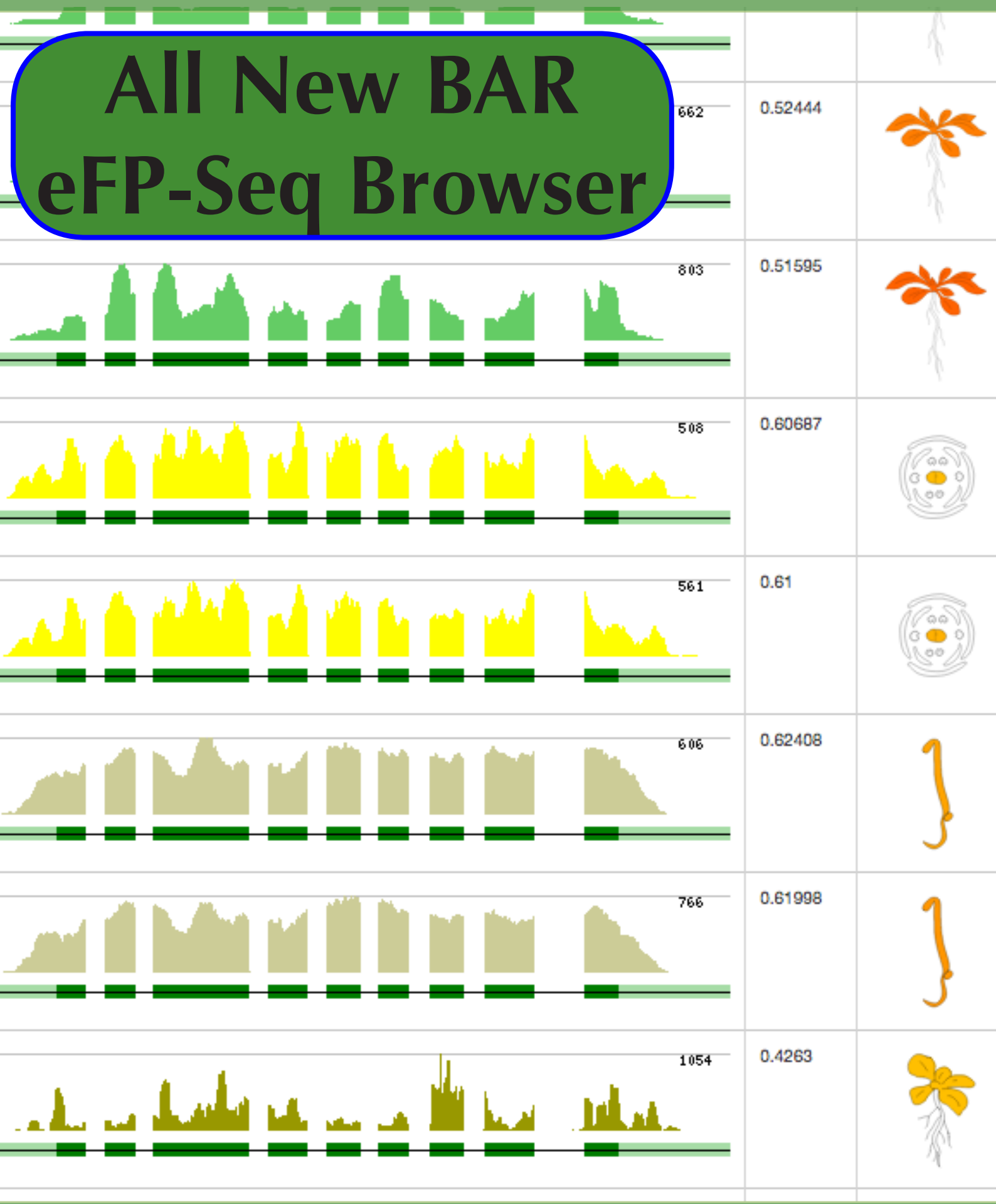
GARNish
December 2016: Edition 26

All New BAR eFP-Seq Browser



Welcome to the December 2016 Issue of GARNish

Geraint Parry

GARNet Coordinator

It has been a worrying six months for UK plant scientists with the uncertainly surrounding the Brexit vote. It remains to be seen how the vote works out for the availability of funding and the implications for collaboration but at GARNet we certainly hope that at the very least something close to the *status-quo* can be maintained. There are a number of UK plant scientists who benefit from individual European Research Council (ERC) fellowships including GARNet committee members Ian Henderson, Sabina Leonelli, Daniel Gibbs and Steven Spoel. These are amongst our best up and coming UK scientists so it is critical that this type of funding remains available for years to come. Time will tell how things work out but it is important that when possible, every scientist impresses on policy makers the critical importance that pan-European collaborations play in maintaining the excellence of UK plant science.

Somewhat surprisingly over the past few months I have heard many scientists talk about 'embracing the opportunities of Brexit'. This demonstrates that people are planning for an uncertain future and shows the adaptability of UK scientists who are ready to look for funding wherever it might appear. One area in which the current UK government might be more supportive than the EU is in the usage of GM technologies. It remains to be seen whether this will provide new opportunities or similar frustrations.

As ever we are delighted to welcome the new arrivals to the GARNet committee, Murray Grant, Jill Harrison and Daniel Gibbs. We are especially grateful to the 150 UK plant science academics who voted in the election, hopefully demonstrating that the work of GARNet is still appreciated by the community.

Contents

Special thanks to: Stuart Casson, Lisa Martin, Jonathan Carruthers, Nick Provart, Sarah Jose, Peter Venn, Nathan Hughes, the BBSRC grant holders and plant science researchers at Sheffield

We are also delighted to announce that Steven Spoel will take the role of GARNet Chairman from January and we look forward to his direction over the next two years. I'm looking forward to working with Steven in the organisation of a SEB symposium entitled '*From Proteome to Phenotype: role of post-translational modifications*' that will take place in Edinburgh next December. This will also feature a GARNet-sponsored plant proteomics workshop led by Dr Alex Jones so please look out for details and consider attending.

David Salt's tenure as GARNet chairman ends in 2016 and we would like to very much thank him for all the work he was done over the past few years. This culminated with the GARNetNatVar16 meeting that took place in mid December (see page 7). Under David's impetus we were able to bring together a world leading set of scientists to discuss the present and future of research on plant Natural Variation. David has recently moved to the University of Nottingham and is already involved with significant grant funding focussed on Food Security. We wish him all the best with that and his many other scientific endeavours. In addition we would like to offer great thanks to Nick Harberd and Anthony Dodd whose time on the GARNet committee also comes to an end.

Please enjoy this edition of GARNish that has reports from a variety of recent meetings, an update about the new BAR eFP Browser, details on recent BBSRC grant funding and a Spotlight article on the University of Sheffield.

Please follow @GARNetweets on Twitter and Facebook and also remember the '**Weeding the Gems' blog** at http://blog.garnetcommunity.org.uk.

Please contact Geraint (geraint@garnetcommunity.org.uk) if you would like to write a guest post.

Views expressed by authors in GARNish are their own opinions and do not necessarily represent the view of GARNet or the BBSRC.

The GARNet Committee

UK Plant Sciences Federation Update

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2016 has seen the initiation and development of a UKPSF project to produce a Roadmap for Plant Sciences in the UK. In March, UKPSF organised workshops in London and Edinburgh, at which experts from academia, industry, public bodies and NGOs identified future directions and challenges for plant sciences. The outputs of these workshops have been crafted into a draft document that will be shared with the community before the end of the year to capture feedback and recommendations. The Roadmap is a collaborative project, intended to serve the needs and reflect the thinking of those working in plant science, so the proactive input of all UKPSF member organisations is vital. The anticipated publication date of the Roadmap is mid-2017.

The PlantSci 2016 conference was also a big event for UKPSF, held in April at the John Innes Centre. Delegates' feedback was positive, but a trend of declining delegate numbers has prompted UKPSF to rethink the meeting design for future events. With work focused on the Roadmap at present, the UKPSF Advisory Group has discussed alternative plans for this year. Wide support from the Group greeted suggestions for a one-day meeting of keynote presentations and a networking reception to launch the Roadmap, in mid-2017, in place of next year's traditional conference.

Since November 2015, Alessandro Allegra has coordinated UKPSF activities with great aplomb as part of his role at the Royal Society of Biology (RSB). Alessandro has now moved on to begin his PhD studies at UCL. We thank him for his work with the UKPSF, and particularly with the Roadmap. Meanwhile, Jonathan Carruthers at RSB has taken on the role of secretariat for UKPSF. With the Roadmap coming together and exciting plans for its launch, UKPSF is looking ahead to a busy 2017, and to engaging with impact across the plant science community and beyond.

Global Plant Council Update

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The GPC held its annual general meeting (AGM) in Brisbane, Australia, in October. After a welcome from our Chair, Professor Barry Pogson from the Australian National University, and a minute's respectful silence to remember our former Board Member Professor Carl Douglas, who sadly passed away earlier this year, introductions were made and we got down to business.

The DivSeek initiative continues to grow in strength and numbers, with 67 partner organizations now committed to working together

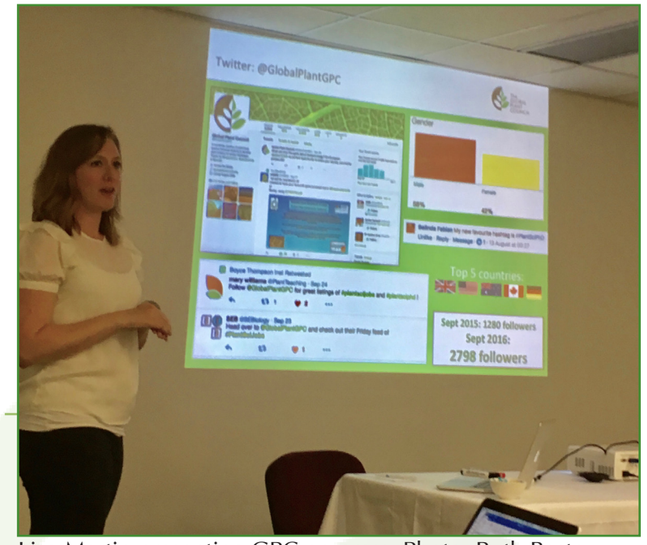
Unsurprisingly this is Brisbane Photo: Lisa Martin

to address genomic and phenomic data challenges in plant science. With funding from the BBSRC, the GPC's Executive Director Ruth Bastow has been providing essential coordination services specifically for this project, and with a Chair and Steering Committee in place, the initiative is making real progress; a number of working groups have been launched to actively engage DivSeek partners and help the initiative advance its mission and aims.

Our other major, current initiative is in the area of Stress Resilience. The GPC held a workshop and discussion forum in 2015 on the subject of 'Stress Resilient Cropping Systems for the Future', in conjunction with the SEB. This successful two-day event brought together experts in this area to share and showcase new research, tools and techniques. We are now turning our discussions from this meeting into a forthcoming white paper, and hopefully a commentary or two for publication in a high impact journal – we'll let you know when these have been launched!

With much help from our New Media Fellow and recent Bristol graduate Sarah Jose, the GPC's social media efforts have been tremendously successful this year. We now have over 3100 followers on Twitter (@GlobalPlantGPC), hundreds of 'fans' on Facebook (www.facebook.com/GlobalPlantGPC), and over 1200 subscribers to our monthly e-Bulletin (http://www.tinyurl.com/GPCsignup). Please follow, like and subscribe if you don't already!

Over the next few months we'll be looking forward to partnering with our friends at the European Plant Science Organisation (EPSO) to help promote the 2017 edition of Fascination of Plants Day (FoPD) around the world. Reflecting on the success of previous FoPDs, our representative from the Chinese Society of Plant Biology remarked that it has almost become like a national holiday in China!



Lisa Martin presenting GPC progress. Photo: Ruth Bastow

We're also hoping to get a new knowledge exchange initiative off the ground in 2017, led by our President Professor Bill Davies (Lancaster University). If successful in securing funding to progress this project, we hope to be involved with the development of an online training platform to transfer knowledge from the laboratory to the field – an exciting idea that will, we hope, be of invaluable benefit to communities in developing regions.

On the subject of funding, this is something the GPC is currently experiencing a shortage of… Our main source of income is its member organizations; yet the economic climate around the world continues to challenge us, and additional sources of funding will be required to ensure the continued sustainability of the GPC.

If you haven't yet bought your colleague a Secret Santa present and would like to help support the GPC, you can gift a one-off or monthly donation via our PayPal giving link here: http://globalplantcouncil.org/donate. Thank you in advance for your support

GARNet-CyVerseUK Workshop

March 20th-21st 2017,
University of York

Importantly this meeting is focused on younger researchers who are either toward the beginning of their studies or have moved onto a new subject area. We will provide a hands-on workshops that will describe the use of software tools that can interrogate RNAseq, imaging or systems biology data. In addition, previous users will provide examples of how the software has been successfully used.

These tools have been developed as part of the CyVerseUK grant (www.cyverseuk.org). We will also highlight the opportunities that exist for the sharing of big data in a meaningful manner.

This workshop is organised by GARNet with Professor Katherine Denby at the University of York.

Registration for this workshop will be through will open in the New Year and will be conducted through EventBrite:
https://www.eventbrite.co.uk/myevent?eid=29982258743

Day One: Monday 20th March

- Introduction to CyVerseUK Tools

- Data Management and Reuse

- Hands-on Workshops
  - Systems Biology tools
  - Conference Dinner

Day Two: Tuesday 21st March

- Hands-on Workshops
  - RNAseq tools
  - SNP discovery and mapping by sequencing tools
  - Image analysis tools

GARNet sponsored session at Monogram2017

April 4th-6th 2017,
University of Bristol

Monogram is the network for cereal researchers that holds an annual meeting bringing together all stakeholders in the organisation. In order to encourage the flow of information from the GARNet community, the majority of whom work with Arabidopsis, into crop plants, GARNet is sponsoring a session of next April's Monogram Meeting.

The GARNet session will include researchers from the labs of Ian Henderson, Zoe Wilson and John Doonan who will highlight research that was developed in Arabidopsis but that has now been now moved into cereals.

In addition GARNet have committeed to provide up to £2000 in travel grants for UK-based researchers and these will be considered to provide a flash talk in our sponsored session.

Therefore if you have any interest in moving your research into cereals then this will be a perfect introduction for you into this community. Regsitration is now open at
http://www.monogram.ac.uk/MgNW2017.php

GARNetNatVar16: Using Natural Variation for Gene Discovery and Crop Improvement

December 12th-13th,
Gonville and Caius College
University of Cambridge

In the world of Plant Science, Dame Caroline Dean, Detlef Weigel and Magnus Nordborg lead some of the most successful and well-respected research groups. Therefore it was with some excitement that GARNet hosted each of these speakers together with a world-leading group of academics for this short but intense meeting.

GARNet committee members David Salt and Ian Henderson were responsible for inviting speakers that covered many aspects of Natural Variation that ranged from fundamental gene discovery, through recent tools and technology and onto use of NatVar to combat the challenges of crop improvement. We aimed to host a meeting that encouraged discussion and the 100 delegates very much appeared to respond to this as we observed many animated conversations both at the conference dinner and during the poster session.

In addition to the well-established academics it was great to listen to up-and-coming researchers describing their research. This included new(ish) arrivals at the John Innes Centre, Kirsten Bomblies and Levi Yant. Kirsten is doing some outstanding work on the mechanisms of recombination, which developed from a genome scan of *Arabidopsis arenosa*. It is a great credit to her that she is tapping into this exciting scientific vein even though it was initially outside of her previous research experience.

Uemit Seren works with Magnus Nordborg at GMI-Vienna and gave an outstandingly prepared talk where he introduced the online tools that he has help to develop. These included the AraPheno database of phenotype data (https://arapheno.1001genomes.org/), the AraGeno tool (https://arageno.1001genomes.org) and the GWAS portal (https://gwas.gmi.oeaw.ac.at/).

GARNet Chairman David Salt addresses the delegates

Levi Yant introduces his exciting future work

The meeting was kindly supported by both PacBio and BioNano so representatives from each of these provided brief overviews of their latest technologies. However it was the talk by Richard Leggett (Earlham Institute) that was perhaps most exciting as he provided an update on the progress of third generation Nanopore minION sequencing. Although there are still issues with this technology Richard suggested it is now ready for analysis of more complex genomes. The community will watch this progress with interest.

The meeting ended with a fascinating session in which Ian Bancroft (University of York), Robbie Waugh (James Hutton Institute) and Cristobal Uauy (John Innes Centre) introduced their work on Brassicas, Barley and Wheat respectively and how taking advantage of the natural variation in these crops will be enormously important to maximise future outputs.

Cristobal Uauy explains the challenges of the large hexaploid Wheat genome

Cristobal is an enthusiastic advocate for wheat research and his lab specifically looks at factors that control variation in seed size. Although they are aiming to identify loci that control a modest 5% increase; over the scale of global wheat farming this would represent a huge increase in absolute productivity. In addition Cristobal's lab established resources either for new wheat researchers (http://www.wheat-training.com/) and/or for those looking to isolate wheat TILLING mutants (http://www.wheat-tilling.com/).

The primary sponsor was the '*Journal of Experimental Botany*' and in 2017, they are publishing a special issue inspired by this meeting. In addition they provided the poster prize that was won by Avichai Amrad (University of Bern) who presented his work on evolution of pollination strategies in Petunia. Another notably excellent poster was that of Dana MacGregor, who is starting her new lab at the University of Durham in January and presented a GWAS study that had identified novel loci involved in seed dormancy. Finally Zsuzsanna Merai (GMI Vienna) presented her interesting work that aims to establish *Aethionema arabicum* as a new system for studying light-inhibited germination. Additionally in this plant the hormones GA and ABA exhibit opposite effects to those seen in many other plants.

The attendees showed a significant appetite for a regular meeting on this topic, which is certainly something GARNet would hope to be involved with in the future. Watch this space for further details.

The abstract book for this meeting can be downloaded from http://www.garnetcommunity.org.uk/reports

eFP-Seq Browser - for Exploring RNA-seq Data from Arabidopsis thaliana and other Species

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Advances in next-generation sequencing (NGS) technologies has led to researchers generating a vast amount of transcriptomics data by conducting RNA-seq experiments to better understand an organism's biology by analyzing the global gene expression patterns in various organs, tissues, or in response to chemical or environmental perturbations. The results of an RNA-seq experiment, the reads mapped to the reference genome, are stored in the BAM file format. Many tools for analyzing and visualizing these large RNA-seq data sets have been developed to allow researchers to explore expression data for hypothesis generation about a gene's function.

We have developed a novel multi-track RNA-seq browser, dubbed the eFP-Seq Browser, that shows the mapping coverage for a selected splice variant along with an "electronic fluorescent pictographic" (eFP) image that serves as a visual representation of the expression level of the gene of interest. The eFP-Seq Browser is also capable of performing statistical analyses by computing the Reads per Kilobase per Million reads mapped (RPKM) value, and the Pearson Correlation Coefficient (PCC) value to sort the experiments based on a gene's expression level across the samples, or based on how similar the read map profile is to a given gene model. The eFP-Seq Browser has been deployed on the Bio-Analytic Resource (bar.utoronto.ca) for Plant Biology and on Araport.org, an international portal with many tools and data sets for plant biology researchers who use *Arabidopsis thaliana* as a reference organism

Background

Once reads generated from an RNA-seq experiment are mapped to a reference genome, such as the TAIR10 (Lamesch *et al.*, 2012) or Araport 11 (Cheng *et al.*, 2016) version of the *Arabidopsis thaliana* genome, the resulting BAM files (Li *et al.*, 2009) may be examined with existing visualization tools such as JBrowse (Skinner *et al.*, 2009), Integrated Genome Browser (IGB; Nicol *et al.*, 2009), or numerous BAM file viewers. However, multitrack tools such as JBrowse and IGB do not perform statistical analysis and display the RNA-seq experiment details simultaneously. Additional sorting and expression level summaries are also not present in the output without further work.

To overcome these shortcomings and to visualize the organism-wide gene expression patterns of the plant Arabidopsis thaliana, the eFP-Seq Browser is capable of displaying: gene splice variants, RNA-seq mapping coverage, gene expression levels as eFP images, statistical analysis results, and the experimental details in an easily sortable and searchable table.

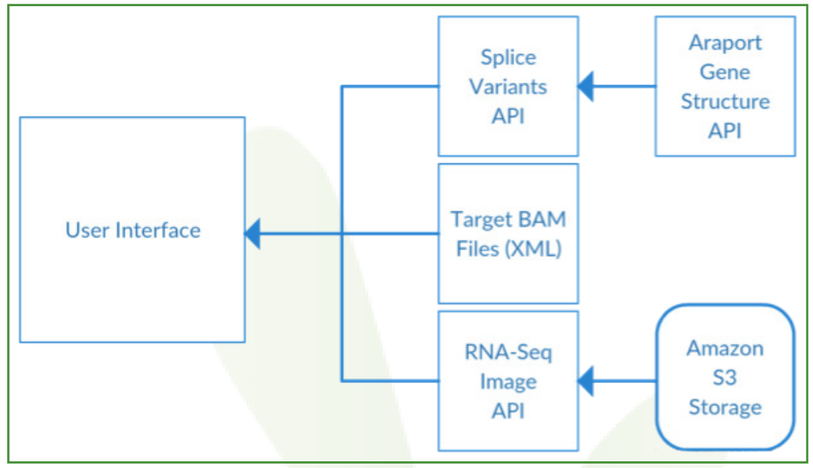

Figure 1: System architecture of the eFP-Seq Browser showing information flow.

Data Sources and Technology

The one hundred and thirteen RNA-seq experiments collected, processed, and organized for the A. thaliana reference genome re-annotation for the Araport11 build (Cheng *et al.*, 2016) form the current basis of the eFP-Seq Browser's expression data. When a user first accesses the tool, the browser populates a table with the information contained in an XML file (see system architecture in Figure 1) which contains details on each of the 113 RNA-seq experiments that are available through Araport's Amazon S3 Storage. The information contained in the 113 BAM files is extracted using SAMtools, an open source utility for sorting, indexing, and extracting information from SAM/BAM files (Li *et al.*, 2009).

The extracted information is analyzed using a server side Python script, and made available to the front-end via the RNA-Seq Image API. The Splice Variants API (using the Araport's gene structure API) provides the necessary information about a given locus' splice variants' structure. The web browser based user interface combines the information from all three data sources to give users access to an open source, customizable, and extensible tool which allows plant researchers to visualize and make unbiased comparisons of a gene's expression level throughout the various tissues of the plant.

eFP-Seq Browser

The eFP-Seq Browser (Figure 2) is capable of displaying the gene splice variants, mapping coverage from all 113 RNA-seq experiments, and electronic fluorescent pictographic (eFP) images for visualization of the expression level of the gene of interest. Furthermore, it is capable for sorting the 113 records based on the relative (to the experiment controls) and absolute expression levels (as determined by the RPKM values), or based on how well they correlate to a specific splice variant (as determined by the PCC values). Taken together with the fact that it displays additional information about each RNA-Seq experiment, the eFP-Seq Browser becomes a complex statistics-based visualization tool that allows its user to make unbiased comparisons across many samples for a gene of interest.

Figure 2: An abbreviated output of the eFP-Seq Browser displaying the RNA-seq mapping coverage and the RPKM-based absolute expression levels (red = 301 RPKM, yellow = 0) for AT2G24270 for the 113 RNA-seq samples used to generate the Araport11 Arabidopsis genome build, sorted here from the highest (meristem) to lowest (pollen) expression level in RPKM expression units.

Discussions and Challenges

The strongest feature of eFP-Seq Browser is its ability to perform statistical analysis in order to make unbiased relative comparisons to sort hundreds of RNA-Seq experiments by expression levels, or by conformity to a specific splice variant structure. The expression level comparisons are done by comparing the RPKM values, and the conformity to a specific splice variant is determined by comparing the PCC values. Integrating these values into a visualization tool allows researchers to get statistical support for their analysis in addition to simply visualizing the expression patterns.

The enormous dataset that forms the basis of this project, pushes it well into the realm of biological big data which introduces significant challenges. For example, network storage space is precious and very costly for academics - Araport is currently hosting the 113 BAM files on Amazon S3 data servers to offer public access quickly at a lower initial cost. Other challenges include processing power limitations, and network transmission rate limitations. We especially have had technical difficulties in getting SAMtools mpileup to produce a reliable output when the BAM file is located on a remote server. This limitations forces the analysis to be conducted on BAM files located on the same server to ensure data can be reliably extracted.

The design of the tool is such that it can be extended to include more RNA-Seq datasets by amending the XML file with another entry, and ensuring that the associated BAM files are accessible locally. This design principle should allow the tool better withstand changes in available data to include (or remove) the experiments analyzed. Furthermore, the BAM file format was designed to be organism and sequence independent – thus this tool can be easily extended to other organisms with minimal effort.

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New Arabidopsis Grants

Over the past two rounds of BBSRC Responsive Mode funding there have been slightly fewer grants that include Arabidopsis research. Whether this is a blip or a trend remains to be seen but at GARNet we continue to strongely support fundamental research that underpins translation into more economically important crops

Seeing the light: automatically identifying key anatomical changes in light sheet microscopy images of plant roots

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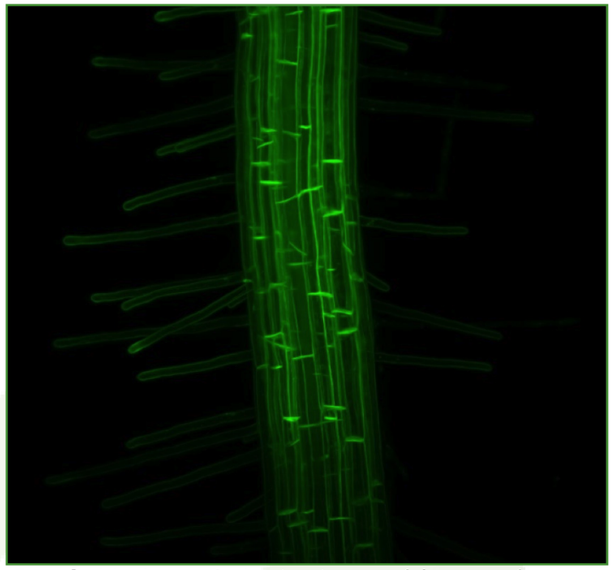
New Investigator Grant

In this new project at the University of Nottingham we will be considering the anatomical changes of roots under low phosphate levels, which give rise to important architectural changes in the root system. With the recent acquisition of a Zeiss Z.1 light sheet microscope, we will be able to capture timeseries data of 3D anatomical events, such as cell division, over long time spans. Whilst a great advance in imaging, the amount of data produced will be large, so a new approach is required to be able to analyse and make sense of the dataset. By combining novel computer science development with cutting-edge bioimaging equipment, we hope to gain new insight into the biological processes at work.

Phosphorus is one of the key macronutrients (alongside nitrogen and potassium) required for healthy plant growth, and is a widely used constituent of fertilizer used in commercial crop production. Phosphorus is a limited strategic resource it is derived from a finite natural supply.



French: Maximum Projection image of the root of an Arabidopsis seedling. Cell membranes are stained with GFP. The image was taken on a Zeiss Z.1 light sheet fluorescent microscope at the CPIB

Understanding phosphorus use and its effects on growth in plants is therefore of key importance to Global Food Security. Phosphorus mobility in the soil is limited by slow diffusion, and so areas of low phosphate concentration are created around root system. Much work has been carried out examining the overall architecture of root systems under differing conditions; however, much less is understood about the anatomical changes, and how they develop. There are only a few instances where root anatomical and architectural traits have been combined in a systematic way to select for plants with enhanced nutrient acquisition, but when this has been done the improvements have been impressive.

There are several reasons for our lack of knowledge in adaptive anatomical responses in roots. First, the equipment has not been available to image the plants growing over the time periods in which these anatomical changes take place. Second, the ability to alter the growth conditions (such as nutrient levels) around the root whilst being imaged has not been possible. Third, discovering subtle anatomical changes in the huge datasets that would be generated is a very challenging manual task. In this new project we will overcome these three challenges, allowing study of anatomical changes in low phosphorus media dynamically.

One key ingredient here is the use of cutting-edge microscope, a light sheet fluorescence microscope (or LSFM) to image cellular anatomy as plants grow. However, a key challenge with LSFM time series data is the huge volume produced – potentially terabytes for a single experiment.

To overcome this, we will develop new computational analysis methods to help make sense of the data sets. One such challenge is identifying formative divisions that give rise to anatomical patterns in 3D datasets of roots resolved over time. We will develop computational methods to automatically identify particular anatomical changes in the large, light sheet datasets.

By developing machine learning approaches and building novel software tools we will automatically identify regions of interest within these datasets. In addition we will build visualisation tools which will display the results of these approaches in intuitive ways, to allow biologists to intelligently navigate the data, rather than manually searching for the needle in the haystack. With new and automated imaging approaches increasing the quality and amount of digital data available, we hope to show that the development of accompanying computational approaches is key to the success of future biological experiments.

Characterisation of a novel Polycomb group protein complex and its effects on the plant epigenome

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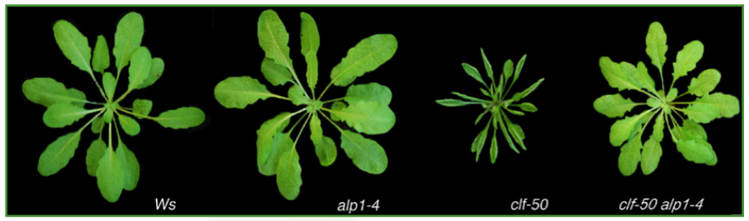
The aim of our project (which was funded under the bilateral UK-Ireland BBSRC-SFI initiative) is to understand how several novel plant-specific proteins called ALPs regulate the activity of Polycomb group (PcG) proteins, which are a conserved group of developmental regulators in plants and animals. The project follows on from a collaboration between the Goodrich group and Franziska Turck's group (MPI Cologne) which was funded through the EU ERA PG initiative. In this we genetically identified the ALP1 gene as an antagonist of the PcG (see Figure).

We anticipated that ALP1 would encode one of the trithorax group proteins, a diverse group of chromatin proteins which function in complexes antagonistic to PcG, for example writing histone marks associated with gene activity. However, we found that ALP1 encodes a novel protein distantly related to a transposase of the PiF/Harbinger class. Most surprisingly, proteomics revealed that the ALP1 protein is a component of a PcG protein complex (PRC2) that writes a repressive mark on histone H3. Our finding that a domesticated transposase (ALP1) is an inhibitory component of a PcG protein complex is intriguing, as the PcG proteins play a role in repressing transposon activity in green algae and some plant cell types (endosperm).



Goodrich: Genetic interaction between ALP1 and Polycomb group genes. The CURLY LEAF (CLF) gene encodes the histone methyltransferase component of the Arabidopsis PRC2 complex. The *alp1* mutation partially suppresses the early flowering and leaf curling phenotypes of the clf mutant. ALP1+ activity is needed for the activation of PcG targets that normally occurs when in *clf* mutants.

One possibility therefore is that the association originally arose as a means for transposons to evade host surveillance. In support of this, the group of Weiqiang Qian (Peking University) recently reported that an ALP1-related domesticated transposase is part of a complex implicated in inhibiting DNA methylation. In fact, bioinformatic studies reveal that PiF/Harbinger-related transposases have been domesticated many other times in plants and animals, but in most cases the function of these novel genes is unknown. An exciting possibility is that they many other of these genes are regulators of the host epigenetic machinery.

The proposal exploits the complementary expertise of the two groups to dissect how ALP proteins antagonize PcG function. Frank Wellmer's group are expert in profiling where developmental regulators bind in the genome and in the developmental consequences of transiently disrupting their binding. His group will tackle the question of where ALP proteins bind and how this affects PcG binding and epigenetic marks at their targets. He will also test whether the ALP proteins may target PRC2 by binding DNA. The Goodrich group will tackle the question of how the ALP proteins affect the biochemical activity of the PRC2 protein complex by purifying the ALP protein complex from plant extracts and assaying their activity on chromatin templates. They will benefit from expertise of groups of Philipp Voigt (PRC2 enzyme assays) and Juri Rappsilber (proteomics) in Edinburgh.

In addition, the Goodrich and Wellmer groups will work jointly to determine the function of additional components of the ALP-PRC2 complex identified through proteomics and to develop resources such as ALP-specific antibodies. To test whether ALP proteins can reverse PcG silencing we will test the effect of tethering ALP proteins to novel targets. Lastly, we will test the role of ALP proteins in the vernalization response, a classic PcG mediated epigenetic pathway in Arabidopsis.

Role of protein phosphorylation in the maintenance of photosystem two in plants

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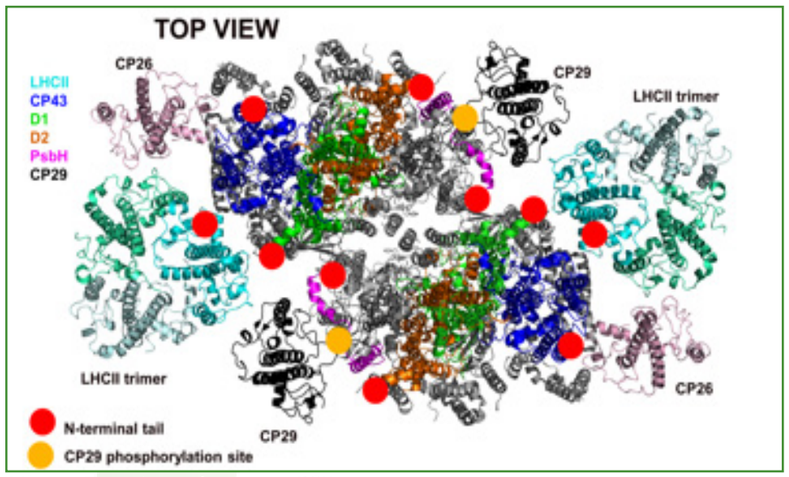
Photosystem II (PSII) is the light-driven water:plastoquinone oxidoreductase of oxygenic photosynthesis found in the thylakoid membranes of chloroplasts. Importantly, PSII is a weak link in the photosynthetic apparatus and is prone to irreversible light damage. Normally, PSII activity is maintained in planta through the operation of an elaborate repair cycle that replaces damaged protein subunits (usually the D1 subunit) by newly synthesised copies. However, when the rate of repair is unable to keep pace with the rate of damage such as at high light irradiances, PSII activity declines leading to reduced plant growth and crop yields. In the case of land plants, the reversible phosphorylation of four PSII core proteins (D1, D2, CP43 and PsbH), all encoded by the chloroplast genome, is thought to fine-tune PSII repair. However, the precise role of PSII phosphorylation *in planta* still remains unclear due to the lack of appropriate mutants. In this project we will address this gap in knowledge by investigating PSII repair in engineered tobacco chloroplast mutants lacking specific PSII phosphorylation sites.

PSII in chloroplasts forms a heterogeneous and dynamic population of larger PSII supercomplexes, containing intrinsic light-harvesting complexes attached to the dimeric core complex (Figure), embedded in the appressed thylakoid membranes of the grana. PSII is therefore physically separated from the repair apparatus located in the granal margins and stromal lamellae. Recent cryo-electron microscopy data has confirmed that the phosphorylated residues of the core subunits lie at the interface between the core complex and the light-harvesting complexes (LHCII trimer, CP29 and CP26) and so ideally located to regulate the disassembly of the damaged PSII supercomplex.

Our application will test three physiological effects attributed to PSII core phosphorylation: (1) disassembly of damaged PSII supercomplexes within the grana to enhance diffusion of smaller damaged PSII core complexes (2) structural reorganisation of the grana to enable a closer approach of the protease complex involved in degrading damaged PSII subunits and (3) inhibition of D1 proteolysis.

We have already used chloroplast transformation technology to generate D1 phosphorylation site mutants in tobacco and plan to construct additional mutants in which the phosphorylation sites within the other chloroplast-encoded PSII subunits (i.e. D2, CP43 and PsbH) are mutated either singly or in different combinations. Established biochemical and physiological approaches will be used to assess the impact on PSII assembly and repair in planta. Changes to the ultrastructure of the thylakoid membrane system will be examined using classical thin-section transmission electron microscopy (TEM) combined with high-resolution freeze-fracture electron microscopy (FFEM) and cryo-scanning electron microscopy (cryo-SEM) and dynamics of PSII migration will be probed by fluorescence recovery after photobleaching (FRAP) experiments, all done in collaboration with Conrad Mullineaux and Chris Duffy at Queen Mary University of London. Effects on the growth of the mutant plants under different light and temperature regimes will be done with Tracey Lawson at the University of Essex.

Overall this collaborative project involving researchers at three UK universities will provide the first detailed examination of the role of PSII core protein phosphorylation in the assembly and maintenance of PSII in land plants.



Nixon: Top view of spinach PSII supercomplex showing approximate positions of the phosphorylation sites in the N-terminal tails of PSII core subunits. Taken from Wei *et al* (2016) Nature 534, 69-74

GARNet2016: Innovation in the Plant Sciences

Sarah Jose
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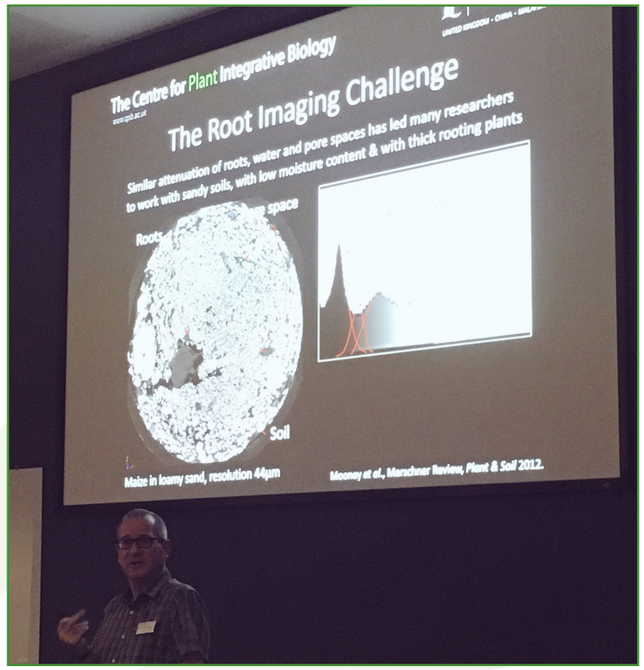
University of Bristol and the Global Plant Council

In September, GARNet hosted a UK Arabidopsis research conference entitled 'Innovation in the Plant Sciences'. The meeting, held at Cardiff University, was attended by over 100 plant scientists for two days of networking, workshops, posters, and plant science research presentations.

The meeting was preceded by an excellent workshop run by Philippa Borrill, Nikolai Adamski and Cristobal Uauy, who guided us through the translation of Arabidopsis research into wheat. While the genetics of this hexaploid cereal were once too tricky for many of us to tackle, the new training materials we were shown along with the improved genetic resources will help researchers to explore their gene of choice in a crop plant.

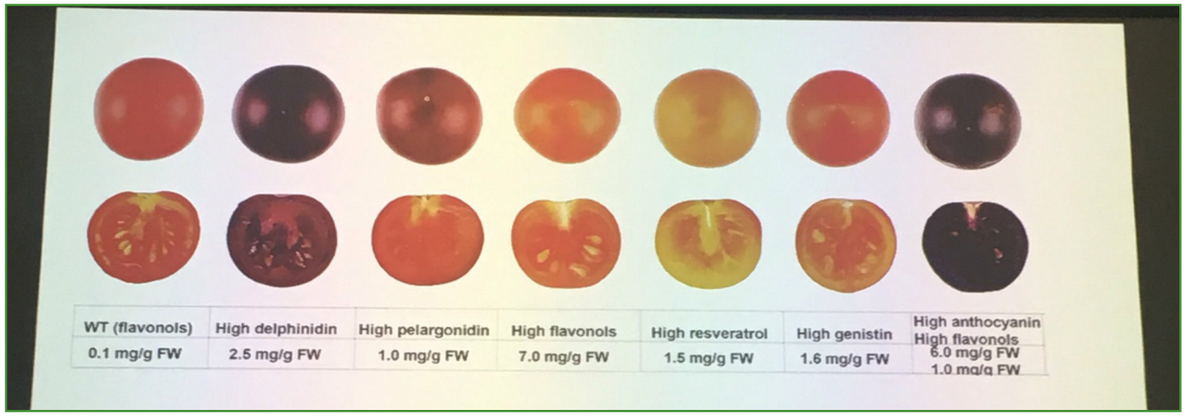
GARNet 2016 then officially began with a welcome from Jim Murray, the GARNet PI, who kicked off the first session, *Frontiers in Plant Imaging*. Ben Scheres from Wageningen University in the Netherlands gave the first plenary research talk, describing the intricate transcriptional regulation governing the formation and maintenance of meristems. He was followed by Jens Tilsner from St. Andrews, who spoke about overcoming the difficulties of visualising RNA in situ to finally observe RNA moving through



Darren Wells: University of Nottingham

plasmodesmata for the first time. Malcolm Hawkesford from Rothamstead described the institute's impressive high-throughput phenotyping technology, which can provide a wealth of information, even down to the number of ears of wheat in a field! The session ended with Nottingham's Darren Wells, whose team developed a novel method to visualise roots in soil using X-ray computed tomography.

After lunch, an unusual but very interesting session, *Enabling the Translational Pipeline*, featured talks from plant scientists who have translated their discoveries into real-world commercial applications. We first heard from Cardiff's Jim Murray, who won the BBSRC Commercial Innovator of the Year in 2012 for his translation of research on bioluminescence into real-time assays for infectious organisms. Next, Lancaster's Mike Roberts explained how simple



Cathie Martin: John Innes Centre

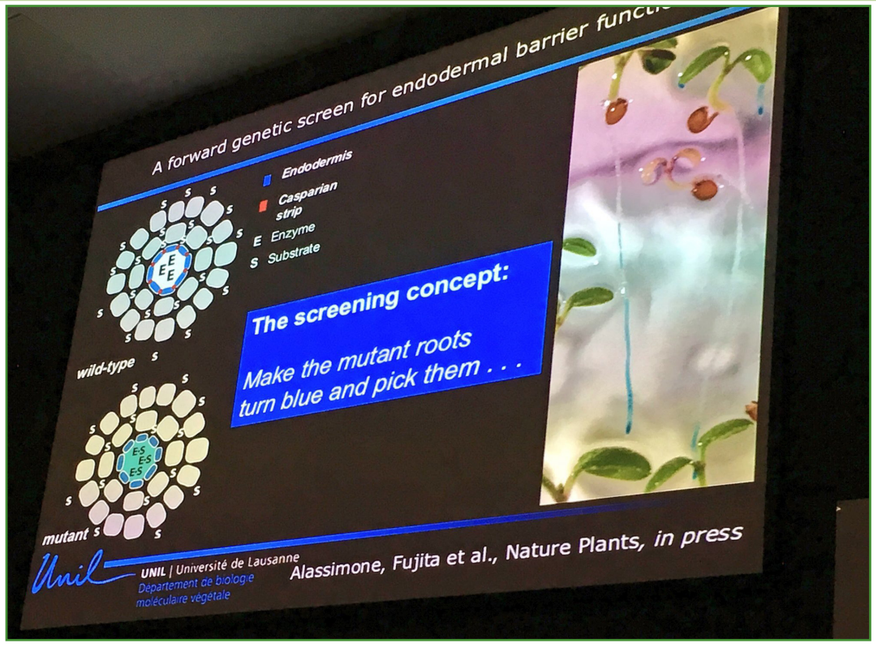
phytohormone experiments in his lab led to the development of a commercial seed treatment that protects against herbivory without the need for pesticides. Neil Bruce from York spoke about engineering switchgrass to remove explosives TNT and RDX from land polluted by munitions testing. These plants can even use RDX as a source of nitrogen – a rather poetic contrast to the use of fertilisers in the manufacture of explosives. The session ended with a discussion panel featuring Jim Murray and Innovate UK's Jon Wood, whose helpful advice included potential funding sources that researchers could use to translate their findings into a commercially valuable product or service.

In the third session, *Plant Synthetic Biology*, Cathie Martin from the JIC spoke about her international collaboration to engineer high levels of polyphenols in plants, overcoming the limitations that typically mean far more modest increases in production. Essex's Christine Raines described her team's development of a transformation pipeline to insert multiple genes to improve photosynthetic efficiency, followed by Anil Day from Manchester, who explained some of the new chloroplast engineering technologies available to researchers. The session was concluded with a look at the standardised automated platforms and other tools that enable the assembly of a range of DNA parts in genetic engineering, outlined by Nicola Patron of the Earlham Institute.

After the day's sessions, we heard several short flash presentations highlighting research posters, which enticed the conference delegates out into the poster session to discuss science over a buffet dinner. This was followed by an evening event at ZeroDegrees in Cardiff's city centre for more networking and a craft beer or two.

The second day began with the *Genomic Tools for Gene Discovery* session, which kicked off with a talk from Chris Town on the array of analytical and data-mining tools available on the Araport platform, along with a wealth of genomic information. The James Hutton Institute's David Marshall then spoke about the powerful bioinformatics tools they've developed for analysing big data from next generation sequencing, with tens of thousands of scientists using them around the world. Next, GARNet committee member Katherine Denby described CyVerseUK, set up to support researchers by

making large-scale data storage and analysis simple to manage both privately and with collaborators. Wageningen's Marnix Medema gave the last talk of the session on a tool with, in my opinion, the best name ever; PlantiSmash! This tool allows users to identify and analyse clusters of genes involved in the same biosynthetic pathway – crucial for the development of efficient product synthesis pathways in synthetic biology. The session concluded with a brief discussion about the needs of the community, with a general consensus that more training should be given to early career researchers to enable them to understand and undertake bioinformatic analyses of big data.



Niko Geldner: University of Lausanne

After coffee, networking and a chance to speak with the exhibitors, the group diverged into two workshops; in one, attendees got to grips with Araport in a hands-on training session from Chris Town and Sergio Contrino from Cambridge. In the other, East Anglia's Amanda Hopes and Vladimir Nekrasov from The Sainsbury Laboratory presented an in-depth look at CRISPR-Cas9, with a guide to mutagenesis, troubleshooting and the verification of mutants.

In the final session of the meeting, *Cell Signalling*, Stefan Kepinski from Leeds described his research into gravitropism and growth angles. He was followed by Glasgow's Eirini Kaiserli, discussing the perception of light and her new finding of a transcriptional regulator of photoperiod and light signalling. Birmingham's Daniel Gibbs told us about oxygen and nitric oxide and their effects on the breakdown of transcription factors in plants. The session, as well as the conference, was concluded by the final plenary speaker, Niko Geldner from the University of Lausanne in Switzerland, who described a series of clever experiments that have greatly expanded our understanding of the Casparian strip in the endodermis of plants.

If you'd like to read more about the work described above, you can find the abstract book for the conference, the workbook for the Araport workshop, and slides for the wheat genomics and CRISPR-Cas9 workshops on the GARNet website: http://www.garnetcommunity.org.uk/reports

Giant Jamboree for the International Genetically Engineered Machine (iGEM)

Geraint Parry

As even the name suggests, the **iGEM Giant Jamboree** is a conference like no other.

The bringing together of a global group of 2500 mostly undergraduate students, the vast majority of them at their first conference where vthat they will each provide presentations that are being critically assessed begins to suggest the kind of frenetic and excited energy that characterises this event.

For those a little confused, the International Genetically Engineered Machine Foundation oversees and organizes iGEM, which is synthetic biology competition for groups who are usually hosted by academic institutions. The basic idea is that a group of students works through the year on a completely novel project that conforms to the principles of synthetic biology, before presenting it in the aforementioned Giant Jamboree.

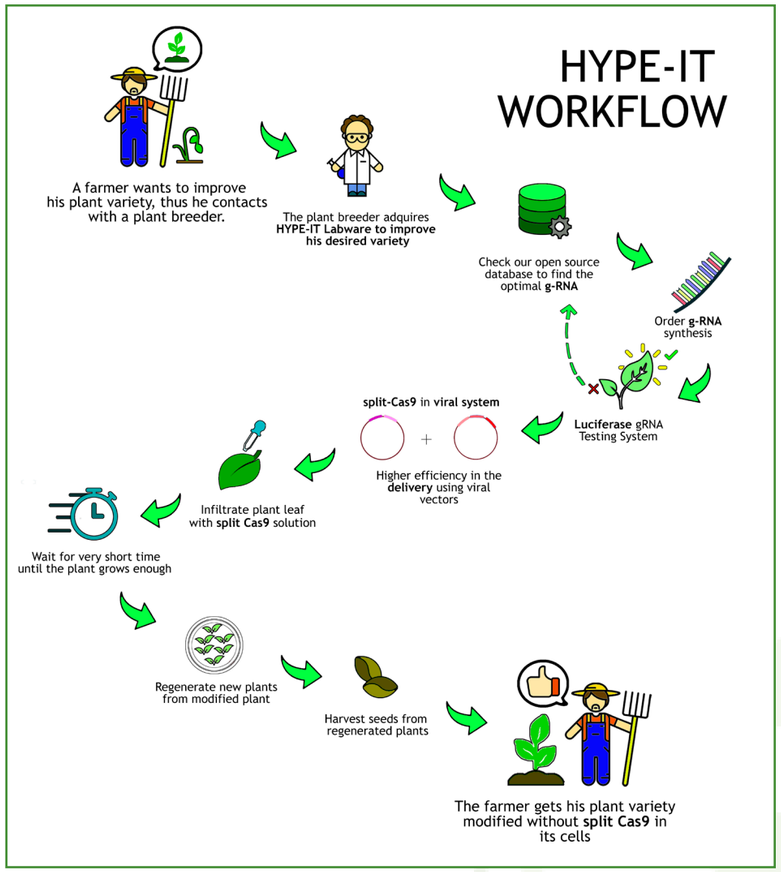

iGEM from above Credit: iGEM Foundation/ Justin Knight

As this is a competition, each project is judged on metrics that assess many aspects of the teams work. These include their contribution of biobricks to the iGEM registry (an enormous selection of molecular parts that are held within a standardised plasmid), the development of the novel project, initiating collaborations with other teams and their attempts to integrate human practices and public engagement into their project. By meeting certain criteria each team is eligible for Gold, Silver or Bronze medals alongside special prizes for different project categories.

Given that registrations, student stipends, research expenses, travel and accommodation costs can stretch to at least £20K, entering even a small team is not something to be taken lightly. To this financial requirement must be added the time donated by a team of instructors and advisors that support the students. However regardless of the cost, one thing is certain; for those students who participate, attend, present and are inspired by the Jamboree, it can be a career-defining moment.

Plant Synthetic Biology can make for a challenging summer!

Plant experimental chassis have not been widely used during the ten years of the iGEM competition where bacteria, yeast, mammalian cell lines or cell-free systems offer time efficient alternatives for the usual 10-week summer research period. However the iGEM foundation, alongside a group of committed advocates have recently developed the Phytobricks cloning standard (http://2016.igem.org/Resources/Plant_Synthetic_Biology/PhytoBricks), which is based on a recently



http://2016.igem.org/Team:Valencia_UPV/Results

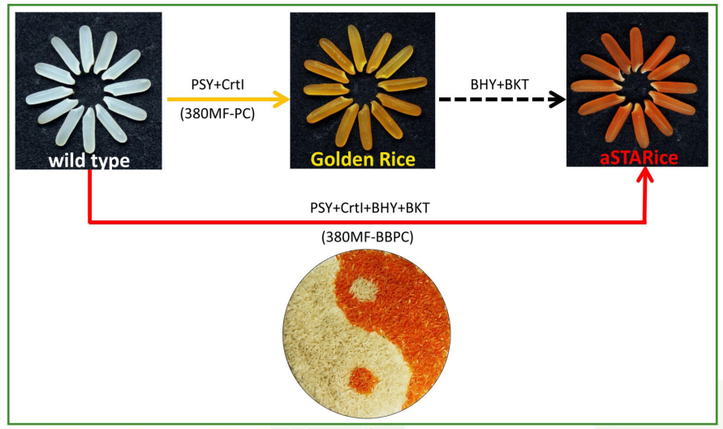
published standard syntax within the Golden Gate cloning system, that was led by Dr Nicola Patron at the Earlham Institute. The aim is to lower the barrier of accessibility for teams to take on plant projects and the evidence from this years competition seems to suggest that this is slowly happening.

The 2016 iGEM team from Valencia-UPV is advised by plant synthetic biologist Diego Orzaez and their project submitted phytobricks for the expression of a split Cas9 system. They showed that the two halves of the Cas9 protein could reconstitute and was active in a tobacco expression system. They have documented this work on their Parts pages and this is hopefully a resource that will be used by future iGEM teams. Their team was very successful at the jamboree, winning a gold medal alongside specific awards for the best hardware (http://2016.igem.org/Team:Valencia_UPV/Hardware) and software (http://2016.igem.org/Team:Valencia_UPV/Software).

Another successful team with a plant project was from SCAU-China who had, over the course of at least two years, added an additional two genes to conventional Golden rice. This produces a '*brown rice*' that additionally produces the natural keto-carotenoid Astaxanthin, which is thought to have beneficial anti-oxidant properties. This is clearly a significant research project that has been badged with the iGEM logo and as such was very positively received by the judges. Although they did not submit parts in the Phytobricks standard it was exciting to see such a potentially high profile plant-project feature at the jamboree.

Although both these projects are well deserving of their awards, their work builds upon years of expertise contained within the supporting labs and highlights one of the challenges for the competitive element of iGEM; namely how teams can be equally judged when they have hugely varying levels of support. Fortunately it appears that this is not a significant issue as each team is able to take positives from their own performances and are happy to celebrate the excellent projects that they each had individually put together.

The team from Pretoria in South Africa took on an extremely ambitious plan to create 'plant batteries' by using short aptamers to attach either photosystem II or a laccase enzyme to



http://2016.igem.org/Team:SCAU-China

either pole of an electrical circuit held within a novel graphene scaffold. The students made some progress with this and the project serves to highlight the blue-sky thinking that undergraduate students undertake as part of this competition.

Algae on the rise.

A number of teams including Cambridge-JIC (http://2016.igem.org/Team:Cambridge-JIC), Linkoping University (http://2016.igem.org/Team:Linkoping_Sweden/Chlamydomonas_reinhardtii) and USP_UNIFESP in Brazil (http://2016.igem.org/Team:USP_UNIFESP-Brazil) used the algae *Chlamydomonas reinhardtii* in their projects. The Cambridge team had most success in their project that generated a set of parts in the Phytobrick standard that can be used in future algal projects. In addition they created a remarkable blueprint and prototype Genegun for plant transformation, costing just £300, making it accessible for less well funded labs. These other two teams were hoping to use Chlamydomonas to produce either biofuels or spider silk protein

Team Cambridge-JIC: http://2016.igem.org/Team:Cambridge-JIC

and although the ambition of both projects outstripped their achievements this year, iGEM is all about thinking big: sometimes it works, sometimes not!

The UK on Top

From a UK perspective the iGEM jamboree was a huge success with Imperial College taking the overall undergradraute award with an exciting project called 'Ecolibrium'. Elsewhere the UK was represented by over 20 teams, the third most numerous behind the USA and China. Aside from Imperial College, the teams from Exeter, Dundee, Dundee Schools, Cambridge-JIC, Oxford, Sheffield, Glasgow and Manchester gained gold medals. There is little doubt that the UK is developing a cohort of talented synthetic biologists who will be the research leaders of the future.

The financial burden is a significant barrier for entry to iGEM but assuming that this can be achieved the development of the Phytobrick standard will hopefully encourage more teams, and UK teams taking on a plant-based project. As most projects as based on a one-year project establishing stably transformed lines is not usually realistic but the use of protoplast or tobacco transient expression systems now allow use of plant as an experimental chassis in the context of iGEM. This is extremely timely given the use of *Nicotiana benthamiana* for production of many heterologous compounds, highlighted by the development of the Ebola vaccine ZMAPP in tobacco.

Overall iGEM has been enormously successful so hopefully it can act as a prelude to increased use of plant systems as a tool for synthetic biology.

GARNish
ICAR2016

International Conference on Arabidopsis Research 2016

Gjeong-Ju, South Korea

Peter Venn,
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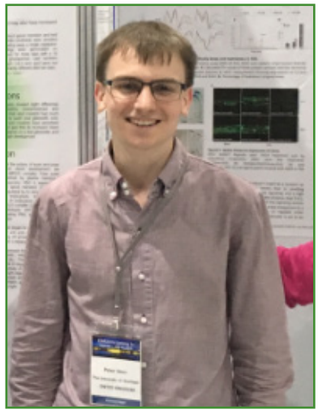
University of Sheffield

Nearly a thousand researchers attended this year's international Conference on Arabidopsis research (ICAR) in South Korea. ICAR2016 was my first experience of a large plant science conference, although it wasn't a completely foreign experience as I recognised a few faces from the 2015 UK PlantSci conference. I was among the hundreds of delegates presenting a poster, and I benefitted greatly from the talks and discussions I had in these sessions. ICAR2016 was a really good opportunity to see what kinds of research being performed across the world and to discover many novel techniques and developments.

With the two concurrent sessions, almost all of the timeslots contained sessions relating to my own interests or the wider interests of the Sorefan lab group. I learnt a lot during the conference, not only new details, but about the high standard of competition in global Arabidopsis research. I also came away with ideas, a potential collaboration and new motivation to get my research published.

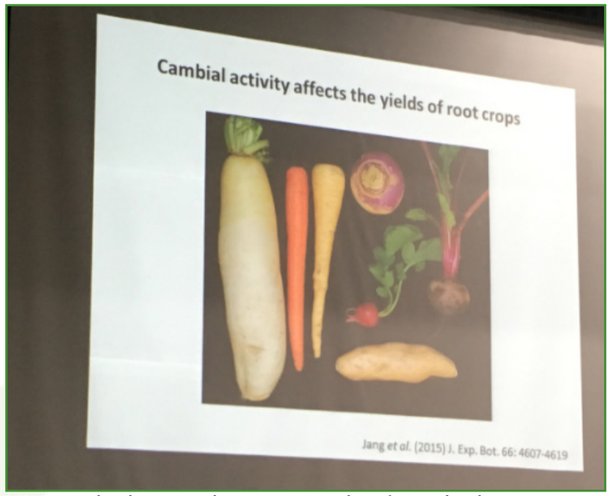


Professor Jen Sheen opened the conference with her latest developments in understanding the sugar signalling function of HEXOKINASE1, the glucose receptor that was first identified and characterised by her lab. Amino acids essential for the sugar signalling function of HEXOKINASE1 but not its catalytic function have been identified and are being characterised. This talk was of particularinterest to me because my research relates to sugar signalling, and Professor Jen Sheen is the leader of the field.

Stem cell development is another interest of my research so it was of interest that a surprising number of talks focussed on cambial development. Professor Hiroo Fukada talked about the role of short peptide ligands and their receptors that regulate xylem formation. Dr Ari Mähönen and Professor Ildoo Hwang talked about different aspects of WOX4 function in cambial development, Whilst Professor Hwang also discussed the role of auxin signalling in cambial development that is mediated through the activity of the BRASSINOSTEROID-INSENSITIVE2-LIKE1 (BIL1) gene.

The meeting also included some excellent research that focussed on shoot apical meristem development. Professor Dave Jackson presented a novel CLAVATA-like peptide that increases floral meristem size and primordia row number in maize and how this peptide was likely linked to the domestication of maize. Professor Naoyuki Uchida presented the role of ERECTA family genes in repressing the cytokinin induction of stem cell maintenance, which is independent of CLV3 signalling.

However it wasn't all science as the long days merged into evening sessions of networking at the local bar. This was situated beneath the intriguing K-pop museum where some very talented locals entertained us with karaoke and dancing! I also used the opportunity to visit several labs in South Korea, including two at Postech in Potang, and two at Seoul National University.



Hiroo Fukada provides an example of Cambial Development. Photo by Geraint Parry

This was a valuable insight into the research culture in South Korea, and an opportunity for further networking. I was really impressed by the scale of some of the research in South Korea as for example, one lab had cloned an entire gene family (of over 70 genes) for a protein-effector interaction screen. I was also able to establish a collaboration with researchers at Seoul National University, inspired by reading a poster from their lab at the conference. I am very grateful to my supervisor, Karim Sorefan and also to the Gatsby Foundation and GARNet for providing the travel grant that enabled me to attend ICAR2016.

ICAR Poster Session: Image from Daniel von Wangenheim twitter.com/DvonWangenheim

Spotlight on:
The University of Sheffield

Kindly compiled by Stuart Casson

Plant Science at Sheffield

Plant sciences at the University of Sheffield span scales from the molecular to ecosystems, with three main focus areas: Photosynthesis, leaf structure and function and the rhizosphere. Our research utilises a range of systems from photosynthetic bacteria to parasitic plants and an array of approaches from biochemistry, molecular genetics, physiology to advanced imaging techniques. Research is concentrated in two cross departmental centres; The Robert Hill Institute, which is focused on fundamental plant science research and P3 (Plant Production and Protection), which is a centre for translational Agri-tech. Our science is supported by state of the art controlled environment facilities, plant phenomic platforms, mass-spectrometry and imaging facilities. Together, our aim is to advance plant science research in order to address and provide solutions for some of our most challenging problems.

Professor David Beerling FRS
d.j.beerling@sheffield.ac.uk

My interdisciplinary research group focuses on fundamental questions concerning how photosynthetic terrestrial ecosystems and the global environment co-evolved over the last half billion years.

David Beerling

Our approach integrates evidence from fossils, experiments with terrestrial organisms, and rigorous theoretical models applied across spatial scales. We focus particularly on key processes and interactions important for revealing insights into the conquest of the land by plants, and the role of terrestrial ecosystems in shaping global ecology, climate and atmospheric composition. Our research findings also inform understanding of current anthropogenic climate change issues facing humanity.

Plants of interest to the Beerling Lab

Professor Michael Burrell
m.burrell@sheffield.ac.uk

My research interest is to understand the control of plant metabolism in non-photosynthetic storage organs, in particular the control of sucrose metabolism and starch synthesis. The sucrose content of crops is central to their harvestable quality. In can be beneficial to have a high content, whereas in other cases such as in potato tubers it is detrimental. Starch quality and quantity are important. Starch is one of the most important plant products used by humans. It provides a large proportion of our calorific intake, is a feedstock for farm animals and has diverse uses in industry such as food processing and papermaking.

Michael Burrell

Dr Pascal-Antoine Christin
p.christin@sheffield.ac.uk

Using phylogenetic frameworks, my research investigates the mechanisms that led to the functional diversity of plants. I combine analyses of gene sequences, genomes, transcriptomes, and ecological and morphological traits to address questions of importance for evolutionary biology in general:

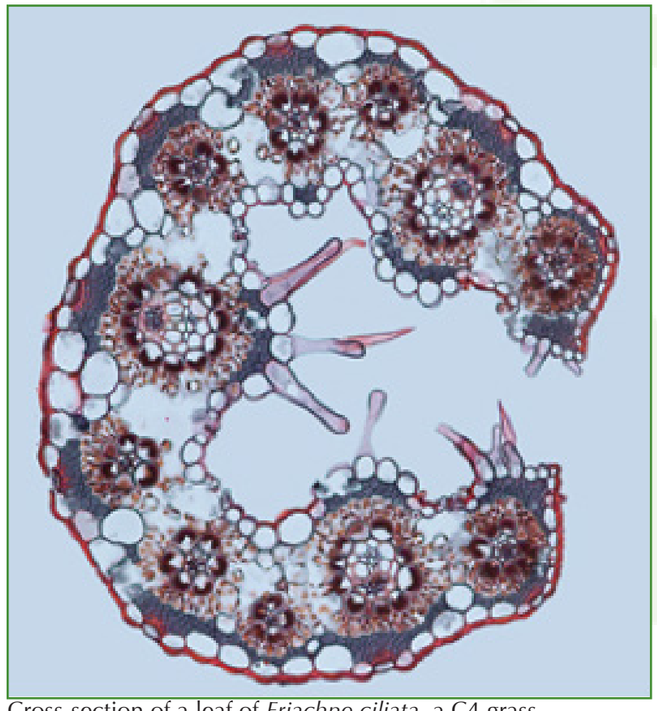
Pascal-Antoine Christin

- How are complex traits assembled during evolution?
- What determines the likelihood of a given lineage evolving novel adaptations?
- How do environmental changes drive the evolution of novel traits?

Professor Duncan Cameron
d.cameron@sheffield.ac.uk

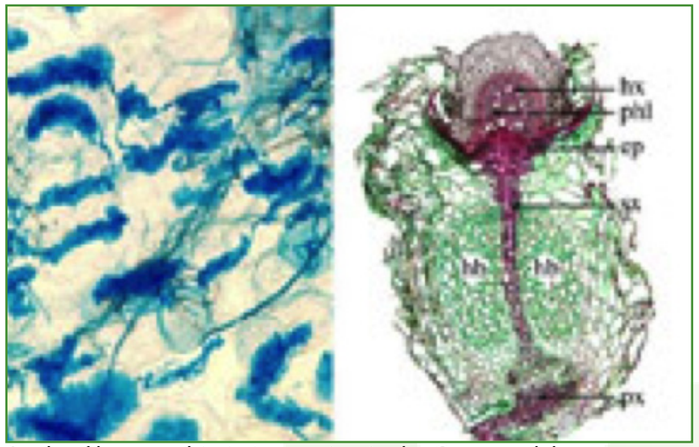
My research seeks to understand how shifts in energy and nutrient flows between symbiotic organisms influences individuals and ultimately communities. My group specialises in the physiology and ecology of host-symbiont interactions, specifically plant-fungal symbioses and plant-parasitic plant symbioses using a combination of metabolomics, isotope tracers and molecular biology to study the following:

Duncan Cameron

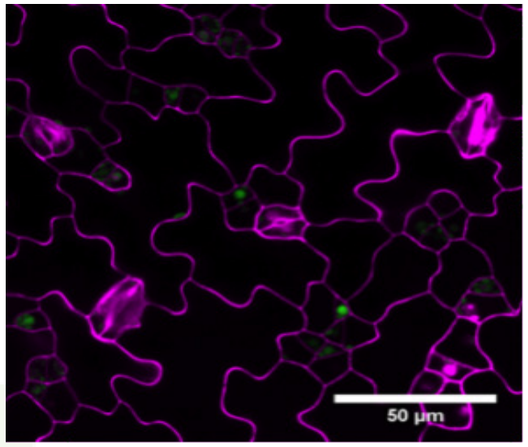


Cross-section of a leaf of *Eriachne ciliata*, a C4 grass

- Soil biology including carbon and mineral fluxes through mycorrhizal networks in natural and agro-ecosystems.
- Microorganisms in sustainable agro ecosystems - Harnessing beneficial microbes for crop production (provisioning of nutrients) and crop protection (inducible plant defenses).
- The physiology and functional ecology of host-parasitic plant interactions.



Study of host-symbiotic interaction in the Cameron lab



Looking at stomata in the Casson lab

Dr Stuart Casson
s.casson@sheffield.ac.uk

Stuart Casson

My laboratory is interested in understanding the mechanisms that regulate plant development and in particular, how environmental signals regulate core developmental pathways. For this purpose I am using stomatal development and photoreceptor signalling as models.

Stomata are microscopic pores on the surface of leaves that regulate gas exchange between the plants and their environment, whilst photoreceptors enable plants to adapt and respond to a dynamic light environment. Understanding how these environmental signals interact to regulate stomatal development is vital if we are to accurately model plant water use and performance in a changing environment.

Professor Andrew Fleming
a.fleming@sheffield.ac.uk

Andrew Fleming

Our research is focussed on plant development. In particular, we are interested in understanding the interplay of cell growth, division and differentiation and how they are incorporated into a developmental program to generate a functional leaf. Using techniques of cell and molecular biology, combined with computational modelling, physiology and biochemistry, we aim to provide an integrated understanding of leaf form and function. Our research includes work on Arabidopsis, Physcomitrella, potato and rice and ranges from single cells to meristems and leaf morphogenesis.

Investigating leaf shape in the Fleming lab

James Locke

Professor Julie Gray
J.E.Gray@sheffield.ac.uk

Julie Gray

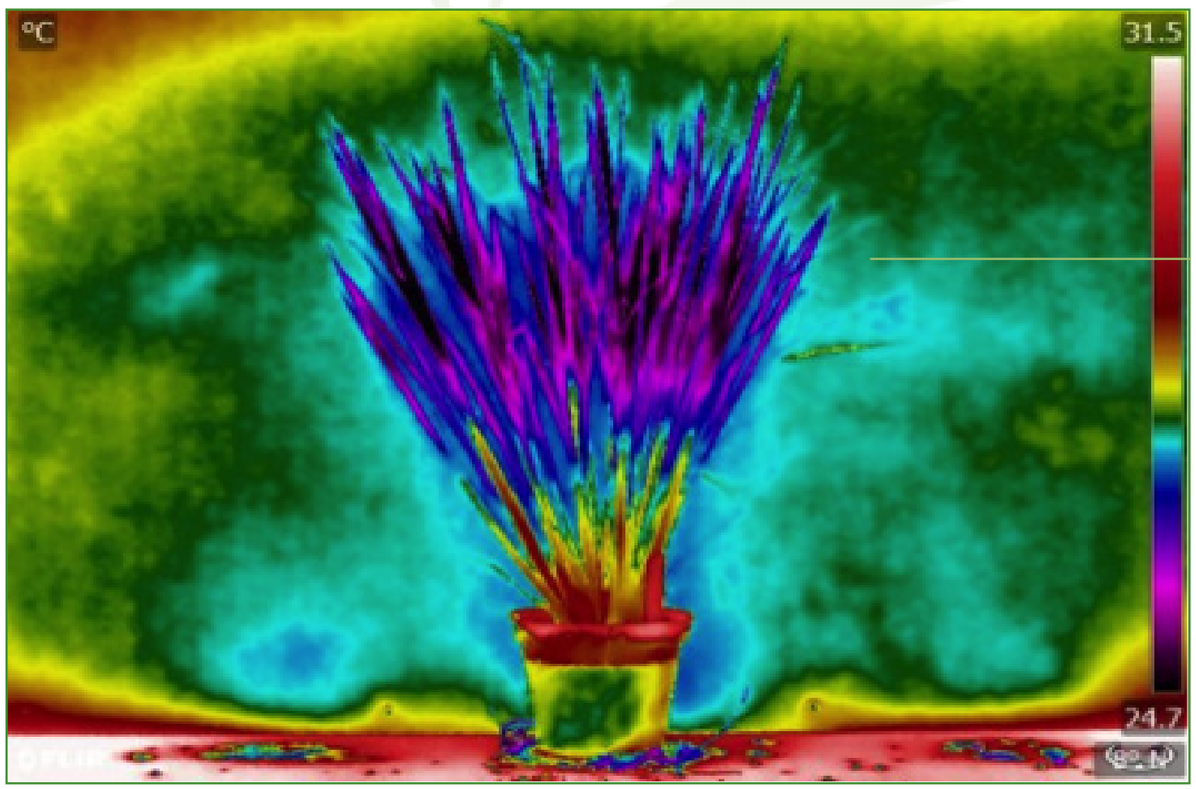
The Gray group studies how stomatal aperture and stomatal development are controlled by the plant, and how environmental change affects both the number of stomata that are produced and their sensitivity.

We use molecular genetic techniques to study the evolution of stomatal signalling pathways which are believed to have been important for the greening of the earth over 400 million years ago. Recently they have begun to translate their findings from Arabidopsis into crops including wheat and rice, to improve drought tolerance and water use efficiency.

Professor Neil Hunter FRS
c.n.hunter@sheffield.ac.uk

Neil Hunter

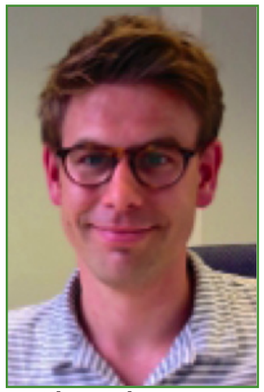
Photosynthesis is essential for life on Earth. It starts with the collection of solar energy by the protein-bound chlorophyll and carotenoid pigments of light-harvesting (LH) complexes, which absorb and transfer this energy to reaction centres (RCs) where it is trapped, before conversion to a form of energy useful for the cell.



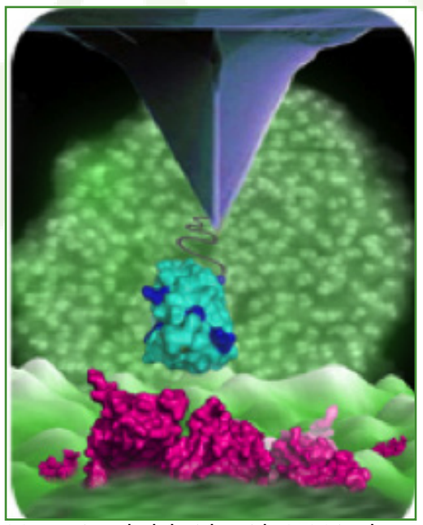
Thermal imaging is used to assess stomatal responses in the Gray lab.

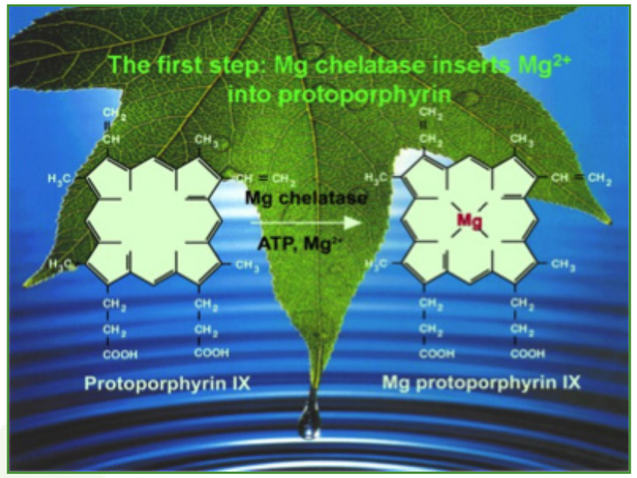
We exploit the relative simplicity of photosynthetic bacteria to study the biosynthesis of these pigments, and the assembly, structure and membrane organisation of LH and RC pigment-protein complexes. We use molecular genetics, protein engineering, atomic force microscopy as well as structural and spectroscopic methods for our studies of photosynthetic membranes.

Dr Matthew Johnson
Matt.Johnson@sheffield.ac.uk


Matthew Johnson

My research is focused on the role of thylakoid membrane organisation in photosynthesis, which uses solar energy to transform water and carbon dioxide into energy and the oxygen. The thylakoid membrane houses several major pigment-protein complexes involved electron transport including photosystem II, the water splitting enzyme, cytochrome b6f, photosystem I and ATP synthase. I combine high resolution imaging techniques such as atomic force microscopy, affinity-mapping AFM and stochastic super-optical microscopy with membrane biochemistry to elucidate how these complexes are spatially organised within membranes. These state-of-the-art single molecule techniques allow me to image membranes in their natural environment thus preserving their native organisation.v


Measuring thylakoids with AFM in the Johnson Lab

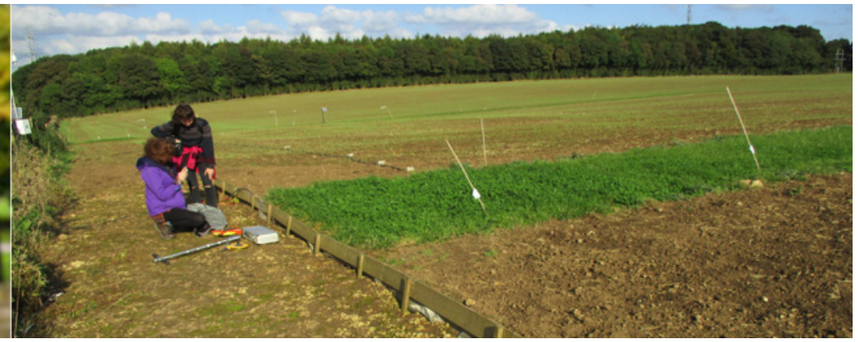


The Hunter lab studies the first step in chlorophyll biosynthesis

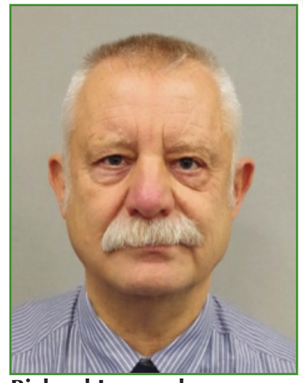
Professor Jonathan Leake
j.r.leake@sheffield.ac.uk

My research investigates how plants interact with soils to affect biogeochemical cycles of carbon, nitrogen, phosphorus; the weathering of minerals; and soil properties. One focus is on the role of plant-to-soil fluxes of photosynthate carbon via roots and mycorrhizal fungi affecting soil processes from the scales of chemical reactions with minerals to global-scale feedbacks on the carbon cycle.

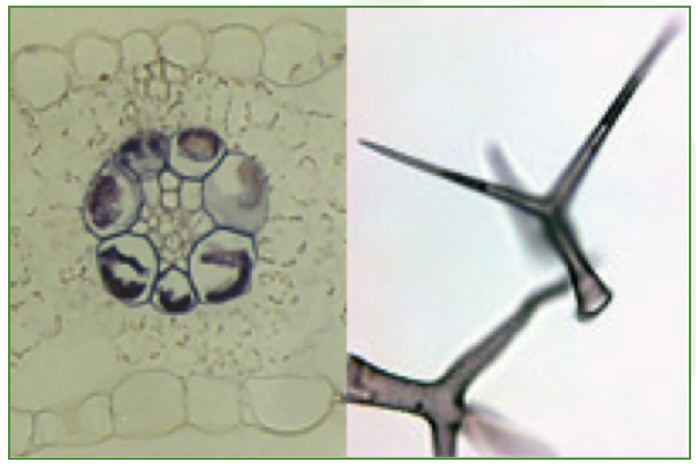
My research on weathering includes studies on the use of calcium silicates as fertilizers and lime-substitutes in acidic soils to help sequester atmospheric CO2 and reduce ocean acidification in the Leverhume Centre for Climate Change Mitigation. I also lead two consortium projects on plant-soil interactions in agriculture studying soil quality in hedgerows, wheat crops, in short-term leys and permanent grassland.



Jonathan Leake, measuring plant-soil interactions in the field.

Professor Richard Leegood
r.leegood@sheffield.ac.uk


Richard Leegood

My research is largely concerned with the regulation and control of photosynthetic carbon and nitrogen metabolism in plants. We have long had a particular interest in an enzyme of primary metabolism, phosphoenolpyruvate carboxykinase (PEPCK) and in the regulation of this area of metabolism (including phosphoenolpyruvate carboxylase and other enzymes of primary metabolism and of the CO2-concentrating mechanism of C4 photosynthesis). Research focussing on PEPCK includes studies of its regulation by phosphorylation, and of its multifarious functions in stomata, in developing seeds, in the vasculature, in trichomes, and in diatoms.


Stomata and trichomes researched in the Leegood Lab

Professor Colin Osborne
C.P.Osborne@sheffield.ac.uk


Colin Osbourne

Our research aims to understand how physiological diversity arises in wild and crop plants. How do evolutionary and ecological processes shape this diversity? What mechanisms underpin the physiological differences among species? We are interested in a number of scientific questions:

- How have plant structure and function adapted to changing ecological conditions on archaeological and geological timescales?
- How do physiological processes and structural traits interact, and how do they contribute to the differences in ecological behaviour among plant species?

• To what extent can the ecological filtering of plant traits explain the structure of communities, the functioning of ecosystems and the assembly of biomes?

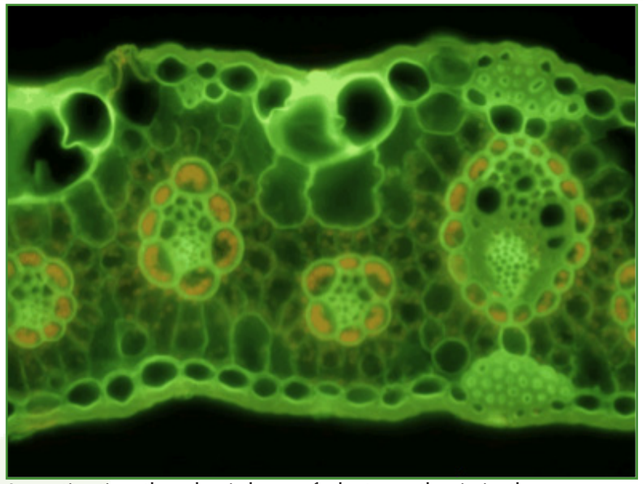
Dr Gareth Phoenix
g.phoenix@sheffield.ac.uk


Gareth Phoenix

My research focuses on the interactions between plants and the environment, particularly in the Arctic, northern boreal and upland ecosystems. I study the impacts of climate change (warming, extreme events, snow regime change, precipitation), UV-B radiation and pollution on ecosystem structure and function.

We are interested in the impacts on biodiversity, on cycling of carbon, nitrogen and phosphorus, and the consequences for feedback to climate (ecosystem carbon balance). We also aim to understand how responses observed at the vegetation/ecosystem level are driven by individual plant, root and leaf responses. Other interests include more applied fields such as understanding nutrient acquisition for sustainable agriculture.


Measuring carbon flux in the Phoenix Lab


Investigating the physiology of photosynthesis in the Osbourne Lab

Professor W Paul Quick
w.p.quick@irri.org


Paul Quick

I am the leader of the C4 research project based at IRRI and am responsible for the day-to-day operation of C4-related research activities in the Institute and for managing and coordinating the global C4 rice program.

Professor Mark Rees
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Our research focuses on plant population biology and in particular, the application of mathematical and statistical approaches to ecological and evolutionary problems, for example:

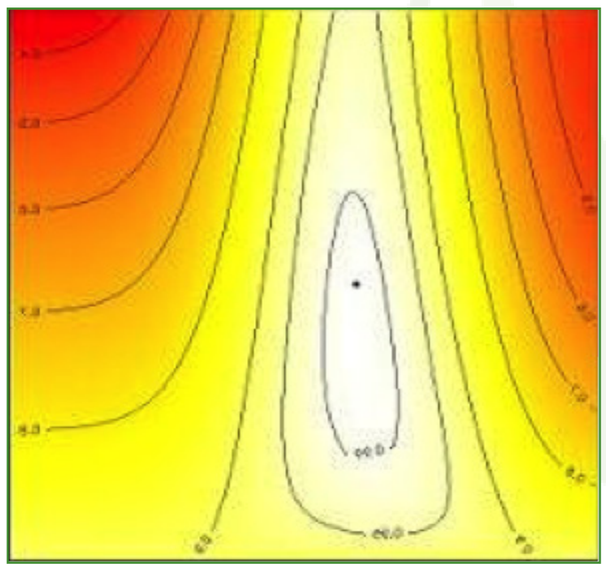
• Evolution of plant reproductive strategies - when and at what size to flower
• Evolution of seed size and dormancy
• Modeling management strategies for weed populations
• Physiology and life history consequences of growth-survival trade-offs
• Population biology of invasive plants
• Modeling structured populations using integral projection models

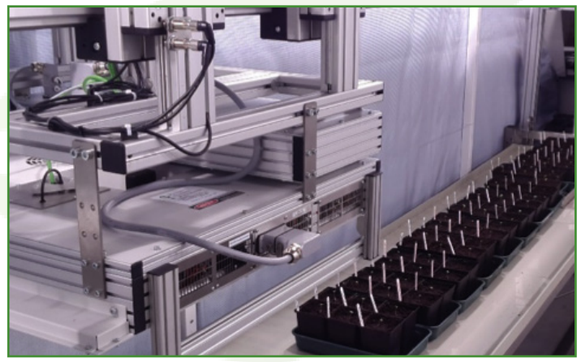

Mark Rees

Dr Stephen Rolfe
s.rolfe@sheffield.ac.uk

Our research focuses on plant responses to biotic and abiotic stresses using biological imaging techniques. I lead the P3 Wolfson Centre for Plant Disease Phenomics where we use high-throughput imaging to explore quantitative resistance to plant diseases. We use chlorophyll fluorescence and multi-spectral imaging to quantify disease development and the impact on host plants. Current projects are focussed on diseases of Brassicas including clubroot and light leaf spot. My group also looks at microbial communities in natural environments using metagenomic approaches. This includes plant pathogen populations but also the root microbiome and degradation of xenobiotics in contaminated environments. Work in my group is funded by the EU, BBSRC, the Wolfson Foundation, Bayer Crop Sciences, Shell Aviation and Airbus.


Stephen Rolfe


Modelling Evolutionary processes in the Rees lab


Bespoke phenomics in the Rolfe Lab

Professor Julie Scholes
j.scholes@sheffield.ac.uk

Our research is focused on understanding the physiological and molecular interactions between plants and their symbionts including parasitic weeds, fungal pathogens and mycorrhizal fungi. Our research focuses on the root parasitic witchweeds and broomrapes of the genera Striga and Orobanche. Striga infects the staple cereal crops of sub-Saharan Africa causing devastating losses in yield. Using both laboratory


Julie Scholes

studies and field trials, my research group exploits genomic, comparative genomic and quantitative genetic approaches to identify mechanisms underlying resistance and susceptibility in cereal hosts to *S. hermonthica* and *S. asiatica* and sunflower to *Orobanche cumana*, with the aim of breeding durable defence against these parasites. We are also taking a population genomics approach to identify virulence loci in Striga in order to understand the basis of host-parasite specificity and we have recently completed sequencing the genome of *Striga hermonthica*.


Studying Witchweed in the field with the Scholes Lab

Dr Lisa Smith
lisa.m.smith@sheffield.ac.uk

My research focuses on plant development and responses to stress. I am particularly interested in the contribution of a group of receptor-like kinases to plant development (fertilisation and drought resistance) and their regulation by environmental signals. The receptor-like kinases are a particular large and diverse group of proteins that detect signals from outside the cell (either from other plant cells or from the surrounding environment) to help the cell and therefore the plant develop appropriately for the given environment. We also examine the developmental roles of a nuclear-localised kinesin as well as the long-term effects of biotic stress on heritable phenotypic and epigenetic variation, the latter in conjunction with Jurriaan Ton's group.


Lisa Smith

Dr Karim Sorefan
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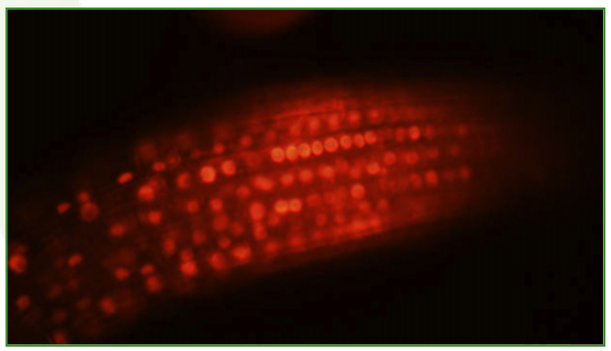
We are interested in the gene regulatory mechanisms controlling plant growth and development. My lab is investigating how plant hormones coordinate gene expression, gene regulatory mechanisms controlling seed size, and how leaf development is regulated. Our group uses next generation sequencing approaches to investigate gene and microRNA expression. Recently, we found that resolving G-quadruplex secondary structures in DNA is important for controlling gene expression and cell growth. G-quadruplexes are of great interest because they regulate fundamental biological processes and are associated with human diseases and cancer. As yet very little is known about their function in any multicellular context or in plant biology.

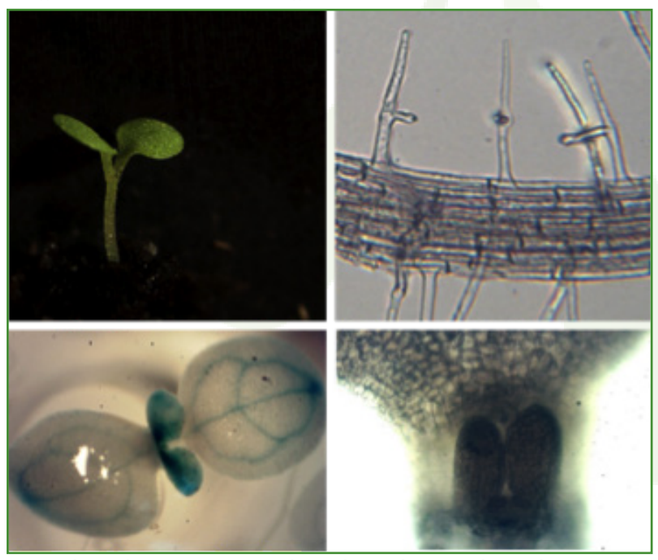

Karim Sorefan


Root tissue expression from the Smith Lab

Professor Jurriaan Ton
j.ton@sheffield.ac.uk

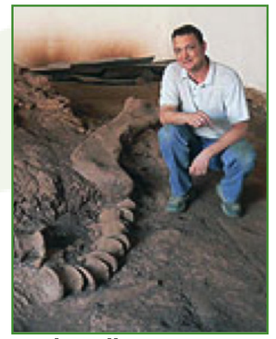
Our lab investigates how plants employ their immune system to adapt to environmental stress. When plants are attacked by pathogens or herbivores, they protect themselves by activating defences. These inducible defences are often costly, due to allocation of limited resources or auto-toxicity toxicity of the defence itself. Plants can also acquire a less costly form of disease resistance after perception of environmental alarm signals that sensitise the plant's immune system. This "immune priming" mediates a faster and/or stronger defence reaction when the plant is attacked at a later stage. We investigate the (epi) genetic basis of priming and the root exudation chemistry that recruits priming-inducing soil microbes. We collaborate with industrial stakeholders to translate this knowledge from model systems (Arabidopsis) into novel crop protection methods for cereals and vegetables.


Jurriaan Ton

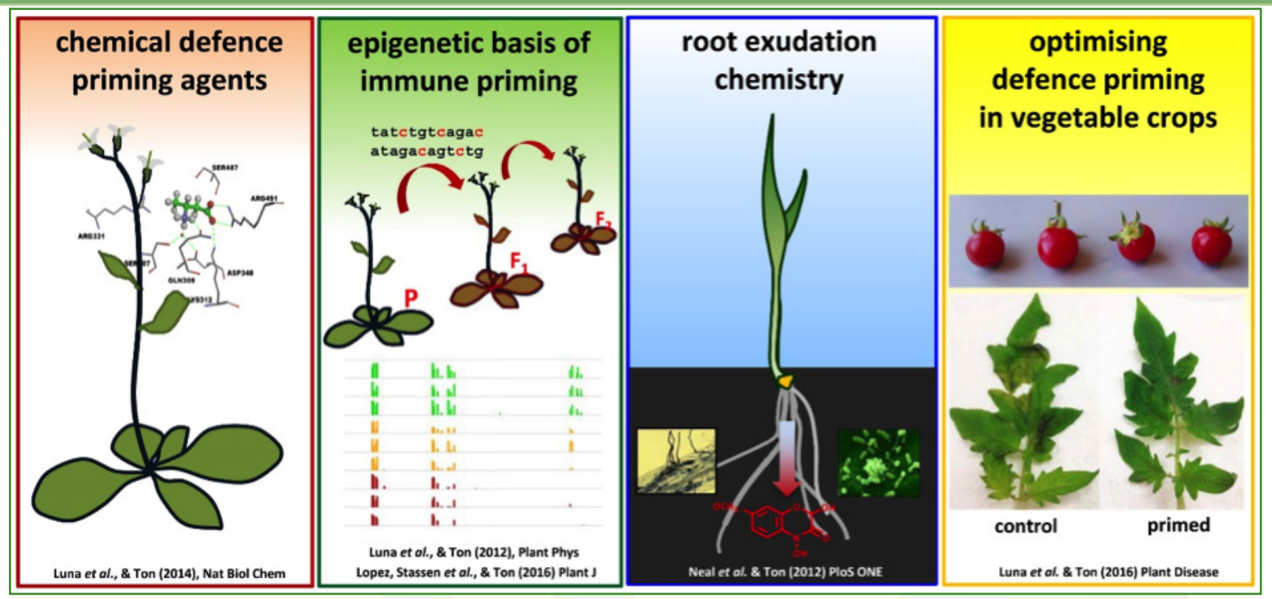

Growth and expression analysis from the Sorefan Lab

Professor Charles Wellman
c.wellman@sheffield.ac.uk

My research aims to shed light on the origin and early evolution of land plants by integrating evidence from both fossil and living plants. Fossil evidence is in the form of early land plant megafossils and microfossils (dispersed spores and fragments). I study living plants in order to interpret the earliest land plant fossils, specifically through: (i) cladistic analyses of evolutionary relationships; (ii) molecular clock analyses of evolutionary divergence times; (iii) analysis of physiological adaptations required for plants to invade the land. We are also exploring the impact of the invasion of the land by plants on global change.


Mark Wellmann

The University Of Sheffield.



Research topics under investigation in the Ton Lab

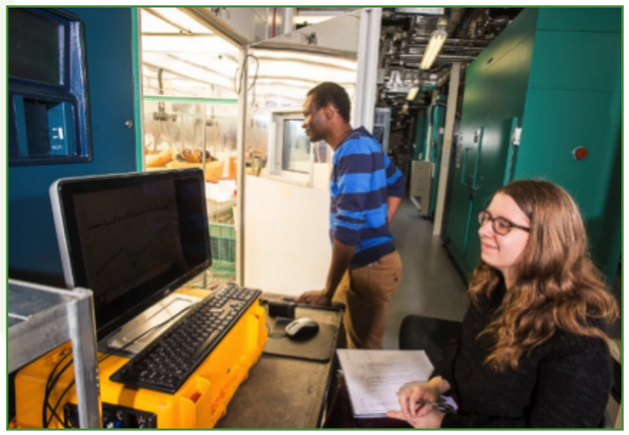
Plant research is blossoming in Sheffield

p3@sheffield.ac.uk

P3 Plant Production and Protection

The Plant Production and Protection (P3) Centre is a translational biology centre encompassing the breadth of plant and soil science expertise within the University of Sheffield. P3's expertise and facilities provides it with a unique capability to work across biological scales, from genome to the global atmosphere.

The P3 Centre has established a comprehensive and state-of-the-art Plant Disease Phenomics Platform based in its controlled environment centres. Combining P3 expertise in plant biology and industrial partner engagement, the Phenomics Platform facilitates rapid selection of robust new crop varieties for use in modern agricultural land management.



Multidiscplinary research is key for the success of the P3 Plant Science Centre

Plants in Computer Vision

Nathan Hughes
nah3l@aber.ac.uk
Department of Computer Science,
Aberystwyth University

Hannah Dee and Jonathon Bell (Aberystwyth University)

Last month I had the opportunity to attend a one day BMVA workshop on plants in computer vision. As I have worked for the past few months in a facility purpose built for analysing and capturing images of plants, I found it to be extremely informative to hear from many other researchers and how they have set about tackling and solving these problems.

Hanno Scharr (Forschungszentrum Julich, Germany) opened the one day meeting by highlighting the importance of purpose built facilities, techniques and equipment for high-throughput plant science; with so many industries depending on the 4 Fs (*Food, Fuel, Feed and Fibre*) there is huge pressure on the efficiency and accuracy of this branch of research. We heard repeatedly from many of the speakers at the meet-up just how vital computer vision, machine learning and automation are for the continuous progression in plant science research. With the majority of research requiring experiments to be done *en masse*, it seems that each project has come-up with their own custom solutions for this. A lot of facilities are making use of "LemnaTec", "Elcom" or similar systems for their automation and image capture and are augmenting these systems to fit their needs. A recurring adaptation to the pre-built systems are higher resolution cameras, which produce larger but clearer data to work with.

Problems of Classification

Whilst I was aware of there being issues with classifications made based on visual analysis in the lab, I had not fully appreciated the problem until listening to Norman MacLeod's talk. The "*Climate leaf analysis multivariate program*" (CLAMP) system is used to identify a leaf by its physical characteristics. This talk in particular highlighted the controversial issue that between two experts (or sometimes even the same expert) the same thing can be identified differently. The red flag here being the lack of consistency tests that have been applied previously to CLAMP data. Norman pointed out that this is an issue that could be rectified/reduced through the use of computer vision, an unbiased system which could routinely and consistently evaluate leaf shape.

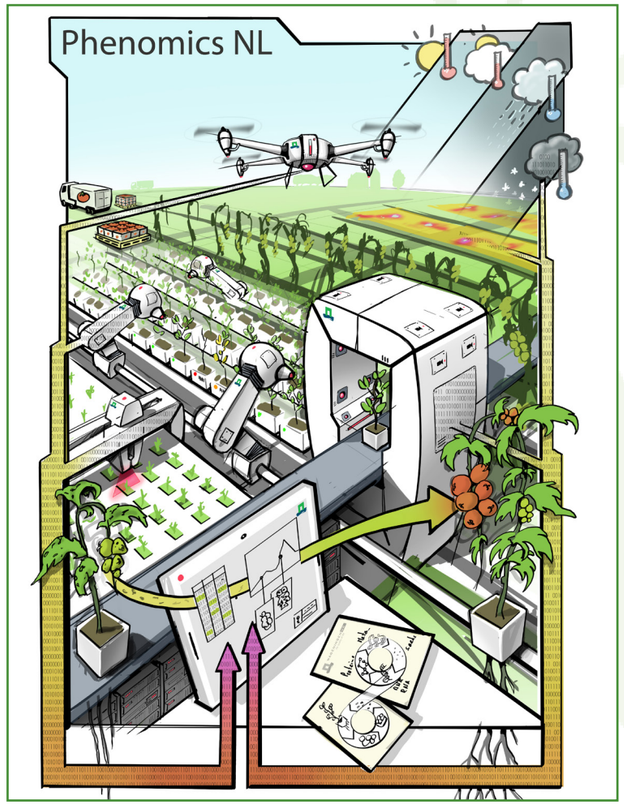
Using Machine learning to aid ID of plants
In order to pull useful information out of images, it is vital to clearly identify regions of a plant and to segment them correctly. This is exactly what Hannah Dee and Jonathon Bell have been using machine learning techniques to achieve. Using Arabidopsis they have utilised convolutional neural networks in order to separate and uniquely identify each individual leaf of a plant, throughout it's life. This allows for the examination of any particular leaf at any stage in growth, and to compare it with any other point.

Building 3D models

As automation overtakes manual tasks in working physically with plants, it becomes incredibly important to have precision. Without this accuracy we would have machines accidentally damaging plants. This presses the importance of building accurate, usable and mapable 3D models for use in directing machines towards their targets i.e. individual leafs, stalks or stems.

Rich van de Zedde's group has done this very successfully with a multi-camera approach in order to build bespoke 3D models, allowing seedlings to be rapidly rendered and processed within milliseconds. They use six cameras and a volumetric insertion technique to carve away anything from the model which is part of the background. This system is currently in use to prune growing roses by cutting away unnecessary parts and allowing the stem to grow unhindered.

In addition to performing clever maintenance for plants, this system is able to capture visual data along with physical tissue information by tactfully sampling leafs without harming the plant. Another interesting approach is Jonathon Gibbs' "Active Vision Cell" which uses a robotic arm with six-degrees of freedom and intelligently captures enough images of a plant, from as many angles as it requires in-order to fully create a 3D model without any gaps.



PhenomicsNL pipeline from Wagenigen University

Hyper-spectral imaging

Another interesting technique is the use of hyper-spectral imaging to filter out different spectra of light. This appears to have huge potential in field work on larger scales as Hanno Scharr pointed out in his keynote. Being able to process entire fields of crops at once could allow identification of diseases, pests or malnutrition effectively reducing the need for wide-scale treatments and allowing for smaller more precise treatments. As Jonathon Gibbs pointed out, hyper-spectral imaging on a plant-per-plant basis has largely been ignored and could allow for easier depth perception in single camera image analysis. Although a lot of work would be needed on identification of spectra of interest per species.

Having said that, Dominic Williams (James Hutton Institute) has found success in identifying raspberry plants in the field using 400-1000 nano-meter (visible/near-infra-red) cameras. He has been able to locate individual plants whilst mostly filtering out backgound foliage / weeds. What's more impressive is that this is being done with mobile equipment moving through a field at ground level. The hope for this research is that it could be easily adapted for other similar field crops such as blueberry plants.

It is clear that this field of research is very much still blossoming, there are so many avenues yet unexplored and a lot of excellent work being produced. As the divide between Biology and Computer Science becomes blurred it is evident that both fields can greatly help and improve the other.

CyVERSE UK WORKSHOP

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