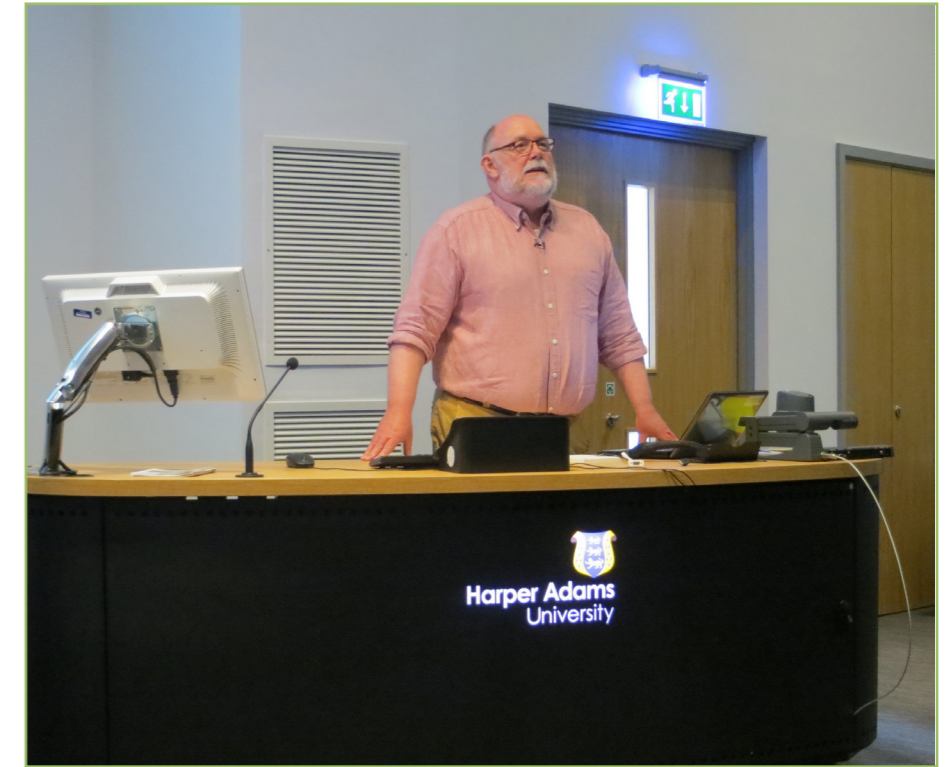
Photo: Lisa Martin

session, in which PhD students and early career plant scientists from all over the UK had the chance to win a cash prize for giving a short talk about their work. The ten-minute presentations covered all sorts of subjects, including how purple tomatoes can reduce the risk of cardiovascular disease, the development of bolt-resistant sugar beet, and a talk from the University of Warwick's very own "rocket scientist" Jemma Taylor!

The prizes, however, went to Kirsty McInnes from the University of Glasgow, who spoke about her PhD project to understand the molecular basis of herbivore resistance in *Brassica napus*, and to Ruth Le Fevre, a postdoc in the Schornack group at The Sainsbury Laboratory, Cambridge, who presented her work on the plant traits underpinning microbial colonisation in barley. Congratulations to both!

The final session of the conference was given to ecology, environment and biodiversity, and was in itself a very biodiverse session! After an introduction by session Chair Kathy Willis from the Royal Botanic Gardens at Kew, we heard from Professor Dieter Helm via video link. Dieter is a Professor of Energy Policy at the University of Oxford – he gave a thought-provoking synopsis of 'natural capital' and its value to the UK economy, suggesting that 'green taxes' on herbicides, pesticides and nitrates may help to fund the restoration of damaged ecosystems and habitats.

Next, Nigel Maxted from the University of Birmingham talked about UK efforts to preserve and conserve crop wild relatives. This linked



Nigel Maxted from the University of Birmingham gave an interesting talk about conserving crop wild relatives in the UK. Photo: Lisa Martin

seamlessly into the next talk by York's Tomasz Czechowski who explained how crop wild relatives are helping to develop resilience in rice to unpredictable water availability. Jonathan Millet then gave a presentation on his research into *Drosera* – a carnivorous plant that was allegedly Darwin's favourite – and finally, to wrap up the conference Mike Garratt talked about bees! Though not strictly plant science, we wouldn't get very far in plant science without pollination, so Mike's talk provided a useful and interesting insight into sustainable crop pollination services.

Thanks to Mimi Tanimoto for organising such a varied, insightful and enjoyable conference, and to Harper Adams University for hosting.

Videos of talks given at the conference can be found online here: <http://tinyurl.com/plantscivids>.

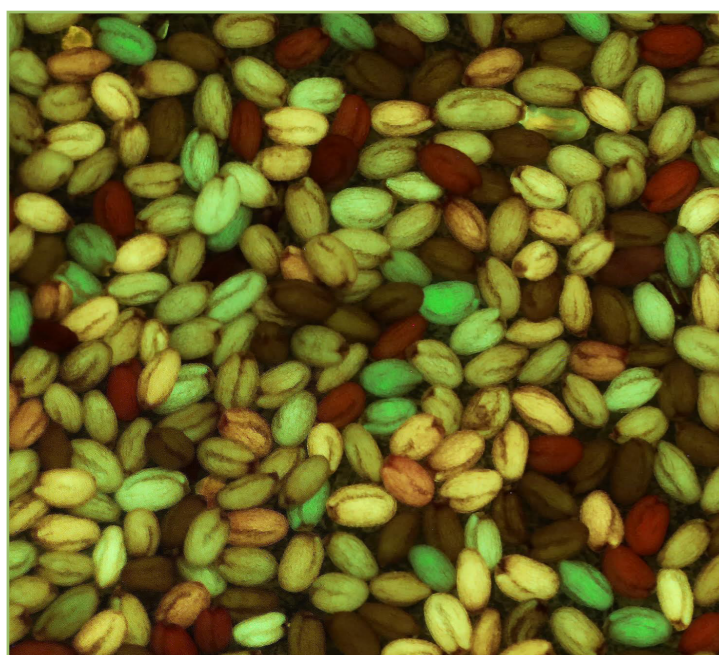


Traffic Lines: New Tools for Genetic Analysis in Arabidopsis

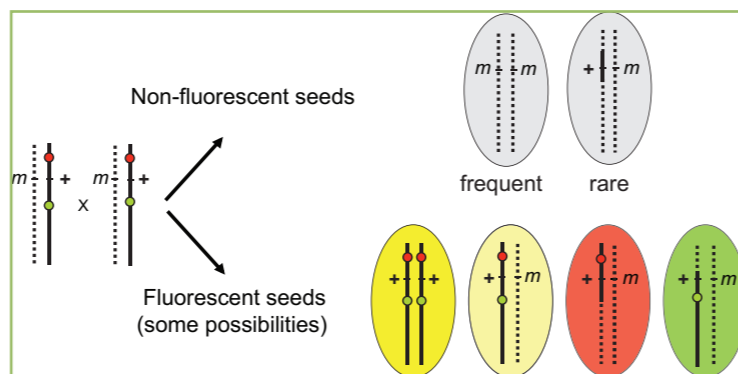
Professor Scott Poethig
University of Pennsylvania, Philadelphia, USA
poethig@sas.upenn.edu

What if you could determine the genotype of a seed before planting? No more PCR genotyping, no more progeny testing, no more dealing with more plants than you actually need! And what if you could identify recombinants in any region of the genome visually, without PCR? This would make it easy to remove unwanted second site mutations from a gene of interest, or to introgress short chromosome segments from one ecotype into another. This is now possible using a tool called a **Traffic Line** (TL).

A Traffic Line is a transgenic stock containing a linked pair of eGFP and dsRED transgenes expressed under the control of a seed-specific promoter. These markers are expressed in dry seeds and are dosage-sensitive, making it possible to identify seeds homozygous or heterozygous for the transgenes by their fluorescence intensity. Mutations placed in trans to a GFP-dsRED



F2 seeds from a plant heterozygous for a TL display a variety of colors and fluorescence intensities.



Some of the expected genotypes in the F2 progeny of a plant heterozygous for a TL.

marked segment can be followed in genetic crosses by selecting seeds with either no, or low, fluorescence.

For example, most of the non-fluorescent F2 seeds produced by a plant heterozygous for a TL will be homozygous for the mutation on the unmarked chromosome, and most of the seeds with low GFP and dsRED fluorescence will be heterozygous for the mutation. In other words, a TL functions like a balancer chromosome, except that it does not block recombination.

Because the segment flanked by eGFP and dsRED undergoes recombination, TLs are useful for a variety of purposes that require the ability to detect recombination within a defined chromosomal region. For example, linked second site mutations can be removed from a mutation of interest by placing the mutation in trans to a TL, and selecting for recombinants within the marked segment. TLs are also useful for QTL analysis because they facilitate the production of near-isogenic lines containing small chromosome segments from a region of interest.

Sets of TLs covering most of the Columbia and Ler genomes are described in Wu et al, 2015, and are available from the ABRC.

Wu G, Rossidivito G, Hu T, Berlyand Y, Poethig RS. Traffic lines: new tools for genetic analysis in *Arabidopsis thaliana*.



TRiP: Rhythms in Plants

Dr Kathleen Greenham, Dr Ping Lou,
Prof. C. Robertson McClung,
Dartmouth College, New Hampshire, USA,
kathleen.m.greenham@dartmouth.edu

One of the most startling examples of plant preparedness is how they anticipate tomorrow. The ability to synchronise physiological processes with the external temporal environment, like dawn, is dependent on an internal biological clock. The first scientific observations documenting the existence of an internal clock were in 1729 by the French astronomer de Mairan who observed the rhythmic leaf movement of a Mimosa plant. The persistence

of this movement in constant darkness provided the first evidence of an internal mechanism that drives this process (de Mairan 1729). A century later, the periodicity of this movement was found to be approximately 24 hours in length, revealing an internal mechanism synchronised with Earth's light/dark cycle (de Candolle 1832). Evidence for endogenous rhythms in animals did not emerge until much later (Kiesel 1894). These early observations of leaf movement paved the way for the emergence of circadian biology. The term 'circadian', coined in 1959 (Halberg et al. 1959) from the Latin words 'circa' (about) and 'dies' (day), is used to describe the 24 h period that signifies one complete cycle of these biological rhythms.

Nearly three centuries later, we are still using leaf movement as a measure of circadian clock periodicity. Confirming evidence is mounting for the importance of the clock in maintaining plant fitness through the temporal gating of physiological responses to the environment (Seo & Mas 2015). In addition, the molecular study of critical traits selected during crop domestication has uncovered variation in circadian clock genes among important cultivars (Nakamichi 2015). The complexity of the circadian oscillator is highlighted by the extensive autoregulatory feedback loops driving the rhythmic behavior of genes, proteins, metabolites and enzymes (Fogelmark & Troein 2014, Hsu & Harmer 2014). To tease apart the components

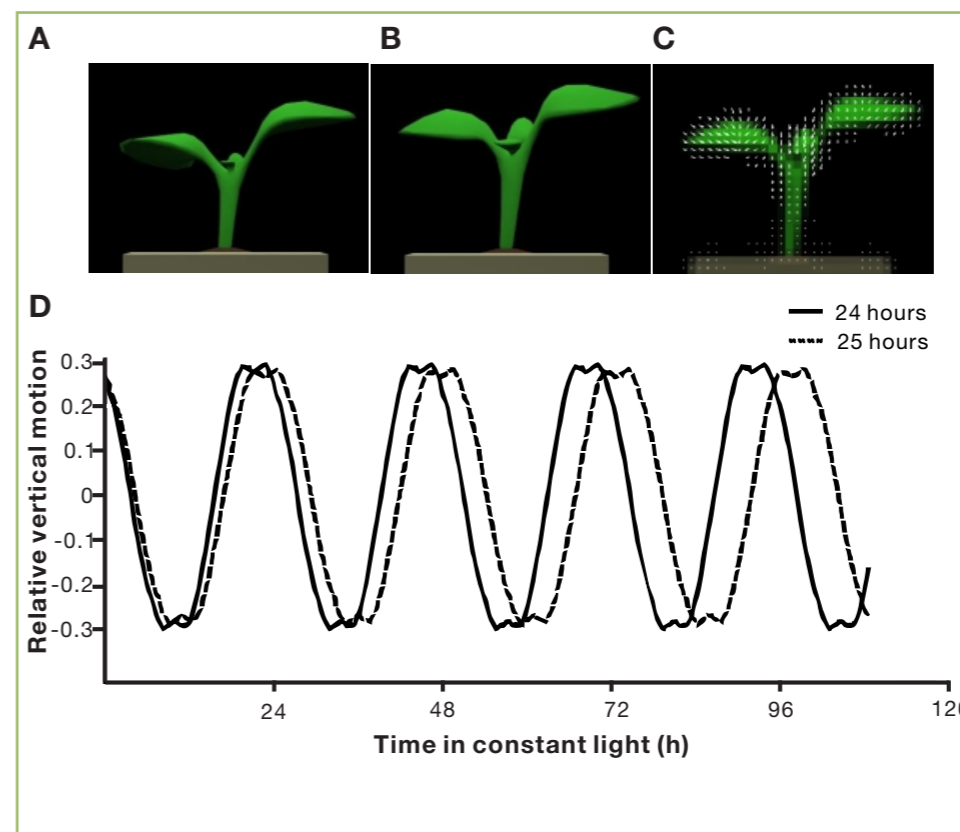


Figure 1. TRiP successfully detects leaf movement from CG plant model with known period. Images of growing Arabidopsis seedlings were digitised and used to animate a time series of leaf movement. (A) A trough (T=12, subjective dusk) and (B) a peak image (T=24, subjective dawn) keyframe were each used to produce two animations that demonstrated plant leaf movement with defined circadian periods, which were assessed using TRiP. (C) Visualisation of motion field at T=24. (D) Traces of simulated leaf movement with periods of 24 h or 25 h measured with TRiP.

driving the response to a given stimuli, existing natural variation in circadian clock performance can be explored. The recent emergence of large mapping populations across diverse plant species has expanded our capacity to assess natural variation of plant responses to the environment. The ability to sequence these populations has led to extensive genetic maps, while the development of cost effective high-throughput phenotyping techniques lags behind. In order to screen these large populations for clock variation, we developed an automated motion estimation program called **TRiP (Tracking Rhythms in Plants)** for estimating circadian period based on leaf movement (Greenham et al. 2015).

TRiP is a Matlab-based program that applies a motion estimation algorithm to a series of plant images and models the vertical motion between sequential frames. To validate TRiP, we used a 3D computer-generated model of a plant with a known motion, reconstructed from top, front and side view images of an *Arabidopsis Col-0* seedling taken every 10 min over 5 days (Figure 1A-C). TRiP successfully estimated the correct circadian period from the 3D model (Figure 1D).

To demonstrate TRiP's versatility we analysed leaf movement of six diverse plant species: *Arabidopsis thaliana*, *Brassica rapa*, *Cleome violacea*, *Glycine max*, *Mimulus guttatus*, and *Solanum lycopersicum* (Figure 2). To apply TRiP to a large population we constructed a step-shaped imaging platform that allowed us to image up to 118 plants per camera. Using 14 cameras we are able to image up to 1652 plants a week. Further, we analysed leaf movement of an *Arabidopsis* RIL population developed from a cross between Col-0 and Jea. The resulting period values were used to identify three putative and two suggestive quantitative trait loci (QTL), several of which contain known circadian clock genes that have been identified

in previous QTL mapping studies for circadian period (Greenham et al. 2015).

The main advantage of using TRiP over other existing methods is the limited amount of user input during the analysis. There is no need to select the leaves to be analysed or to attach polystyrene balls to the tips of the leaves for tracking. TRiP detects both cotyledons and tracks their movement simultaneously, thereby generating one motion trace per plant. In addition, TRiP has tracked *Arabidopsis* plants from cotyledon through the emergence of true leaves. This convenience is necessary when the growth rate in a population varies, something commonly encountered in collections of natural accessions, and within and between RIL populations.

As well as the motion estimation algorithm, we also applied a grid-based cropping function that takes each camera image stack as input and crops the images using the grid coordinates supplied, and outputs the image stack for each plant to a directory to be analysed by the motion estimation function. TRiP will work on any jpeg image with a minimum per plant pixel count of 10,000 (100 x 100 pixels).

TRiP source code is available as a supplemental file with the manuscript (Greenham et al. 2015) or can be downloaded from GitHub (<http://github.com/KTgreenham/TRiP>). Instructions for running TRiP on a sample set of images provided with the code can be found in the README file. The circadian period is estimated using a single frequency fast Fourier transform-non-linear least squares (FFT-NLLS) method; however, the output from the motion estimation step can be analysed using other methods (see BioDare: Moore et al. 2014). Included with the manuscript are video files for the six plant species imaged, as well as the 3D model used for TRiP validation (Greenham et al. 2015).

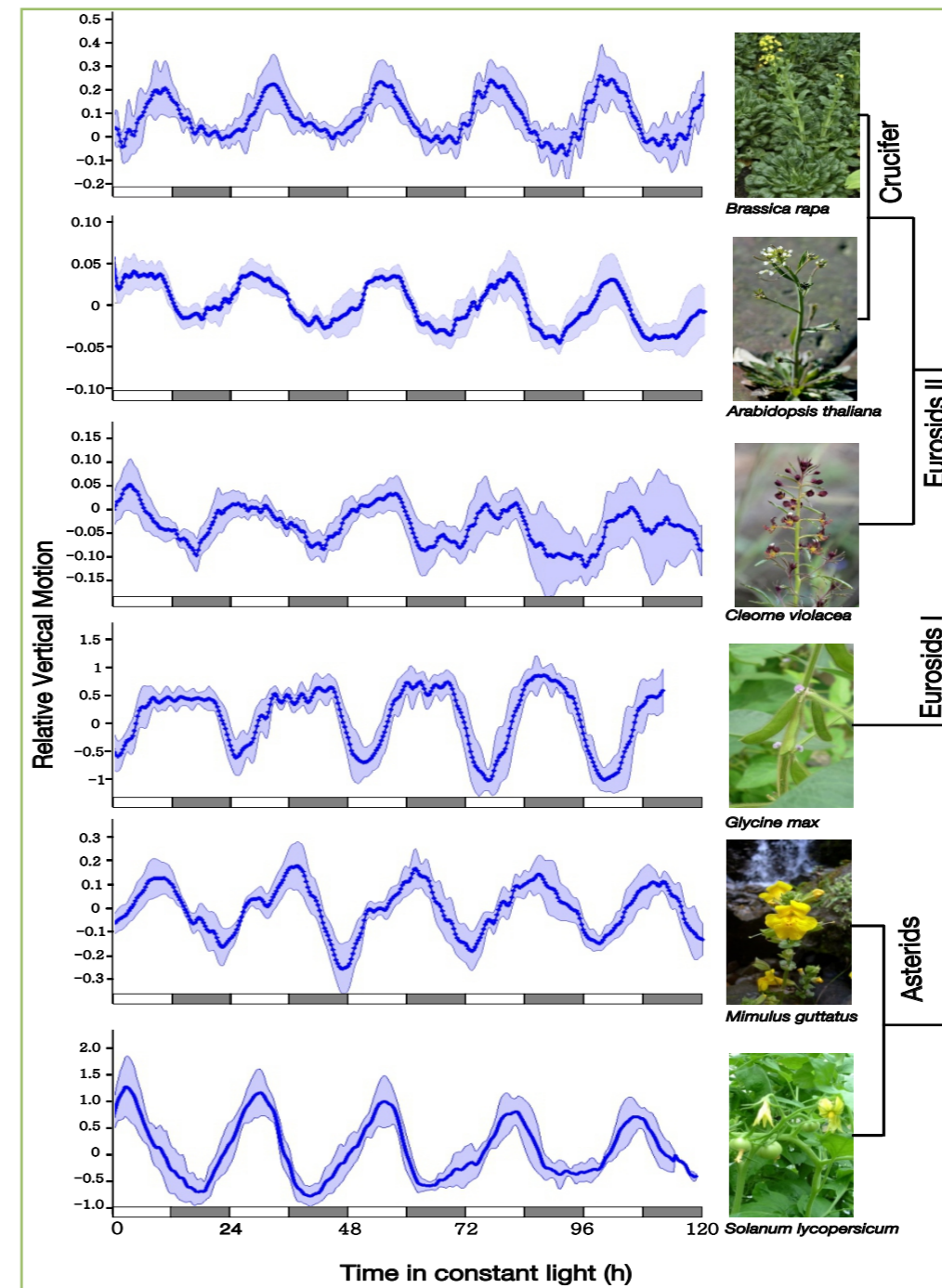


Figure 2. TRiP can be applied to a wide range of plant species with varying leaf morphologies. Plants were imaged every 20 minutes for 5 days under constant light and temperature at 20°C except *Glycine max*, which was imaged at 25°C. For each species, the relative vertical motion traces are an average of 8 individual plants (except *Solanum lycopersicum*, where n = 5) analyzed over 5 days. Shading indicates the standard deviation. White and grey bars below each trace indicate subjective day and subjective night, respectively, defined by the entraining photocycle. The phylogenetic relationships among the species are indicated at the right.

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 Fascination of Plants Day, Liverpool World Museum



Geraint Parry
GARNet Coordinator
geraint@garnetcommunityorg.uk

On May 16th the World Museum in Liverpool hosted its third Fascination of Plants Day (FoPD) in collaboration with scientists and educators from the University of Liverpool (UoL).

The organisation of an indoor event is one of the few reasons to hope for a rainy day but sadly it was nice and sunny in Liverpool! Nevertheless, about 500 visitors passed through the exhibition, each spending at least 20 minutes learning about many aspects of plant science.

Everyone likes a free gift so the exhibit from Ness Botanic Gardens was very popular! Rose Froud and Andrew Lambie showed visitors of all ages how to make pots out of newspaper, then helped them plant either sunflowers or runner beans.

Dr Geraldine Reid from the Museum's Botany Department set up a microscope where the visitors



Rose Froud from Ness Gardens helping the Beavers to make plant pots out of newspaper!

Photo: Donna Young, World Museum.



Before the doors opened!



Extracting strawberry and blackberry DNA with members of the Life Science Outreach Team. *Photo: Donna Young*

were challenged to try and find locally discovered new varieties of diatom. Chris Jones, a researcher at the museum manned the 'scope and wowed the kids as they searched for little creatures in seawater collected from the Mersey.

The botanical collection at the World Museum is an (mostly) undiscovered wonder of Liverpool, with 400,000 specimens kept in the basement



A young Beaver trying to find diatoms with Chris Jones: *Photo Donna Young*

repository. Elsewhere, techniques of plant science ancient and modern collided as dried and mounted specimens from the botanical collections were situated alongside a DNA sequencer that UoL researchers had used to sequence the wheat genome.

This machine has recently been donated to the museum for future exhibitions and formed the second stage of an exhibit that allowed (mostly) kids to extract strawberry DNA and then 'load' it onto the sequencer! Rumours are unfounded that this is the start of the octoploid Strawberry Genome Project...

The group from UoL was led by Dr Geraint Parry and Professor Anthony Hall, ably helped by members the Life Science Outreach team.

Overall it was extremely gratifying to see plenty of smiling faces leaving the exhibition clutching their DNA extractions and temporary plant pots! Hopefully they'll be back again next year!

 Spotlight on the University of Warwick

Katherine Denby,
kj.denby@warwick.ac.uk



Plant science research at the University of Warwick is characterised by the breadth of expertise - from fundamental molecular mechanisms to projects with direct application to industry. We have world-class basic science in signalling, gene regulation, development, plant-environment (microbes, virus, soil) interactions, and evolution through to pest management, crop genetics and genomics underpinning the development of new varieties.

Warwick has a strong interdisciplinary tradition leading to expertise in bioinformatics, systems and synthetic biology. We lead a project to establish the iPlant cyberinfrastructure in the UK and plant science is an integral part of the new Warwick Integrative Synthetic Biology Centre. The Warwick Crop Centre is an internationally recognised centre for strategic and applied research focusing



Robin Allaby uses a variety of techniques from biology and archaeology to study the evolution of plants.

on sustainable agriculture, horticulture and food security.

Warwick offers a range of excellent plant science training opportunities through MSc courses in environmental science, sustainable crop production and food security, our BBSRC PhD training programme (Midlands Integrative Bioscience Training Partnership), and the new Innovative Food Systems Teaching and Learning programme.

Robin Allaby
rg.allaby@warwick.ac.uk
www.warwick.ac.uk/lifesci/research/archaeobotany

Plant Evolution

My research focus is the evolutionary dynamics associated with the domestication process on several levels of organisation: the gene, the genome, the population and the selective environment in which the population exists.

Genetic information is utilised directly from both archaeological and modern samples, and bioinformatic approaches developed for high throughput analysis. The empirical work is balanced by a theoretical approach, through computational biology, in which the complex evolutionary system is studied which gives rise to the patterns of genetic diversity observed.

Using this *in vitro* and *in silico* two-pronged approach questions about where crops come from are answered, and how plants such as crops become locally adapted to environmental conditions. Such information may help in the future to produce crops which are better adapted to a wider range of conditions: the key to a sustainable future is to understand the past.

Charlotte Allender
charlotte.allender@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/callender
www.warwick.ac.uk/golgru
www.warwick.ac.uk/vegin

Genetic diversity in crops and related wild species and conservation of plant genetic resources

Understanding and conserving genetic diversity in crop gene pools is essential in order to ensure novel allelic variation is available for the development of improved crop varieties, as well as aiding the understanding of issues in gene flow, evolution and domestication.

A major focus is the management of the UK Vegetable Genebank, part of Warwick Genetic Resources Unit, which actively conserves and makes available the genetic diversity in vegetable crops such as Brassica, onions, lettuce and carrots. Almost 14,000 seed samples are conserved under long-term storage conditions and supplied upon request to researchers, breeders and growers across the world. Seed requests are welcomed – see the website www.warwick.ac.uk/go/gru for details.

The seed collections have formed the basis of a linked research programme with projects investigating gene pool diversity and the origin of *Brassica napus*, domestication of carrot (*Daucus carota*) and methods for maintaining F1 hybrid varieties in seed collections.

Material from the UK Vegetable Genebank is also the basis of the resources developed within the Vegetable Genetic Improvement Network (VeGIN) project, which aims to develop a pre-breeding



Charlotte Allender is Manager of the Warwick Genetic Resources Unit - follow her on Twitter @WarwickGRU.

pipeline which facilitates the identification and deployment of novel traits in the improved crop varieties required to support sustainable production systems.

Guy Barker
Guy.Barker@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/gbarker

Comparative genomics and genome organisation and identification of genes underlying quantitative traits.

Our interests include exploring the genes and mechanisms underlying quality and other traits related to health and wellbeing. Recently we have also been interested in looking at the isolation of secondary metabolites from different plant species for both pharmaceutical and nutraceutical use.

Other research interests include sustainable production, exploring the genes and mechanisms underlying fatty acid quality and utilising diversity within the gene pool to understand gene expression and regulation of biodiversity as well as recovery of bio-energy from ligno-cellulolytic waste. We have been involved in sequencing Brassica genomes and have used transcriptomics to study crop evolution in *Brassica oleracea* crops.



The Barker lab looks at the genetics of healthful traits in crop plants.

Gary Bending

gary.bending@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/gbending/

Plant-soil-microbe interactions in the rhizosphere

The Bending group studies the factors that shape the assembly and function of microbial communities (including fungi, bacteria and protists) in the rhizosphere, and how this influences plant growth and nutrition. They use largely field and landscape based approaches coupled with next generation sequencing, including metatranscriptomics, to unravel plant-microbe interactions. They are also interested in understanding the plant genetic determinants of microbial community assembly in the root zone.



Gary Bending: how do microbial communities form and function in the rhizosphere?

This includes traits controlling the composition of root exudates, presenting the potential for engineering communities to promote beneficial functions.

John Clarkson

john.clarkson@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/jclarkson/

Plant pathology

John's research team is focused on investigating some of the more intractable soil-borne diseases caused by fungi/oomycetes such as *Sclerotinia*, *Fusarium* and *Pythium* species, primarily on vegetable crops. Various aspects of pathogen biology, epidemiology, ecology and diversity are being studied with the aim of improving the understanding of these pathogens and finding solutions for their control.



John Clarkson

Identifying plant resistance to many of these diseases is challenging, but in a recently awarded BBSRC HAPI project, the genetics of onion lines highly resistant to *Fusarium oxysporum* is being examined as well as developing an understanding of the effectors involved in pathogenicity.

Similarly, John is involved in another HAPI project led by Katherine Denby where resistance to *Botrytis cinerea* and *Sclerotinia sclerotiorum* is being investigated in lettuce. This is aligned to John's recent research work on *Sclerotinia* resistance in Brassica and on defining pathogen population structure, which also resulted in the

first report of the related species *S. subarctica* in the UK.

The team also carries out more applied research, most often with industry involvement, and includes developing soilborne disease management approaches such as biofumigation, disease forecasting and biological control.

Rosemary Collier

rosemary.collier@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/rcollier/

Development and application of Integrated Pest Management strategies for horticultural crops.

Rosemary undertakes research on the development and integration of components of Integrated Pest Management (IPM) strategies for crop pests, particularly field vegetables. She works on a range of pest insects including Diptera, Lepidoptera, Coleoptera and Homoptera and has also worked on slugs.

Her work includes the development of pest monitoring systems and weather-based forecasting systems, based on mathematical models, to support the application of control measures. In



Rosemary Collier's research focuses on the development of Integrated Pest Management strategies.

this respect she is currently leading a collaborative project on the migrant Lepidoptera infesting Brassica crops.

Rosemary and her group undertake research on a variety of control methods which include identification of novel uses for insecticides and biopesticides on horticultural crops, often in collaboration with the companies who have developed the products. She also collaborates with colleagues at the University of Warwick and elsewhere to identify novel sources of resistance to pest insects in plant material obtained from the Warwick Vegetable Gene Bank as part of VeGIN.

Rosemary works on other non-insecticidal methods of control such as cultural control (e.g. crop rotation, companion planting) and physical control using barriers (e.g. crop covers, insect fences). She is also interested in how growers use information to enable them to manage crop pests more effectively and this includes the dissemination of information, for example, via the HDC Pest Bulletin: <http://www3.syngenta.com/country/uk/en/AgronomyTools/HDCPestBulletin/Pages/HDCPestBulletin.aspx>.

Katherine Denby

k.j.denby@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/kdenby/denbylabwordpress.com

Systems biology of plant disease resistance

The Denby group is interested in plant disease resistance and using systems biology approaches to unravel how plants respond to pathogen infection, and the regulatory networks underlying the defence response. We have focused on transcriptional networks driving the response to the fungal pathogen *Botrytis cinerea*, identifying key regulators of the defence response and how they interact.



Follow Katherine on Twitter @Katherine_Denby.

We have developed computational tools to analyse large transcriptome data sets and to integrate information from multiple plant environmental stress responses. As part of the Warwick Integrative Synthetic Biology Centre we are re-engineering plant stress regulatory networks to enhance disease resistance.

We are also interested in translating our work into crops and have recently started a project to improve disease resistance against necrotrophic fungal pathogens in lettuce.

Bill Finch-Savage

bill.finch-savage@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/bfinch-savage/
www.warwick.ac.uk/lifesci/research/seeds/science/

Understanding seed behaviour in response to environmental signals.

Seeds are the means of delivering genetic information across space and time, not only in natural systems and conservation programmes, but also, crucially, in agriculture where the world seed market is predicted to soon exceed US\$40 bn. Our interests focus on



Bill Finch-Savage's group seeks to improve seed performance in terms of environmental signals.

understanding and enhancing the performance of seeds in this process and subsequent seedling establishment. The latter phase is fundamental to efficient crop production and the species balance of natural plant communities.

Of particular interest is the response of seeds to environmental signals and the control of seed germination timing through dormancy, which dominates seedling establishment. To understand these responses it is essential to integrate knowledge from laboratory studies with understanding of the soil surface complex in which the seed functions. Nevertheless, seed ecology, physiology and molecular biology have tended to be studied by separate scientific communities and with limited reference to the soil physical environment. The vision behind this work has been to bring these different disciplines together to develop a better understanding of seed behaviour.

Lorenzo Frigerio

l.frigerio@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/lfrigerio/

Biogenesis of organelles of the plant secretory pathway.

Most plant proteins that are essential for animal nutrition are synthesised and stored in the secretory pathway - a system of dynamic, membrane-bound organelles, which comprise the endoplasmic reticulum (ER), Golgi complex, endosomes and vacuoles. In many seed crops, proteins are stored in special



Lorenzo Frigerio

vacuoles called protein storage vacuoles (PSV). In the seeds of several cereal species, the ER itself can accumulate large amounts of storage proteins.

The Frigerio team is currently addressing the following questions: 1) What determines the distinctive shape of the endoplasmic reticulum? 2) How does ER shape relate to its biosynthetic function? 3) How do protein storage vacuoles originate in dicot seeds?

The group is using a combination of genetics, biochemistry and live confocal and electron microscopy. Recent efforts include the study of proteins of the reticulon family, which have a key role in shaping the tubular ER network, and the use of an inducible system to stimulate the biogenesis of protein storage vacuoles in non-seed tissues.

Miriam Gifford

miriam.gifford@warwick.ac.uk
www.warwick.ac.uk/lifesci/research/systemsdev
 Twitter: @giffordlab

Molecular mechanisms of plant developmental plasticity

Plant plasticity is amazing! Plant development is highly tuned to the environment both at the whole plant or systemic level, and at the local cellular level. Gifford lab work seeks to understand how plasticity of gene expression responses in specific cell types underlies root responses to nitrogen in the environment. To do this a key method is Fluorescence-Activated Cell Sorting (FACS) to enable isolation of specific populations of cells - single cells, cell types or cells in a particular response state. Transcriptomics, and more recently cell type specific proteomics is used to identify the molecular responses to symbiosis and environmental perturbations. High resolution temporal and spatial molecular profiling enables



Follow Miriam's lab on Twitter @giffordlab.

regulatory network inference, and recent work has shown the importance of gene expression pattern specialization in both cell type-specific and coordinated environmental responses.

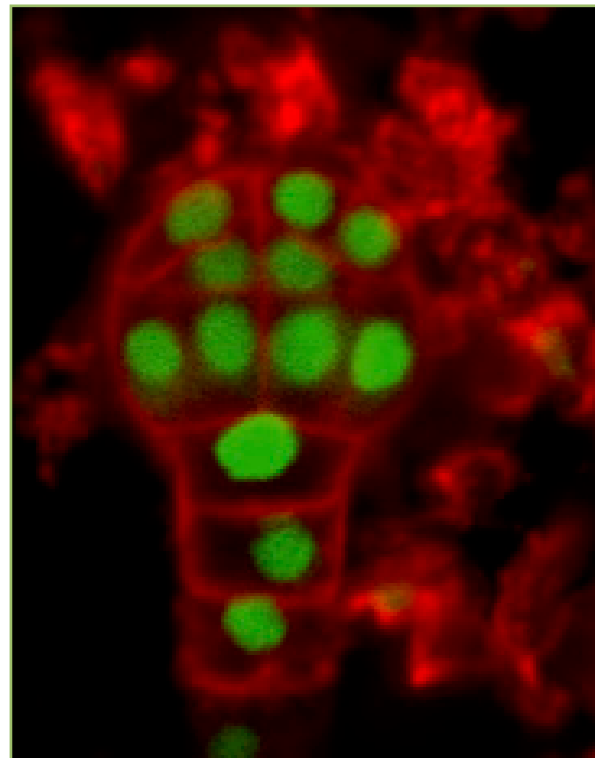
At the phenotypic level the lab is investigating connections between plant root architecture forms: lateral root development and nodule formation during nitrogen-fixing symbiosis. Work in crops includes analysis of abiotic responses in tomato and Brassica roots. The long-term aim is to combine genomics, plant development and plant-microbe interactions with synthetic biology tools to transfer nodulation to staple crop species.

Jose Gutierrez-Marcos

j.fgutierrez-marcos@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/jgutierrez-marcos/

Genetic and epigenetic control of plant development

We are interested in understanding how development and environmental responses are



The Gutierrez-Marcos lab uses model plant species to understand the coordination of developmental environmental and responses.

coordinated across diverse cell types in multicellular organisms.

We use plants (Arabidopsis, maize, rice and Lotus) as model systems to address this question by investigating (i) the role of cell-cell communication in coordinating development, (ii) how parasitic organisms can manipulate developmental programmes and (iii) how the environment influences the epigenome and establishes short term stress adaptation.

Eric Holub
eric.holub@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/eholub/

Molecular genetics of plant-microbe interactions, pathogen genomics, and translation into crops for sustainable disease control

The Holub group has a keen interest in translating advances from molecular genetics into crop



Though his roots are in the USA, Eric Holub's research now has a focus on British beans.

varieties that are better adapted for secure food supplies. Our research focuses mainly on elucidating the molecular basis of broad-spectrum disease resistance, which stems from Eric's training in the US as a legume pathologist where he developed tetraploid alfalfa with multi-genic resistance to a root-rotting complex of oomycete pathogens.

This has had major impact on the dairy industry from widespread cultivation of durable resistant cultivars, which are extending productivity of an important perennial crop for soil conservation in rotation with maize under stress of harsh winters and wet soils.

Eric's postdoctoral research began as a geneticist at what is now East Malling Research, where he established Arabidopsis as an experimental model for investigating the molecular basis of resistance to natural parasites (*Hyaloperonospora arabidopsidis* and *Albugo laibachii*). This

generated seminal publications with UK and international collaborators on R-gene mediated resistance, defence-signalling and pathogen avirulence.

Eric then moved to HRI-Wellesbourne where he used Brassica pathogens (*Albugo candida* [White Rust], and *Xanthomonas campestris* [Black Rot]) to investigate non-host resistance in Arabidopsis. This will translate into crop solutions with BBSRC funding to exploit the advances from Arabidopsis in oilseed mustard (*B. juncea*), through collaboration with scientists in India and the UK and with English mustard growers.

In 2011, Eric renewed his legume research by reviving heritage germplasm from UK Phaseolus (haricot) bean breeding programmes, with the aim of improving sustainable production systems for UK growers by the addition of a novel short season, UK-adapted legume into rotations.

Stephen Jackson
stephen.jackson@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/sjackson/

Control of flowering time in model and crop plants

Current research activities in the Jackson lab include: i) Identification of key genes controlling flowering time in crop plants in the field. Delayed bolting (flowering) is desirable in leafy vegetable crops to increase yield, holding ability in the field and reduce wastage due to premature flowering. NextGen sequencing is being used together with bulk segregant and co-segregation analysis in lettuce and rocket to identify SNPs affecting flowering time in mutant populations.

ii) Functional mutation scanning of the FT protein *in vivo*. Viral expression has been used to express the Arabidopsis FT gene in tobacco and induce flowering. It has also been used as a rapid method to test the *in vivo* functional effect of individual amino acid substitutions at every position in the



The Jackson lab explores the control of flowering time in both models and crop plants.

FT protein, this has revealed novel functions of FT which could potentially make a significant contribution towards greater Food Security in the future.

iii) Gene-editing of flowering time genes. CRISPR/Cas9 approaches have been used to mutate a key flowering time gene in *Arabidopsis*. These approaches are being further developed and tailored for their use in manipulating flowering time in crop plants.

Alex Jones

alex.jones@warwick.ac.uk

www.warwick.ac.uk/lifesci/people/alexjones/

Signalling and trafficking



To flourish, plants need to adapt their growth and development in response to changes in their environment. Great progress has been made in determining the mechanisms of signal perception and an essential part of subsequent responses is rapid alteration of cellular membranes and

the cell wall. Beyond transport of materials, the internalisation and secretion of plasma membrane receptors can regulate signal duration and create local signal gradients. Thus, the trafficking of vesicles within a cell can be viewed as regulating overall cellular output. The mechanisms of how signal pathways and membrane trafficking events interact are largely unknown, but substantial rearrangements occur quickly, likely using pre-formed proteins.

Alex Jones

The question my research seeks to address is 'how are the mechanisms of membrane trafficking involved in normal growth and development, adapted for responses to biotic and abiotic stress?' My objective is to identify components recruited to responsive trafficking mechanisms and provide targets to uncouple negative impacts on plant growth during adaptation to stress.

My group uses the model plant *Arabidopsis thaliana*, advanced proteomics, confocal microscopy and biochemistry to identify protein complexes and post-translational modifications at the interface between signal perception and responsive trafficking.

Richard Napier

richard.napier@warwick.ac.uk

www.warwick.ac.uk/lifesci/research/molrecognition

Molecular recognition and transport: receptors and biosensors

The Napier group has a focus on hormone recognition, binding and selectivity and seeks to answer the question: how is TIR1 selective for IAA and not tryptophan?

The auxin receptor TIR1 and the related AFBs are expressed from insect cells and binding is explored in terms of kinetics (SPR Biacore), thermodynamics (ITC) and structure. This project is joint with Stefan Kepinski (University of Leeds) and Syngenta UK. It is known that auxin completes a nascent substrate binding pocket as it binds to TIR1. The substrates are the Aux/IAA transcriptional regulators which, on binding, become ubiquitinated through the ubiquitin E3



Richard Napier

ligase activity of TIR1. We are exploring how selectivity is conferred for different auxins and different Aux/IAAs, and how these two variables affect each other.

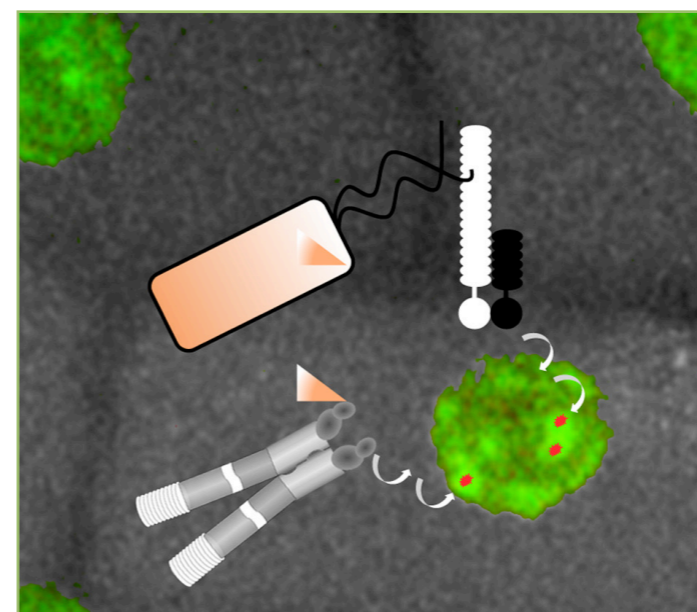
We also work with Noel Ferro Diaz (Institute for Physical and Theoretical Chemistry, University of Bonn) on the chemical definition of auxins and receptor selectivity profiles.

Biosensors, like receptor proteins, need to recognise analytes with appropriate sensitivity and selectivity. The group is developing hormone sensor domains in order to generate experimental plant hormone biosensors. We are working with Prof Nick Dale, a neurobiologist at Warwick and specialist in the development of purine biosensors, to develop a microelectrode-based biosensor for cytokinins. This work is in collaboration with Ivo Frebort (University of Olomouc). We have also developed SPR as a generic immunosensor platform for real-time phytohormone sensing, testing it for both ABA and auxin.

Dr Vardis Ntoukakis

v.ntoukakis@warwick.ac.uk

www.warwick.ac.uk/lifesci/people/vntoukakis/



Ntoukakis lab: how do plants respond to pathogens?

Plant-Microbe Interactions

Plants are constantly exposed to a variety of environmental challenges, including pathogen infection that can cause substantial agricultural losses. Minimising agricultural losses from pathogens holds the potential to substantially increase crop yield and decrease the cost of food production. Despite the scientific advances in the identification of immune receptors involved in pathogen perception a gap exists in our knowledge between recognition and downstream functional immunity. The Ntoukakis lab address the fundamental questions of how plant cells transduce receptor activation to downstream signalling in order to express a subset of genes and respond appropriately to pathogen infection. They employ transcriptomics and proteomics both in *Arabidopsis* and crop plants to dissect immune signaling cascades. They are particularly interesting in the role of chromatin remodeling, a conserved mechanism though out all plant lineages, in regulating gene expression during pathogen infections.

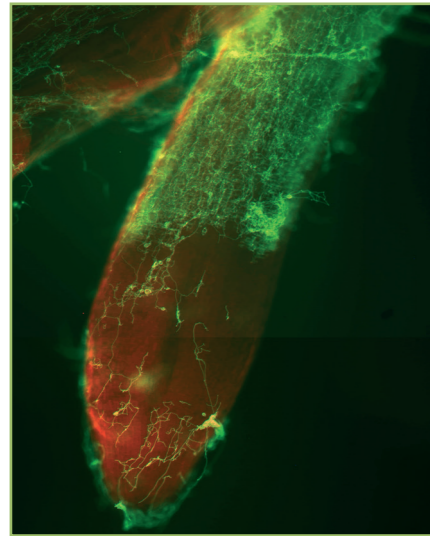
Dr Patrick Schäfer

pschafer@warwick.ac.uk

www.warwick.ac.uk/lifesci/people/pshafar/

Exploring beneficial symbioses to engineer customised plant signalling pathway

Research in the Schäfer lab aims at improving plant stress signalling and plant growth under stress. The mutualistic symbiosis of plant roots with the beneficial fungus *Piriformospora indica* is a powerful system for this purpose. As a result of root colonisation the fungus reprograms host signalling, which is the source of observed beneficial effects. The current focus is on understanding how individual cell types contribute to stress resistance and how *P. indica* modulates



Schäfer lab: plant stress signalling.

cell-cell communication to support growth under environmental stress. This knowledge will allow the targeted engineering of plant signalling to generate crops with improved stress characteristics. In this respect,

the broad host range of *P. indica* including all major crops and all members of the Brassicales is very significant.

Graham Teakle

graham.teakle@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/gteakle/

Crop genetic improvement

The Teakle group focuses on trait genetics and crop improvement. A key activity has been the development and curation of a range of genetic resources in the form of diversity sets and segregating mapping populations. These are derived from crop varieties, also also crop wild

relatives, which provide the opportunity to find novel alleles not present in crop gene pools. The genetic diversity encompassed by these resources is assessed using the latest genotyping and sequencing technologies.

The group extensively collaborates in the assessment of this germplasm, including currently funded projects with the Bending, Barker, Clarkson and Walsh groups at Warwick, and with external academic and industrial partners. Traits of interest include Turnip Yellows Virus resistance in Brassicas, rhizodeposition in oilseed rape in the context of its relationship to the interaction with the soil microbial community and yield, mineral nutrition of oilseed rape, Fusarium resistance in onions and insect pest resistance in lettuce and Brassicas. PhD students in the group are also carrying out work on pest and disease traits.

Brian Thomas

brian.thomas@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/bthomas

Genetic and environmental control of plant and crop development



Graham Teakle

The Thomas lab is particularly interested in the role of light and photoperiod in modulating developmental transitions,



The Thomas lab's interests are in the mechanisms by which plants interact with the environment and the practical consequences of these interactions.

such as juvenility, flowering and bulb initiation. Current research includes a study of the control of juvenility, which is defined as the phase of early development during which plants are not competent to respond to environmental signals that induce flowering in adult plants. The lab mainly works with *Antirrhinum* and *Arabidopsis*, where flowering is photoperiod-sensitive, and with Brassica where flowering is usually low-temperature-dependent. An important finding is that the length of the juvenile phase is regulated by light quantity, pointing to links between assimilate resource management and flowering capacity.

A second area of research is the genetic control of bulbing in *Allium* species in response to daylength. The working hypothesis is that the mechanism is analogous to the photoperiodic regulation of flowering. The group was the first to identify components in onion linked to the circadian clock that may be related to daylength sensitivity. Current work concentrates on the role of CO and FT-like genes in varieties with different daylength requirements. Brian also leads the VeGIN project.

John A. Walsh

john.walsh@warwick.ac.uk
www.warwick.ac.uk/lifesci/people/jwalsh/

Plant-virus interactions

Plant viruses are a major constraint on food production worldwide. Once crop plants are infected there are no effective ways of eradicating the virus. Most plant viruses are transmitted by insects and insecticides are rather inefficient in stopping the insects transmitting viruses to plants.

In the Walsh group, research is currently focused on exploiting natural plant resistance to viruses. In



John Walsh explores plant resistance to viruses.

order to understand such resistances and identify sources that are potentially durable, it is important to study virus diversity.

Broad-spectrum, recessive, potentially durable resistance has been identified to the potyvirus Turnip mosaic virus. The plant genes involved in the resistance and the novel mechanism underlying the resistance have been identified. This has allowed the development of within gene molecular markers which are now being used in marker-assisted selection to speed up the introgression of the resistance in to commercial plant varieties.

Integrated control strategies for controlling plant viruses infecting field crops are also being developed. These involve combining partial plant resistance to the virus, crop planting date, seed treatments and the monitoring of aphid vector activity and virus carrying status of aphid vectors to inform the timing of insecticide sprays. The group has particular expertise in arable and vegetable Brassica crop plant species and has released a number of plant lines with resistance to different viruses. They have also commercialised a number of diagnostic reagents for different plant pathogens.

SEB Plant Section Symposium

Stress Resilience Forum

Save
the date!
23 – 25
October 2015
Iguassu Falls,
Brazil

Achieving food security in a changing and unpredictable climate urgently requires a better understanding of the mechanisms by which plants interact with and respond to their environments. FAO predicts that the world population is estimated to rise to 9.6 billion by 2050. In order to feed an expanding global population in the next decades, it is crucial to increase food production in a sustainable manner, and to improve crop resilience to the stresses generated by reduced inputs and changes in climate.

In collaboration with the Global Plant Council, this SEB Symposium aims to bring together experts from across the world to discuss current research efforts in stress resilience, show case new approaches and technologies and build new networks and collaborations that will contribute to global efforts to develop crops that are better able to deal with fluctuating and stressful environmental conditions.

Confirmed speakers

Matthew Reynolds (CIMMYT, Mexico)
Sarah Gurr (University of Exeter, UK)
Jean Marcel Ribaut (CGIAR, Argentina)
Martin Parry (Lancaster University, UK)
Andrew Borrell (University of Queensland, Australia)
Vincent Vadez (ICRISAT, India)
Francois Tardieu (INRA, France)
Scott Chapman (CSIRO, Australia)
Ariel Orellana (Universidad Andrés Bello, Chile)
Katherine Denby (University of Warwick, UK)
Xin-Gunag Zhu (Shanghai Institute of Biological Sciences, China)
Bill Davies (Lancaster University, UK)
Jianbo Shen (China Agricultural University, China)
Roberto Tuberosa (University of Bolgna, Italy)



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