

The Sainsbury Laboratory Summer School 2017

Contents

Welcome to The Sainsbury Laboratory Summer School 2017	5
Course schedule	7
Monday 31st July	7
Tuesday 1st August	7
Wednesday 2nd August	8
Thursday 3rd August	8
Friday 4th August	9
Saturday 5th August	9
Sunday 6th August	9
Monday 7th August	10
Tuesday 8th August	10
Wednesday 9th August	11
Thursday 10th August	11
Friday 11th August	11
Resistance Proteins	13
Keynote Lecture	13
Practical Session - Model pathosystems and effector triggered immunity readouts	14
Genomic Resources and Bioinformatics for Plant Microbe Interactions.	17
Keynote Lecture	18
Practical Session - From Sequence Data to Candidate Gene	19
Effectors and Immunity	21
Keynote Lecture	23
Practical Session - Studying Effector Function <i>in planta</i>	24
Surface Immunity	25
Keynote Lecture	26
Practical Session - Analysing Surface Immunity	27
Cellular Defence	29
Keynote Lecture	30
Practical Session - Live Cell Imaging and Investigation of Subcellular Membrane Trafficking	31

Functional Plant Genomics	33
Keynote Lecture	34
Practical Session - Functional Plant Genomics	36
Application of Discovery and Targeted Proteomics in Plant Pathogen Interactions	37
Keynote Lecture	37
Practical Session - Analysing and interpreting mass spec data	39
Translations and Tipping the Balance	41
Keynote Lecture	41
Practical Session - Practical Session Title	43
General Information	45
Arriving into Norwich	45
Checking into Accomodation	47
Meals	47
Getting Around	47
Site Registration	48
Emergency Contacts	48
Transport	48
Norwich and Norwich Research Park	49
Wifi Connections	49
Social Media	49
Map	49
Excursion	50

Welcome to The Sainsbury Laboratory Summer School 2017

The last 20 years have provided a sophisticated understanding of how plants recognise relatively conserved microbial patterns to activate defence. In recent years DNA sequencing has allowed genomes and transcriptomes of eukaryotic rusts and mildew pathogens to be studied. High-throughput imaging advances have made possible the study and visualisation of intracellular interactions during pathogenesis and defence.

We will present and teach on these many aspects of plant-microbe interactions from the fundamental genomic, cellular and molecular processes to translational activities about how we convert basic discovery to real world impact.

The TSL Summer School will focus on dynamic and interactive practical sessions will naturally promote strong interactions between speakers and participants.

Over the next two weeks we will cover a wide range of topics, including:

- Pathogenomics
- Effectors
- Surface Immunity
- Bioinformatics
- Resistance Proteins
- Cellular Defence
- Proteomics
- Functional Plant Genomics
- Translation to the field

We hope that you will find the whole exercise enlightening and educational and perhaps also a little fun.

Course schedule

Monday 31st July

Introductions

Time	Activity	Venue
1000	Welcome to TSL and housekeeping	
1015	Introductions by TSL Staff	
1030	Participant Introductions	
1100	PEDAGOGICAL LECTURE - Introduction to Plant Microbe Interactions	
1200	Lunch	NRP Venues
1300	Poster Session	John Innes Centre Conference Centre
1500	Tour of The Sainsbury Laboratory and Norwich Research Park	Meet at John Innes Reception

NB All activities will take place in the Training Suite, unless otherwise stated

Tuesday 1st August

Resistance Proteins. Led by Jonathan Jones

Time	Activity	Venue
0930	PEDAGOGICAL LECTURE - Jonathan Jones	
1100	Tea Break and Discussion	
1130	Practical Session	
1230	Lunch	NRP Venues
1330	KEYNOTE LECTURE - JIJIE CHAI - Structural Study of Plant Receptor Kinases	Jane Rogers Seminar Room at EI
1430	Tea Break and Discussion	

Time	Activity	Venue
1500	Practical Session	

Wednesday 2nd August

Resistance Proteins. Led by Jonathan Jones

Time	Activity	Venue
930	Practical Session	

Genomic Resources and Bioinformatics for Plant Microbe Interactions. Led by Dan MacLean

Time	Activity	Venue
1130	PEDAGOGICAL LECTURE - Dan MacLean	
1230	Lunch	NRP Venues
1330	KEYNOTE LECTURE - DIANE SAUNDERS - Developing new tools for interrogating cereal invaders	Jane Rogers Seminar Room at EI
1430	Tea Break and Discussion	
1500	Practical Session	
1800	Social Session with TSL Students	

Thursday 3rd August

Effectors and Plant Immunity. Led by Sophien Kamoun

Time	Activity	Venue
930	PEDAGOGICAL LECTURE - Sophien Kamoun	
1100	Tea Break and Discussion	
1130	Practical Session	
1230	Lunch	NRP Venues
1330	KEYNOTE LECTURE - JENS BOCH - A battle for life and death - Evolution of TALEs in plant-pathogenic <i>Xanthomonas</i> bacteria	JIC G34/35
1430	Tea Break and Discussion	
1500	Practical Session	

Friday 4th August

Effectors and Plant Immunity. Led by Sophien Kamoun

Time	Activity	Venue
930	Practical Session	

Surface Immunity. Led by Cyril Zipfel

Time	Activity	Venue
1030	Practical Session	
1230	Lunch	NRP Venues
1330	Pedagogical Lecture - Cyril Zipfel	
1430	Tea Break and Discussion	
1500	Practical Session	
1530	KEYNOTE LECTURE - STEFANIE RANF - Specificity of LORE-dependent lipopolysaccharide immune sensing in <i>Arabidopsis</i>	
1630	Practical Session	
1900	Conference Dinner	Sainsbury Centre for Visual Arts

Saturday 5th August

Surface Immunity. Led by Cyril Zipfel

Time	Activity	Venue
930	Practical Session	

Sunday 6th August

Excursion

Time	Activity	Venue
1100	Board Coach to Cromer	JIC Reception
1600	Board Coach to Blakeney	Cromer Coach Park - TBC
1645	Bean's Seal Trip Departs	Quayside Blakeney
1845 (approx)	Arrive back at UEA	

Monday 7th August

Cellular Defence. Led by Silke Robatzek

Time	Activity	Venue
930	PEDAGOGICAL LECTURE - Silke Robatzek	
1100	Tea Break and Discussion	
1130	Practical Session	
1230	Lunch	NRP Venues
1330	KEYNOTE LECTURE - PAUL BIRCH - The Delivery and Activity of late blight effector proteins that suppress plant immunity	JIC G34/35
1430	Tea Break and Discussion	
1500	Practical Session	

Tuesday 8th August

Cellular Defence. Led by Silke Robatzek

Time	Activity	Venue
930	Practical Session	
1230	Lunch	NRP Venues

Functional Plant Genomics. Led by Ksenia Krasileva

Time	Activity	Venue
1330	KEYNOTE LECTURE - DANIEL CROLL - Retracing genome evolution of pathogens during rapid disease emergence in agricultural ecosystems	JIC G34/35
1430	PEDAGOGICAL LECTURE - Ksenia Krasileva	
1530	Tea Break and Discussion	
1600	Practical Session	

Wednesday 9th August

Proteomics. Led by Frank Menke

Time	Activity	Venue
930	PEDAGOGICAL LECTURE - Frank Menke	
1100	Tea Break and Discussion	
1130	Practical Session	
1230	Lunch	NRP Venues
1330	KEYNOTE LECTURE - DELPHINE PFLEIGER - Quantitative phosphoproteomic analysis reveals shared and specific targets of Arabidopsis MAPkinases MPK3 MPK4 and MPK6	Jane Rogers Seminar Room at EI
1430	Tea Break and Discussion	
1500	Practical Session	

Thursday 10th August

Translations and Tipping the Balance. Led by Matt Moscou and Peter Van Esse

Time	Activity	Venue
930	PEDAGOGICAL LECTURE - Matt Moscou	
1100	Tea Break and Discussion	
1130	Practical Session	
1230	Lunch	NRP Venues
1330	KEYNOTE LECTURE - BEAT KELLER - Molecular analysis of wheat – fungal pathosystems and applications in resistance breeding	JIC G34/35
1430	Tea Break and Discussion	
1500	Practical Session	

Friday 11th August

Translations and Tipping the Balance. Led by Peter Van Esse and Matt Moscou

Time	Activity	Venue
930	Practical Session	
1030	Tea Break and Discussion	
1100	PEDAGOGICAL LECTURE - Peter Van Esse	
1200	Concluding Remarks	

Resistance Proteins

Led by Jonathan Jones

Plant Resistance Genes, Proteins and Mechanisms

The plant immune system contains both cell surface and intracellular receptors. Cell surface receptors often confer broad spectrum recognition to conserved pathogen-associated molecular patterns (PAMPs), and upon recognition the plant mounts an immune response termed PAMP-triggered immunity (PTI). Pathogens co-evolve with their hosts and can overcome PTI through the evolution of proteins they secrete into plants, termed effectors, which suppress components of the PTI machinery. Plant intracellular receptors can detect effectors by binding them directly or by indirectly recognising their activity; this recognition triggers a strong immune response (effector-trigger immunity; ETI) that shares molecular components with PTI but is often stronger and is characterized by a cell-death response termed the hypersensitive response (HR). A recognised effector leads to loss of virulence in resistant plants with the cognate intracellular receptor, and is hence termed an avirulence factor (Avr) in this case. The intracellular receptors are encoded by Resistance (R) genes that have been strongly selected for by plant breeders for the strain-specific resistance conferred to pathogens that have broken other resistance mechanisms. This co-evolution of pathogen virulence versus plant immunity is encompassed by the zig-zag model of plant immunity (Figure 1)

Keynote Lecture

Jijie Chai - Structural Study of Plant Receptor Kinases

Max Planck Institute for Breeding Research, University of Cologne

Plant receptor kinases (RKs) are a large family of single transmembrane proteins that play important roles in diverse biological processes including development, growth and immunity. RKs are characterized with diversified extracellular domains (ECDs) and conserved intracellular kinase domains. Recognition of their cognate ligands by ECDs of RKs initiates activation of RKs. The molecular mechanisms underlying this process remained poorly defined. We recently solved the crystal structures of the ECDs derived from several RKs in complex with their respective ligands. These structures define the molecular mechanisms by which these RKs recognize their specific ligands. More importantly, a general mechanism underlying ligand-induced activation of RKs can be formulated. In the current talk, I will briefly review what we have done on structural study of RKs and present two examples of how RK activation and ligand recognition mechanisms were used for the matching of receptor-ligand pairs.

About Jijie Chai

Jijie Chai was born on April 16, 1966 in Liaoning province, China. He received his bachelor's degree in chemical engineering from Dalian Light Industry College, master's degree in applied chemistry from the Research Institute of Petroleum Processing (Beijing) and Ph.D. in analytical chemistry from the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

From 1999 to 2004, he worked as postdoctoral fellow at Princeton University, where he started his research in structural biology.

In July 2004, he joined the National Institute of Biological Sciences as an independent investigator, where he established his own research programs, structural study of plant receptor kinases and NOD-like receptors. After working there for six and half years, he moved to Tsinghua University and continued his research as a full professor.

Early last year, he was awarded with the Alexander von Humboldt Professorship, and he moved to Cologne late March of 2017. Jijie has published a number of papers on RLKs and NLRs, advancing our understanding the mechanisms of RLK activation and NLR inhibition and activation.

Jijie is happily married and the father of a daughter. He is currently living in Cologne.

Practical Session - Model pathosystems and effector triggered immunity readouts

Led by Zane Duxbury

Aims and Objectives

1. Become familiar with using model organisms to probe the plant immune system
2. Understand and recognise the lifecycle and symptoms of some common diseases
3. Understand transient expression systems for determining relationship between **R** and **avirulence** genes

The aim of this practical session will be to familiarise you with the use of model organisms to probe the plant immune system. The practical will include a general introduction to the oomycete pathogens of *Arabidopsis*: *Albugo* spp and *Hyaloperonospora arabidopsidis* (white rust and downy mildew respectively), the bacterial species *Pseudomonas syringae* and *P. fluorescens*, and the pathosystem of potato and the oomycete *Phytophthora infestans* (late blight) (Figure 2).

We will familiarise you with the life cycle, pathogenesis and symptoms of these pathogens. We will use various techniques to assess the growth of pathogens on their hosts and to assess immune responses mounted against these pathogens. For example, we will use light microscopy of trypan-blue stained *Arabidopsis* infected with downy mildew to qualitatively assess the success of infection and resistance of different genotypes of the pathogen and plant. We will introduce transient expression systems such as bombardment

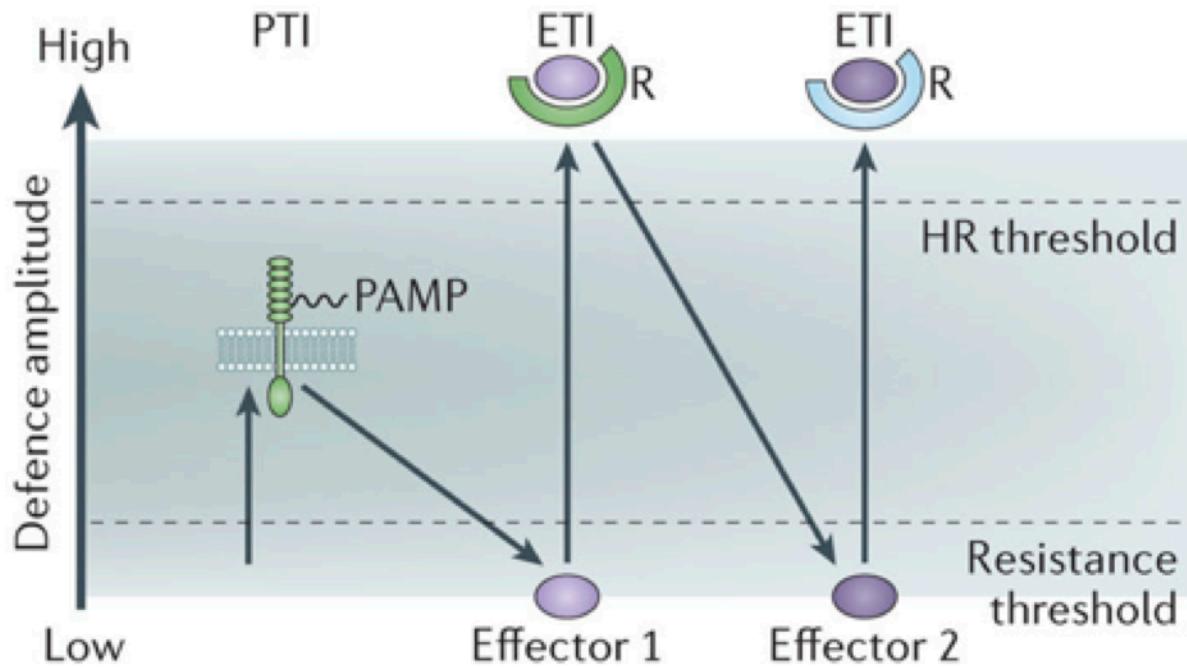


Figure 1: The zig-zag model of plant immunity (Jones and Dangl, 2006). The ultimate amplitude of defence is the combined sum of resistance output (ETI+PTI) and the difference of the effect of pathogen effectors (-ETS; effector triggered susceptibility). This diagram captures the observation that many PTI and ETI outputs are similar, but HR is associated specifically with successful ETI, and that virulent pathogens with specific effectors are able to suppress immunity to compromise immunity. Image is from Pumplin and Voinnet (2013).

and *Agrobacterium tumefaciens*-mediated *Nicotiana tabacum* transformation (agroinfiltration) to determine the relationship between R- and avirulence-genes responsible for compatible (resulting in disease) or incompatible (resulting in healthy plants) interactions.

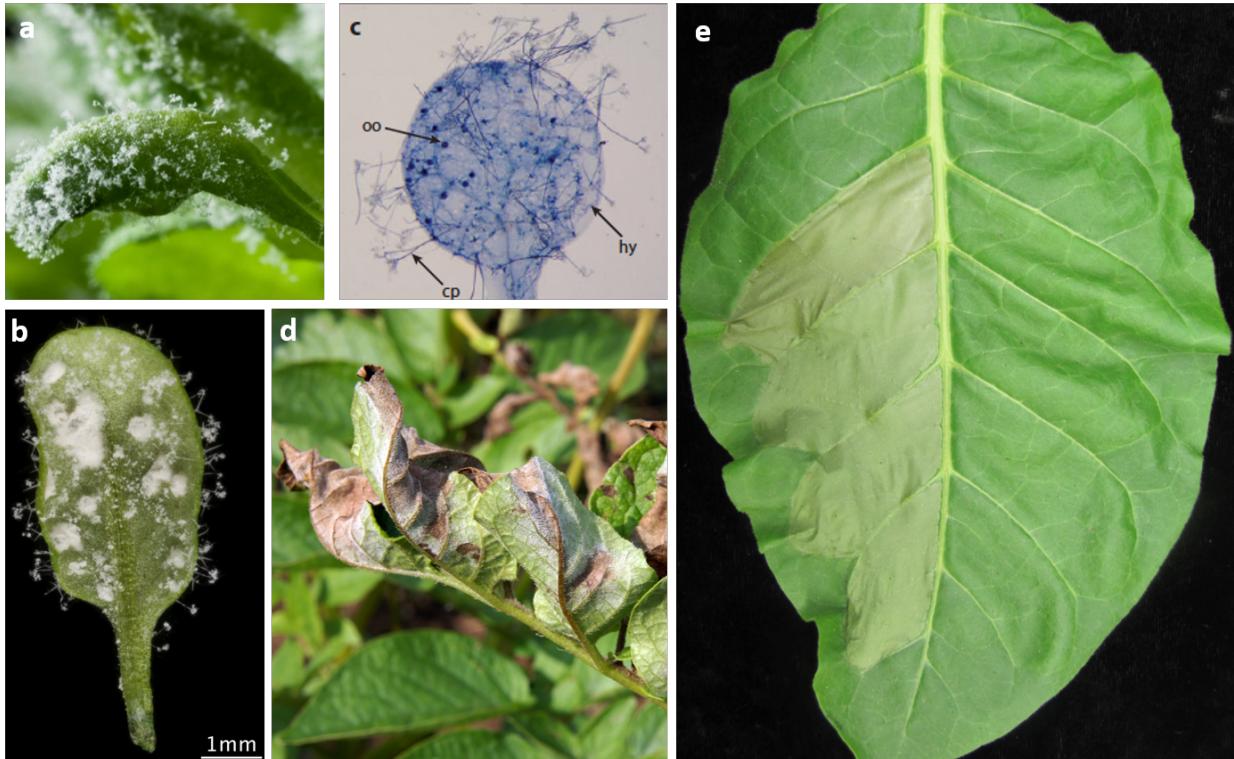


Figure 2: Macroscopic characteristics of plant-pathogen interactions. a, b) Sporulating *Hyaloperonospora arabidopsisidis* (seen on the edges of the leaf in b) growing on *Arabidopsis thaliana* leaves. Albugo is also growing on the abaxial surface of the leaf in b. c) Trypan blue staining of a *H. arabidopsisidis*-infected leaf of *Arabidopsis*. d) Foliar symptoms of *Phytophthora infestans* infection of potato. e) Hypersensitive cell death in tobacco leaf resulting from *Agrobacterium tumefaciens*-mediated transformation with cognate R gene and Avr gene.

Genomic Resources and Bioinformatics for Plant Microbe Interactions.

Led by Dan MacLean

The increase in the generation and analysis of sequence data in the last ten years has had a profound effect on plant and microbe interaction research. The genomes of the wide range of host and pathogen's of interest are now open to study in a way that is within reach of most scientists - not just large genome sequencing institutes - and many laboratories are now undertaking genomics as a routine approach.

The deluge of data created by new sequencing approaches has been collected into a wide range of general and domain specific databases, each of which contain different information accessed in different ways. Knowing which are the most useful databases in a given context is therefore a tricky question and in this session we will take a tour of the most widely-used including Ensembl¹, PhytoPath², SolGenomics³, TAIR⁴ and AraPort⁵.

Sequence data are used in a wide range of applications and the source molecule will be selected in an application specific way. Genomic DNA is used for assembly of draft genomes, RNA is used for gene expression analysis, genome annotation and exome construction. Both DNA and RNA get used to identify genetic polymorphisms. Mixed populations of nucleic acids from environmental (e.g soil or pathogen/host interaction sites) are used to study species compositions. A wide range of bioinformatics tools have been developed and are in common use for these approaches, so in this topic we will study briefly the tools and their core algorithms and competencies with the aim of helping you to decide on the right tools for any particular analysis that you may wish to do outside of the course. A useful guide is available in MacLean et al. (2009). In particular we will look at algorithms and tools for *de novo* assembly of sequence including SOAPdenovo (Luo et al., 2012) and Celera Assembler⁶. We will study tools for RNASeq expression and annotation analyses including Tophat (Trapnell et al., 2009) and Bowtie (Langmead and Salzberg, 2012) and DESeq (Anders and Huber, 2010) and edgeR (Robinson et al., 2010).

¹<http://ensembl.org>

²<http://phytopathdb.org>

³<http://solgenomics.net>

⁴<http://arabidopsis.org>

⁵<http://araport.org>

⁶http://wgs-assembler.sourceforge.net/wiki/index.php?title=Main_Page

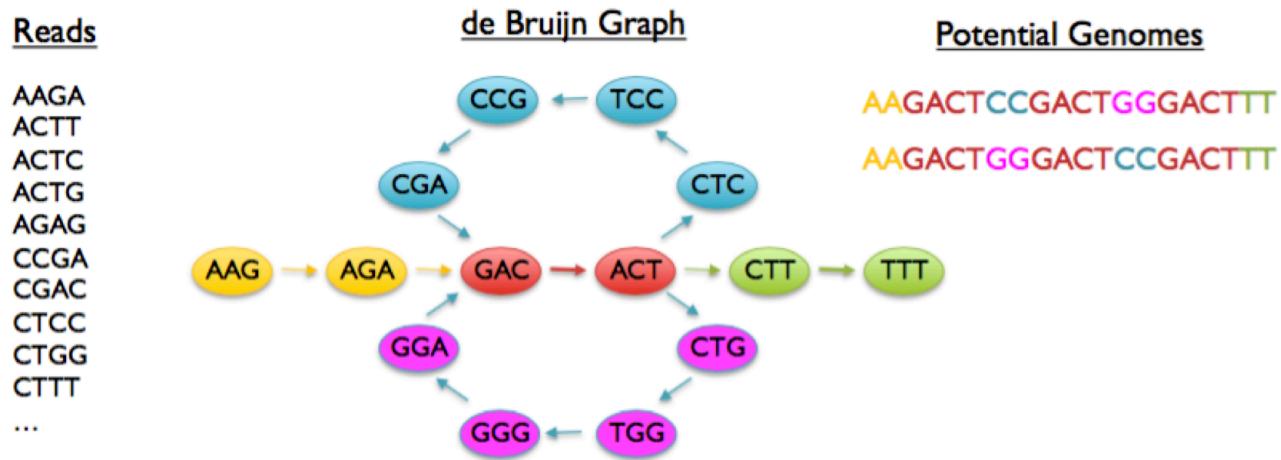


Figure 3: Graphical summary of a *de novo* assembly algorithm. Sequence reads are broken down into constituent k -mers and a network of overlapping k -mers is produced. The paths in the graph are traversed and the k -mers collected into a growing string representing a long sequence in the original data and therefore genome.

Keynote Lecture

Diane G.O. Saunders - Developing new tools for interrogating cereal invaders

John Innes Centre, Norwich, UK

Emerging and re-emerging pathogens pose a continuous threat to food security and human health. Recent advances in next-generation sequencing technologies have provided new opportunities to integrate genomics into the tracking of emerging filamentous plant pathogens. This approach is particularly valuable for analysing rust pathogens, which are frequently obligate biotrophs that cannot be axenically cultured in the laboratory environment. Accordingly, we are leading the genome sequencing of hundreds of isolates of the wheat yellow rust pathogen *Puccinia striiformis* f. sp. *tritici* (PST), aimed at improving our understanding of the molecular mechanisms that drive PST evolution. Furthermore, we have developed a robust and rapid “field pathogenomics” strategy to improve filamentous pathogen surveillance. Using gene sequencing of PST-infected wheat leaves taken directly from the field, this technique enabled us to gain insight into the population structure of PST over successive seasons. Our analysis uncovered a dramatic shift in the PST population in the UK and supports the hypothesis that a recent introduction of a diverse set of exotic PST lineages may have displaced the previous populations. Furthermore, we uncovered potential seasonal and varietal specificity for specific genotypes of PST. Our discovery of wheat variety susceptibility to specific *P. striiformis* isolates that are prevalent at certain times of the year could have considerable impact on directing future disease management approaches.

About Diane Saunders

Dr. Saunders received her BSc degree from Exeter University where she continued her studies to PhD level in the pioneering laboratory of Prof. Nick Talbot. After receiving her PhD in

2009 she joined Prof. Sophien Kamoun's group at The Sainsbury laboratory (TSL) to continue to pursue her interest in the molecular mechanisms that underpin plant-pathogen interactions. Following an early career Leverhulme fellowship at TSL she was awarded a research fellowship from the Earlham Institute and the John Innes Centre to develop her own research group with a focus on emerging and re-emerging plant pathogens. She was then appointed as Project Leader at the John Innes Centre in 2017 where she continues to pursue a multi-disciplinary approach to her research, integrating molecular genetics, microbiology, cell biology, biochemistry, genomics and data mining. In addition, she has worked on three of the most important plant diseases in the world: rice blast, potato late blight, and cereal rusts.

Practical Session - From Sequence Data to Candidate Gene

Led by Dan MacLean

Aims and Objectives

1. Understand Strengths and Weaknesses of High Throughput Sequence Data
2. Know how to call SNPs from HTS data on
3. Categorise SNPs according to an expected genetic background

Genomics has come a long way. We can now sequence genomes quickly and to a reasonable degree of accuracy. We can create in a high-throughput manner an inventory of sub-regions in a genome that we think are genes. We know the functions (or some of the functions) of lots of genes and we can infer functions of newly discovered genes by comparison of sequence or structure, basically by seeing whether our new thing looks like something else.

These methods are actually only PREDICTIONS of function. Looking a bit like something else is only a clue to what something does. It frequently fails us.

In this practical we will look at the powerful technique of mutational genomics. This is possibly the coolest thing ever as it involves mutating a living organism so that it is different from other things and then sequencing the genome to pinpoint the exact changes that cause the difference. We don't have the scope or chemicals to do the mutation bit, so we'll pick up with the genomics and use Galaxy and Galaxy tools to carry out the analysis that takes us from sequence data to actual candidate mutations in the genome sequence.

With mutational genomics we deal initially with the effect of the gene on the whole organism. By performing mutagenesis on our favourite organism then carrying out a genetic screen (Page and Grossniklaus, 2002) that selects individuals that have changed in the phenotype we are interested in, we have our first foothold on function. We can study those individuals and apply the principles of genetics, use modern high-throughput sequencing and bioinformatics tools to identify the gene causing that phenotype change (or at least ones involved in the process we have messed up).

We will use tools in the Galaxy (Goecks et al., 2010) framework including FastQC for quality control of sequence data (Andrews, 2017), BWA for read mapping and alignment (Li and Durbin, 2009) and CandiSNP (Etherington et al., 2014) to identify candidate mutations (Figure 4).

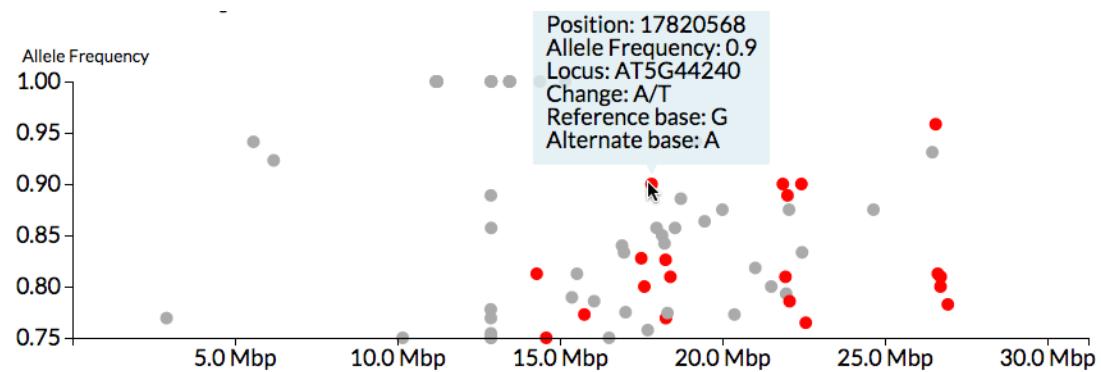


Figure 4: CandiSNP visualisation of SNPs

Effectors and Immunity

Led by **Sophien Kamoun**

One of nature's many secrets in biology is how filamentous plant pathogens cause disease on their hosts. We are making tremendous progress towards understanding this process largely due to breakthroughs in "effector biology" (Hogenhout et al., 2009; Win et al., 2012). Effectors are proteins secreted by pathogens to suppress host immune systems and manipulate plant physiology to enable pathogen colonization (Figure 5A).

We can predict an "effectorome", or effector repertoire, of a pathogen from its genome sequence based on several criteria that we know about effectors (Saunders et al., 2012). These criteria include, presence of a secretory signal peptide, lack of transmembrane domains, lack of mitochondrial targeting signals, presence of known/conserved motifs such as RXLR, upregulation of their transcripts during infection, specific localization in host cells, similarity to known effectors of other plant pathogen species, etc. However, one persistent question is: What are the intrinsic functions of these effectors? To find out, we express tagged effectors in plants and observe their effect on plant morphology and physiology [such as hypersensitive response (HR) cell death] using a technique called the agrobacteria-mediated transient transformation procedure (Kaipala et al., 1997), also known as agroinfiltration. In addition, to work out what these effectors might be doing in plants, we derive clues from the effector-associated plant proteins that we identify by performing co-immunoprecipitation (co-IP) of tagged effectors followed by mass spectrometry (MS) (Win et al., 2011). These plant proteins constitute potential targets of the effectors. We confirm the association between the effectors and the potential targets in an independent system known as yeast-two-hybrid interaction assays (Fields and Song, 1989), or in vitro interaction assays. Once we know the effector targets, we investigate them to find out if they play important roles in plant-pathogen interactions by knocking down their transcripts in plants by virus-induced gene silencing (VIGS) (Lindbo et al., 1993) and observing the effects on plant responses to infection.

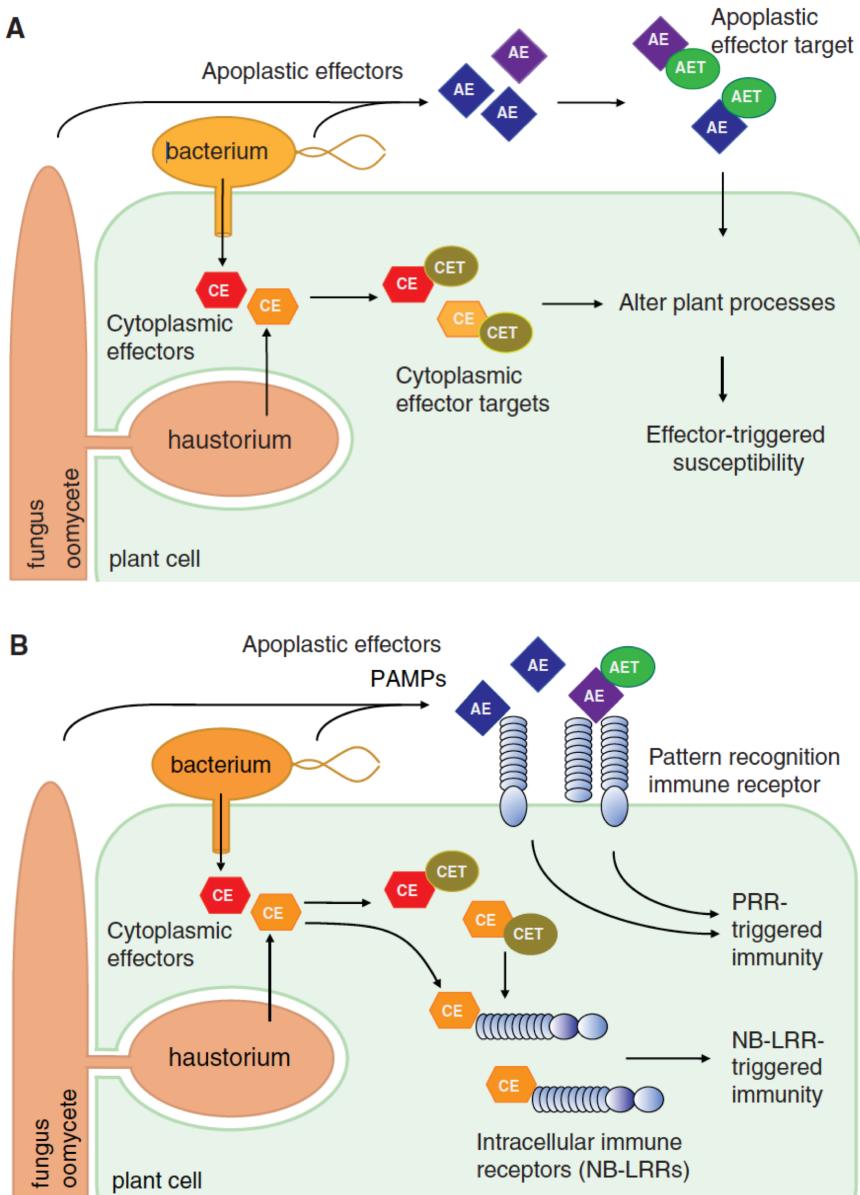


Figure 5: The concept of effectors in plant immunity. Infectious pathogens such as bacteria, fungi, oomycete, and nematodes deliver effectors at the interface of the host plant (apoplastic effectors, AE) or inside the cell (cytoplasmic effectors, CE). Host-translocated (cytoplasmic) effectors are delivered into the host cytoplasm through a type-III secretion pilus or specialized infectious structures called haustoria that form within the cell. Pathogen effectors traffic to various compartments, bind, and manipulate different host proteins called targets. Depending on their localization in the cells, these targets are designated as apoplastic effector target (AET) and cytoplasmic effector target (CET). Effector-target interactions impact the outcome of the interaction between the pathogen and its host. In susceptible genotypes (A), these molecular interactions can alter plant cell processes and suppress immune responses, leading to effector-triggered susceptibility (ETS) and host colonization. In resistant genotypes (B), these interactions are perceived by key sensing receptors of the immune system that, in turn, stop pathogen growth. Cell surface pattern recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs), apoplastic effectors, and/or apoplastic effector-target interactions to initiate PRR-triggered immunity (PTI). Intracellular nucleotide-binding receptors (NB-LRR) induce NB-LRR-triggered immunity (ETI) on recognition of cytoplasmic effectors and/or cytoplasmic effectors-target interactions (Reproduced from Win et al. (2012)).

Keynote Lecture

Jens Boch - A battle for life and death - Evolution of TALEs in plant-pathogenic *Xanthomonas* bacteria

Leibniz Universität Hannover, Institute of Plant Genetics, 30419 Hannover, Germany

Plant pathogens embark upon a fierce battle with their plant hosts over tissue colonization and nutrient acquisition. This has deadly consequences for both partners of the interaction, but also for us if crop plants are involved. Plant pathogenic *Xanthomonas oryzae* bacteria can cause substantial losses in rice, making this pathogen a key threat to our food production. Like many other plant pathogens, *Xanthomonas* bacteria translocate a collection of effector proteins into eukaryotic host cells to function as potent weapons. Among these effectors, TALEs (transcription activator-like effectors) play a pivotal role. TALEs act as transcription factors within the plant cell to induce expression of specific target genes and support the bacterial infection. The DNA-binding mechanism of TALEs is unique and based on a number of characteristic 34-amino acid tandem repeats. Each repeat recognizes one base in the target DNA via one precisely positioned amino acid of a repeat-variable diresidue (RVD). This modular architecture allows the rearrangement of TALE repeats to evolve new DNA-binding specificities matching new virulence targets. Whole genome sequences of several *Xanthomonas oryzae* strains recently revealed both, the diversity and conservation of TALEs in *X. oryzae*. In combination with plant transcript analyses and TALE target prediction we now have an outstanding opportunity to identify TALE virulence targets and decipher the pathogen's strategy. The constant evolutionary battle between pathogen and host has caused evasive mutations in plant promoters that preclude TALE-binding. Matching modifications in TALEs lead to the development of aberrant repeats with novel DNA-binding behaviours. This exemplifies the power of the molecular arms race between pathogen and host. The simple way of generating any desired DNA-binding specificity has initiated a worldwide use of TALEs for biotechnological applications. Designer TALEs and TALE nucleases (TALEN) have successfully been used for highly efficient genome reprogramming and genome editing. Today, crops and livestock with novel traits as well as powerful tools for molecular medicine have already been developed using TALEs.

Further reading:

1. Boch et al. (2009) Breaking the code of DNA binding specificity of TAL-type III effectors.
2. Richter et al. (2014) A TAL effector repeat architecture for frame shift binding.
3. Grau et al. (2016) AnnoTALE: bioinformatics tools for identification, annotation, and nomenclature of TALEs from *Xanthomonas* genomic sequences

About Jens Boch

Professor for Plant Biotechnology (2015-present), Leibniz Universität Hannover, Germany
Assistant/Associate Professor (1999-2015), Martin Luther University Halle-Wittenberg, Germany
Postdoctoral Fellow (1997-1999), Washington University in St. Louis, USA Postdoctoral Fellow (1996-1997), MPI for Terrestrial Microbiology, Marburg, Germany Ph.D. (1996), Philipps University Marburg, Germany

Practical Session - Studying Effector Function *in planta*

Led by Joe Win

We are interested in how plants defend themselves against pathogens, and how the pathogens suppress these defences. Plant immunity is governed by recognition of effectors by plant immune receptors (Figure 5B). Once recognized, several signalling processes are initiated by the receptors and defence is mounted against the invading pathogen. We have identified a novel network of plant immune components that is involved in defence signalling of Solanaceous plants (Wu et al., 2016). We study their function and activities by deploying the methods such as co-IP, HR assays and VIGS as described above.

In these practical sessions, we will guide you through the techniques and methods described above to probe plant-microbe interactions with a focus on effectors and plant immunity.

Aims and Objectives

1. To be familiar with current knowledge of effectors and their roles in infection of host plants.
2. To understand the roles of helper components in plant immune signalling network.
3. To understand the theory and practice of methods and techniques that are routinely used to study effectors and plant immunity

In this practical session, you will be invited to choose one of the following techniques for hands-on experience guided by the instructors.

1. In planta co-immunoprecipitation of effectors and associated plant proteins, and analysis of effector-plant protein interactions *in vitro*
2. Yeast two-hybrid assay to study protein-protein interaction
3. Agroinfiltration and virus-induced gene silencing to study gene functions in plants

Surface Immunity

Led by Cyril Zipfel

The first layer of plant innate immunity depends on the recognition of microbes via the perception of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) by plasma membrane-localized pattern recognition receptors (PRRs). Plant PRRs are ligand-binding receptor kinases or receptor-like proteins that exist in multi-protein complexes to transduce intracellular immune signaling by triggering downstream phosphorylation cascades (Couto and Zipfel, 2016). The *Arabidopsis* leucine-rich repeat receptor kinases (LRR-RKs) FLS2 and EFR are well-studied PRRs that bind the bacterial PAMPs flagellin and EF-Tu (or their immunogenic epitopes flg22 and elf18), respectively. In both cases, ligand-binding triggers rapid hetero-oligomerization with SERK co-receptors. Of these, BAK1/SERK3 and its closest paralog BKK1/SERK4 are the major regulators of FLS2- and EFR-dependent signaling. Intriguingly, BAK1 and other SERKs are also involved in various non-immune signaling pathways (Ma et al., 2016). An allele of *BAK1* specifically affected in PTI, *bak1-5*, enabled the uncoupling of BAK1's functions in PTI, brassinosteroid (BR) signaling and cell death control (Schwessinger et al., 2011), allowing a detailed characterization of BAK1 in immune signaling without the influence of the morphological defects observed for other *BAK1* alleles.

The plasma membrane-associated receptor-like cytoplasmic kinase (RLCK) BIK1 is an immediate convergent substrate of several different PRRs as well as BAK1 (Couto and Zipfel, 2016). In response to PAMP perception, BIK1 is phosphorylated and released from the receptor complex and subsequently phosphorylates the NADPH oxidase RBOHD, which is required to produce reactive oxygen species (ROS), one of the first hallmark responses triggered after PAMP perception (Kadota et al., 2014; Li et al., 2014). Another early PTI response is the influx of apoplastic Ca^{2+} via yet unknown channels, a response that is also BIK1-dependent (Li et al., 2014; Ranf et al., 2014; Seybold et al., 2014). Subsequently, PAMP perception results in the activation of MAP kinase cascades and calcium-dependent protein kinases, which ultimately results in the transcriptional reprogramming of the cell mostly via WRKY transcription factors and ultimately to the restriction of pathogen growth (Couto and Zipfel, 2016). Later responses associated with FLS2 and EFR activation include increased ethylene biosynthesis, callose deposition, and inhibition of seedling growth (Boller and Felix, 2009).

Keynote Lecture

Stefanie Ranf - Specificity of LORE-dependent lipopolysaccharide immune sensing in Arabidopsis

Phytopathology, TUM School of Life Sciences, Technical University of Munich, Freising-Weihenstephan, Germany

Innate immunity, triggered upon recognition of microbe-associated molecular patterns (MAMPs) by specific host receptors, is crucial for animals and plants. Cell surface components such as lipopolysaccharide (LPS), peptidoglycan and flagellin are typical MAMPs as they are vital for microbial survival and common to whole microbial classes. LPS, in particular the endotoxic lipid A moiety, of Gram-negative bacteria is sensed as MAMP in mammals through different extra- and intracellular LPS receptors. Recently, we found that the receptor-like kinase LORE (LipoOligosaccharide-specific Reduced Elicitation), which belongs to the plant-specific class of bulb-type lectin S-domain-1 kinases (SD1-RLKs), mediates sensitive perception of Pseudomonas LPS in Arabidopsis. The lipid A moiety from Pseudomonas LPS alone is sufficient to induce LORE-dependent immune responses. Interestingly, LORE specifically senses Pseudomonas and Xanthomonas but not the typical enterobacterial LPS e.g. of Escherichia coli. In mammals, on the contrary, enterobacterial LPS is the most potent immune activator, whereas Pseudomonas LPS is only a weak agonist due to structural differences within the lipid A. Thus, both mammals and plants evolved to sense LPS via its lipid A moiety but, apparently, with distinct epitope specificities and through structurally unrelated receptors.

About Stefanie Ranf

Since March 2016 - Independent Emmy-Noether group leader at the Technical University of Munich, Chair of Phytopathology

Jan. 2013 – Feb. 2016 - Junior group leader at the Technical University of Munich, Chair of Phytopathology, Prof. Dr. Ralph Hückelhoven

May 2011 – Dec. 2012 - Postdoctoral research with Prof. Dr. Dierk Scheel and Dr. Justin Lee, Department of Stress and Developmental Biology, Institute of Plant Biochemistry (IPB), Halle (Saale)

Jan. 2005 – Apr. 2011 - Ph.D. thesis with Prof. Dr. Dierk Scheel and Dr. Justin Lee, Department of Stress and Developmental Biology, Institute of Plant Biochemistry (IPB), Halle (Saale)

Oct. 2003 – Dec. 2004 - Research fellow with Prof. Dr. Johannes Stratmann, University of South Carolina, USA

Oct. 1998 – Feb. 2003 - Studies of Biochemistry at the Universität Regensburg

Practical Session - Analysing Surface Immunity

Led by Martin Stegmann

Aims and Objectives

1. Understanding of and hands on experience in classical methods to analyse PTI responses in the model organism *Arabidopsis thaliana*
2. Testing the importance of critical PTI regulators, mainly by analysing the impact of PRRs and PRR-associated RLKs on the activation of PTI signalling
3. Understanding the contribution of PTI on plant immunity against adapted bacterial pathogens

The practical session “Surface Immunity” will consist of a pedagogical lecture on the biochemical and molecular biological techniques used to investigate the molecular basis of PTI signaling, and to identify novel PAMPs or PRRs. We will demonstrate some of the classical methods used to analyze downstream PTI responses, such as ROS production, MAPK activation, seedling growth inhibition and surface immunity upon spray infection with bacterial pathogens.

For measuring the PAMP-triggered ROS burst, we will make use of a luminescence-based assay, which enables the detection of apoplastic ROS that can be monitored live with a charge-coupled device camera. This is a fast and easy quantitative assay that can be used, in many cases, to study early PTI signalling in a given mutant compared to a wild type control.

In addition, we will assay for the induction of a MAPK cascade, specifically for the PAMP-induced phosphorylation of the four *Arabidopsis* MAPKs MPK3, MPK4, MPK6 and MPK11. Their activation can be detected within a few minutes after PAMP treatment by western blot analysis using a well-established commercial antibody that was raised to detect phosphorylated MAPKs in mammalian systems. Prolonged exposure to PAMPs results in a growth arrest of seedlings, which can be used as a quantitative measure for the capability of a given genotype to respond to different stimuli. We will look at different *Arabidopsis* lines exposed to PAMPs for several days and assess the resulting growth differences compared to mock grown seedlings.

Finally, PAMP-triggered immunity contributes to the basal resistance of plants against adapted pathogens. In the frame of the practical session we will assess the differences in susceptibility of known PTI pathway mutants to demonstrate the importance of PTI for plant resistance.

Cellular Defence

Led by Silke Robatzek

Many microbes that colonize plants invade extracellular spaces. A physical barrier is thus present between the two organisms. Thus, the signal exchange between plants and microbes must occur across their cell surfaces. Pattern recognition receptors (PRRs) are primary sensors of the plant's immune system, and presented at the plasma membrane to recognize pathogens and trigger immunity. Recent research has revealed the machinery that is essential for routing of PRRs and that endocytosis of PRRs is a mechanism for sustaining cellular responsiveness to confer long-term anti-bacterial immunity.

The FLAGELLIN SENSING 2 (FLS2) receptor for bacterial flagellin (flg22) is a prime example to investigate PRR trafficking. Plasma membrane-localized FLS2 is internalized in a clathrin-dependent manner and targeted towards distinct subcellular fates in dependence of its activation status. At the *trans*-Golgi network (TGN), non-activated FLS2 is recycled back to the plasma membrane. Activated (flg22-bound) FLS2 is ubiquitinated and sorted into multi-vesicular bodies (MVBs) of the late endosomal pathway destined for vacuolar degradation, which is regulated by the endosomal sorting complexes required for transport (ESCRT) machinery (See Figure 6a). In light of this, pathogen virulence effectors target TGN-localized trafficking regulators, and deregulation of the trafficking machinery i.e. mutants in clathrin or ESCRT components, enhances infection.

Following recognition of extracellular pathogens by PRRs, defence components must be delivered across the cell surface to inhibit pathogen growth. This involves significant reprogramming in secretory and endocytic trafficking, enabling focal accumulation at infection sites. Small Rab GTPases and syntaxins act at multiple steps of membrane trafficking, including cargo selection, vesicle formation, vesicle movement, tethering and membrane fusion. Distinct members of these families are associated with specific membrane compartments. Therefore, these trafficking regulators, i.e. the Rab5 GTPases ARA6 and ARA7 or the secretory ARA5 found at the MVB, TGN and Golgi, respectively, represent molecular probes not only to visualize focal accumulation and PRR trafficking along the endocytic route, but also to identify defence-related cargoes that are transported to pathogen infection sites.

Keynote Lecture

Paul Birch - The Delivery and Activity of late blight effector proteins that suppress plant immunity

James Hutton Institute, Dundee, UK

The oomycete *Phytophthora infestans* causes late blight, the most significant disease of potato and tomato. As such, it is a threat to food security. It has been shown to secrete so-called effector proteins to suppress the host immune system. Some of these effectors, notably the RXLR class, are delivered (or translocated) inside plant cells where they interact with host proteins, manipulating their function to promote disease. We have found that some of these RXLR effectors target and suppress the activity of plant proteins that are positive regulators of immunity. In contrast, other RXLRs target negative regulators of immunity, presumably supporting or promoting their activity to create a susceptible environment. This presentation will review both classes of RXLR effector activity, and where this occurs within the plant cell. In addition, we will look at how these effector proteins are translocated into the host cell from finger-like structures called haustoria that are formed by the pathogen during the early stages of infection.

About Paul Birch

After a PhD and post-doc in University of Manchester on the molecular biology of lignocellulose degradation by fungi, Paul Birch took up a Principal Investigator position at the Scottish Crop Research Institute (now James Hutton Institute) in Dundee in 1995. Since then he has studied the potato late blight pathogen, *Phytophthora infestans*, with a particular interest in the molecular and cellular processes governing the development of disease or disease resistance. His group has been instrumental in discovering effector proteins from the pathogen that enter plant cells and manipulate host defences. In 2007, he became 'Professor of Plant Pathology' at the University of Dundee. In 2016 he was made Fellow of the Royal Society of Edinburgh. His main interests are in understanding:

- How pathogen effectors are delivered inside plant cells;
- What host proteins and processes they target;
- How they are recognised by host resistance proteins; and
- How the complex co-evolution between host and pathogen dictates the outcomes of attempted infection.

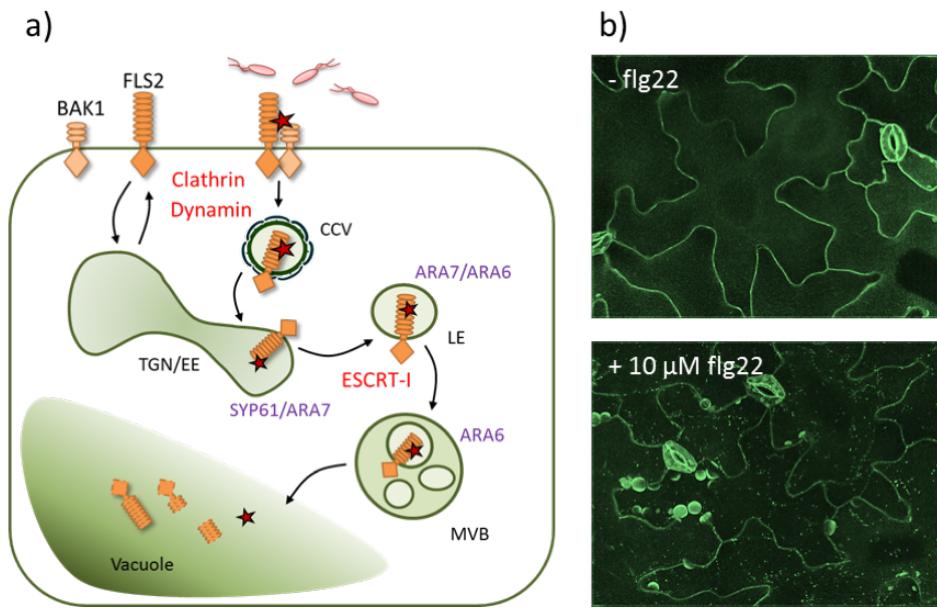


Figure 6: FLS2 endocytosis. **a)** The PRR FLS2 and its co-receptor BAK1 localise at the plasma membrane. Upon flg22 perception, a receptor complex is formed and the activated receptor is internalised into clathrin-coated vesicles (CCV) and sorted into intraluminal vesicles of multi-vesicular bodies (MVBs) via the trans-Golgi-network (TGN)/early endosomes (EE) and the late endosomes (LE). Components required for FLS2 internalisation and sorting are indicated in red, endosomal marker proteins are indicated in lilac. **b)** Confocal micrographs (spinning disc microscopy) of FLS2-GFP before and after elicitation with flg22.

Practical Session - Live Cell Imaging and Investigation of Subcellular Membrane Trafficking

Led by Gildas Bourdais⁷, Michaela Kopischke⁸, Agnieszka Siwoszek⁹, Jelle Postma¹⁰, Katarzyna Rybak¹¹, Janina Tamborski¹²

Aims and Objectives

1. Perform advanced fluorescence imaging using confocal laser scanning and spinning disc microscopy
2. Generate images suitable for qualitative and quantitative analyses
3. Provide a tool-box to understand and study the plant's endomembrane trafficking machinery

In the practical sessions we will investigate the (co-)localisation of FLS2-GFP and various endosomal

⁷gildas.bourdais@sainsbury-laboratory.ac.uk

⁸michaela.kopischke@sainsbury-laboratory.ac.uk

⁹agnieszka.siwoszek@sainsbury-laboratory.ac.uk

¹⁰jelle.postma@sainsbury-laboratory.ac.uk

¹¹katarzyna.rybak@sainsbury-laboratory.ac.uk

¹²janina.tamborski@sainsbury-laboratory.ac.uk

marker proteins following flg22 treatment using live-cell imaging approaches (See Figure 6 b). Chemical inhibitors and genetic interference with the endomembrane trafficking machinery will help us to dissect and understand the route of activated FLS2 in a temporal and spatial resolution.

Fuctional Plant Genomics

Led by Ksenia Krasileva

Adopting new wheat genomic tools to dissect plant innate immunity

Almost every week a new plant genome becomes available. Expansion of genomic data is mostly due to recent advances in sequencing technologies that have greatly leveraged the field between model and non-model organisms. Bread wheat (*Triticum aestivum*) is a plant of immense agronomic value with a highly complex 17 Gb allohexaploid genome. Recent development of new sequencing and genome assembly approaches greatly reduced the time and resources needed to assemble wheat genome and 6 genomes of different wheats are already available, allowing for in depth comparative analyses. Moreover, development of exome capture assay (targeted sequencing of gene space) allowed establishing reverse genetics resources for wheat as well as rapid mapping-by-sequencing identification of mutations from forward genetic screens.

Availability of genome of wheat and many other plant species allowed us to elucidate evolutionary history of plant immune receptors of Nucleotide-Binding Leucine Rich Repeat class (NLRs). A recent paradigm in NLR-based recognition of pathogen derived effectors involves NLRs with exogenous integrated domains (NLR-IDs) that can serve as baits for effectors by mimicking plant proteins that pathogens target. We have shown that NLR-IDs are prevalent across flowering plants and identified their ID repertoires. This information now allows us to a) search for new effectors b) predict host susceptibility genes normally targeted by pathogens (Figure 7).

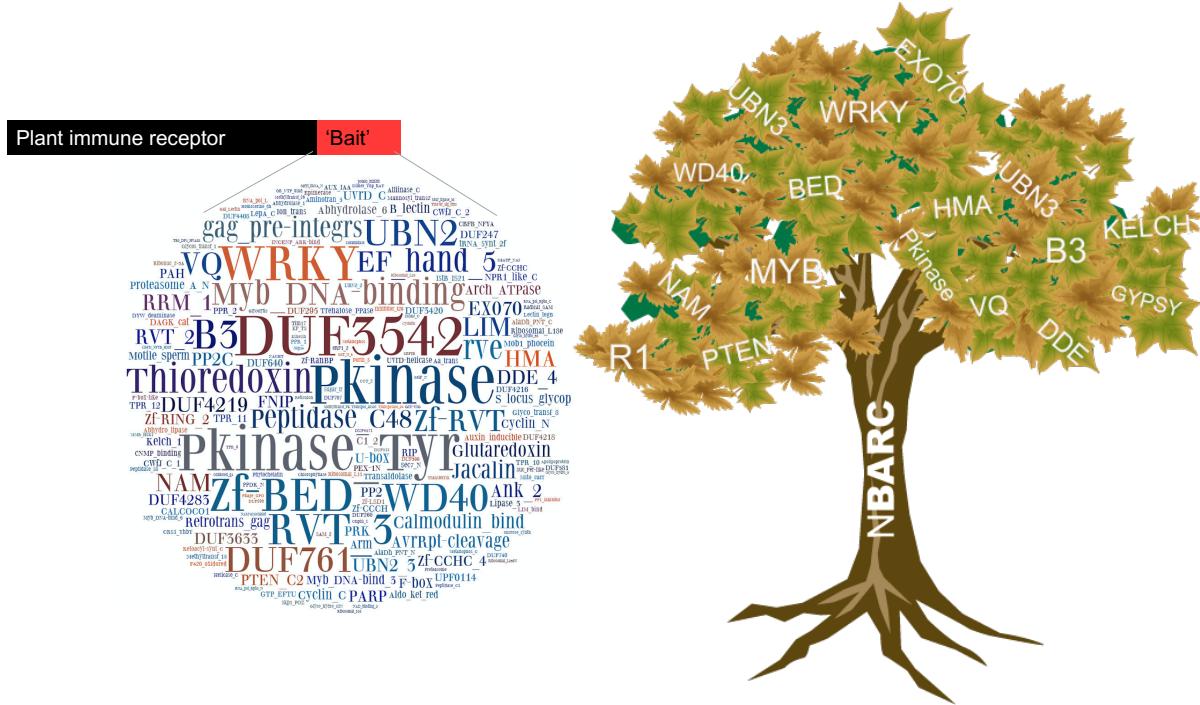


Figure 7: The NLR Integrated Domain Paradigm

Keynote Lecture

Daniel Croll - Retracing genome evolution of pathogens during rapid disease emergence in agricultural ecosystems

University of Neuchâtel, Switzerland

Most plants face attacks by pathogens. In agriculture, outbreaks of fungal diseases are frequent and pose a significant threat to sustainable food production. What enables pathogens to overcome host defenses and cause damage is poorly understood. A key evolutionary step for pathogens is to evolve effectors that specifically target and disable the plant immune system. We use experimental and population genomics tools to identify the genes underlying pathogenicity. Our main model is the fungus *Zymoseptoria tritici*, which causes one of the most important diseases on wheat. Based on large collections of sequenced pathogen genomes, we performed genome-wide association studies (GWAS) to identify the genes linked to the breakdown of host resistance. These genes encoded small secreted proteins that were highly expressed during plant infection. Then, we assembled reference-quality genomes to analyze the chromosomal regions surrounding effector genes. We found that these regions were undergoing rapid chromosomal sequence evolution driven by repetitive elements. We found substantial gene deletion polymorphism segregating in pathogen populations and, hence, functional differences among pathogen strains. Genes located in highly dynamic chromosomal regions provide pathogen populations with evolutionary potential to rapidly adapt to environmental changes or new hosts.

About Daniel Croll

Daniel Croll joined the University of Neuchâtel, Switzerland, in 2017 where he leads the Laboratory of Evolutionary Genetics as an Assistant Professor. Daniel Croll received his MSc in Biology in 2003 and his PhD in Life Sciences in 2009 from the University of Lausanne, Switzerland. He then joined the ETH Zürich as a postdoctoral fellow. Later, he received an Advanced Postdoctoral Fellowship from the Swiss National Science Foundation to work 2013-2014 at the University of British Columbia in Vancouver, Canada. In 2015, Daniel Croll was appointed as an Oberassistant (group leader) and lecturer at the ETH Zürich. At the University of Neuchâtel, Daniel Croll continues to investigate the evolutionary dynamics of disease emergence in agricultural ecosystems. The main interests include the dissection of phenotypic traits using genome-wide association mapping, the mechanisms of rapid genome evolution and the signatures of recent adaptive evolution.

Practical Session - Functional Plant Genomics

Led by Erin Baggs and Elisha Thynne

Aims and Objectives

1. Become familiar with web-servers and command line tools for protein family analyses
2. Understand how to produce and critically assess a local sequence alignment and phylogeny
3. Be able to scan genomes for sequences with particular domains and understand their evolutionary origin

The aim of this practical session is to analyse given NLR-IDs as examples of proteins with complex evolutionary histories and be able to robustly identify the origins of integrated domains. Each student will be given an NLR-ID sequences previously identified from our genomic screens, and will analyse the origin of IDs through genomics and phylogenetics, predicting what parental gene it might have originated from. We will also guide you through how to run our r_gene NLR-ID scan pipeline (Sarris et al 2016) on a new genome which will allow you to identify all NLRs and all NLR-IDs from a given proteome. By the end of the practical we hope you should be able to investigate evolutionary history of a protein and gain skills in protein family analyses.

The practical will be centred around a biological question of identifying the origin of integrated domains and predicting parental genes from which they are derived. The parental genes may in some instances represent potential pathogen targets, in which case they could be considered host susceptibility genes. The practical will be organized in exercises in different levels to match your skillsets. Our exercises will take you through the steps of identifying conserved domains present in a protein of interest and show you how to identify all sequences in an organism of interest with that domain. Then we will show you how these sequences can form the basis for an alignment from which you can generate a phylogeny. At all steps we will highlight tools, such as BioMart, which will allow you to complete the task with little bioinformatic skill required. The session will also offer the chance to use command line tools and scripts available on github which allow for greater flexibility and control over the data you generate. The practicals modular composition should allow you to apply many of the tools introduced to you to your own biological questions.

At the end of the session, we can demonstrate how you can obtain a knockout of your identified putative susceptibility gene in wheat by using www.wheat-tilling.com resevre genetics resource.

Application of Discovery and Targeted Proteomics in Plant Pathogen Interactions

Led by Frank Menke

The field of proteomics is rapidly advancing, driven in part by technological innovation and the design of sophisticated approaches to target a specific biological question. Proteomics is a broad term that covers the large-scale analysis of proteins and their modifications (the proteome) in a given cell, tissue or organism under a defined condition. While this could involve a diverse set of approaches including molecular biology, biochemistry and genetics, in practise it mostly refers to protein purification and analysis by mass spectrometry (Aebersold and Mann, 2016).

Current developments in the proteomics field are very exciting and discovery (or shotgun) proteomics data sets are becoming more comprehensive with more sensitive hybrid mass spectrometers. Furthermore, targeted proteomics approaches, such as Selective Reaction Monitoring (Picotti et al., 2013) have made it possible to reproducible and accurately test a biological hypothesis (impossible with shotgun proteomics at the moment) and bring hands-on proteomics to biologists.

This session will cover the basics of proteomics, including introduction to mass spectrometry and experimental design. We'll look at both discovery and targeted proteomic workflows and how these can be used in studying plant pathogen interactions. See Figure 8.

Keynote Lecture

Delphine Pflieger - Quantitative phosphoproteomic analysis reveals shared and specific targets of *Arabidopsis* MAPkinases MPK3, MPK4 and MPK6

Commissariat à l'énergie atomique et aux énergies alternatives (CEA), Grenoble, France

In *Arabidopsis*, mitogen-activated protein kinases MPK3, MPK4 and MPK6 constitute essential relays for a variety of functions including cell division, development and innate immunity. While some substrates of MPK3, MPK4 and MPK6 have been identified, the picture is still far from complete. To identify the substrates of these MAPKs in cell division, growth and development we compared the cytosolic and chromatin-linked phosphoproteomes of wild-type and mpk3, mpk4 and mpk6 mutant plants. To study the function of these MAPKs in innate immunity, we analyzed their phosphoproteomes following activation by a microbe-associated molecular pattern (MAMP). We identified 152 differentially phosphorylated

peptides in cytosolic fractions, in response to MAMP treatment and/or when compared between genotypes. 70 of these could be classified as putative MAPK targets, with phosphosites that are specific to one or shared by several MAPKs. Biochemical analysis of a number of putative MAPK substrates by phosphorylation and interaction assays confirmed our global phosphoproteome approach. Finally, we examined in detail the unknown function protein AYL1 (AT5G43830), confirming that it is a MAPK target and plays a role in defense responses. The results obtained on the fraction of chromatin-linked phosphoproteins confirm the picture obtained in the cytosol: in particular, the most dramatic effect on the phosphoproteome is observed in the mpk6 deletion mutant. In conclusion, partially overlapping substrate networks were retrieved for all three MAPKs, showing target specificity to one, two or all three MAPKs in different biological processes. Interestingly, we unveil the fact that within a given protein substrate, different phosphosites may be modified by one specific MAPK or by several of the three MAPKs. Our study also expands the set of novel MAPK substrates and functions to other involved protein kinases, including calcium-dependent (CDPK) and sugar non-fermenting (SnRK) protein kinases.

About Delphine Pflieger

Delphine Pflieger is a French researcher who followed the boom of proteomics since the early 2000s, by defending a PhD in 2004 on the use of the coupling between liquid chromatography and mass spectrometry (LC-MS) as a very robust and versatile approach for protein sample characterization. After completing her PhD of Analytical Chemistry in Paris (University Paris VI), she joined the group of Prof. Ruedi Aebersold, starting at the Institute for Systems Biology in Seattle and then participating in the establishment of a new laboratory in ETH Zürich. During this post-doctoral experience, she was in particular interested in developing a proteomics method to better study imperfectly affinity-purified protein complexes and their phosphorylations. In 2006, she got a researcher position at CNRS (Centre National de Recherche Scientifique) in Evry, in the Parisian suburb. In tight collaboration with the group of Heribert Hirt, she developed there phosphoproteomic analyses to decipher the signaling cascades triggered in *Arabidopsis thaliana* when the plant is subjected to a simulation of pathogen attack. The team analyzed the cytosolic part of the cascades but also gave a special focus on the events occurring in the vicinity of chromatin, to connect the very end of the signaling pathways with the rewiring of the transcriptional program (Bigeard et al., 2014b,a; Rayapuram et al., 2014). This collaborative work is still on-going. Since her move to Grenoble in September 2014, Delphine Pflieger however developed new interests in the more specific analysis of histones, key constituents of chromatin, both in terms of variants and of their multiplicity of post-translational modifications.

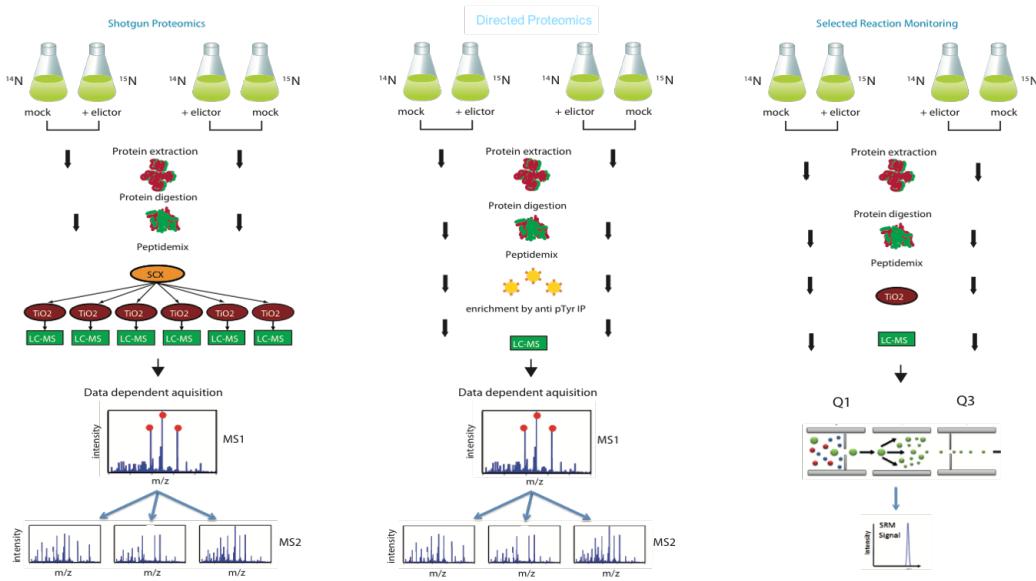


Figure 8: Discovery and targets proteomics workflows

Practical Session - Analysing and interpreting mass spec data

Led by Frank Menke

Aims and Objectives

1. Understanding how mass spec data analysed
2. learning how to interpret targeted proteomics data

Extracting raw data acquired by mass spectrometry to obtain an interpretable protein list is achieved by step-wise processing of the data files through a variety of purposely built open source and commercial software. The first tutorial will cover discovery proteomics and introduces basic concepts and frequently used data pipelines. Using actual data collected on TSL mass spectrometers, we will introduce the most important parameters and variables in data processing, and describe individual steps of the processing pipeline. We will create textual input for the search engine, and commence the search to interpret tandem mass spectra. This will result in a list of identified peptides, their sequences, and list of proteins. We will then interpret the results using several software that include web based search engine output, Scaffold, and data export to tabular format for further processing and visualization (Figure 9).

The second tutorial will cover targeted proteomics and analysis of Selected Reaction Monitoring (SRM) data in Skyline. In this elementary introduction to Skyline, students will build a spectral library, create a background proteome, import SRM data files and adjust settings to allow data refinement, analysis and interpretation.

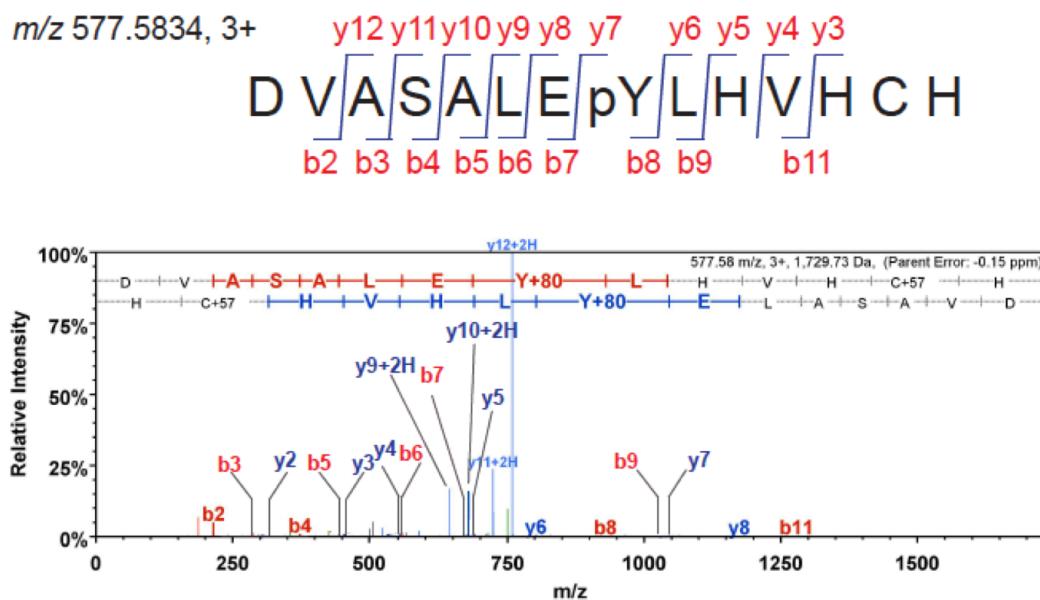


Figure 9: Interpretation of a MS2 spectrum

Translations and Tipping the Balance

Led by Matt Moscou and Peter Van Esse

Exploiting Knowledge of Plant Pathogen Interactions for Durable Disease Resistance

Lorem ipsum dolor sit amet, consectetur adipiscing elit. In iaculis sagittis metus quis malesuada. Vestibulum laoreet vel tortor at tempor. Mauris blandit volutpat risus, et placerat odio varius eget. Cras vitae diam sollicitudin justo vehicula ultrices. Curabitur consequat ornare odio ac hendrerit. Mauris bibendum diam nec gravida molestie. Nullam interdum, nulla eget sollicitudin elementum, ante lorem auctor odio, sit amet varius diam magna et lectus. Sed vehicula velit velit, convallis mollis erat laoreet nec. Cras semper blandit felis vel iaculis. Sed in mollis nulla. Nulla sed egestas odio, nec finibus lectus. In vel porttitor lacus, nec tincidunt neque. Suspendisse at magna non neque congue fermentum at ut augue. Vivamus suscipit finibus tortor, ut accumsan dui pretium nec. Integer luctus eros non convallis ornare.

Pellentesque dapibus arcu rutrum neque tempor, ac consequat nisi consectetur. Nunc eu tortor sed lorem vehicula finibus. Sed sed lacus at erat fringilla auctor. Aenean malesuada mi est, sit amet molestie ante malesuada in. Aenean elit risus, imperdiet quis pretium ac, imperdiet a turpis. Praesent cursus, orci a viverra tincidunt, augue tortor volutpat erat, a elementum nisi ante vel nisl. Vestibulum purus nisl, imperdiet egestas ultricies nec, tempor vitae ligula. Integer dapibus magna vitae orci pharetra rhoncus. Vestibulum in mauris feugiat, pulvinar massa ac, convallis lacus. In hac habitasse platea dictumst.

Aenean sed leo sit amet dolor congue semper. Curabitur non malesuada erat. Phasellus scelerisque rutrum tristique. Duis posuere quam tellus, eu posuere lorem convallis facilisis. Duis euismod tincidunt neque, eu pellentesque nisi mollis sed. Suspendisse eu nunc rutrum massa iaculis rhoncus rutrum nec velit. Nulla et erat quis urna malesuada auctor. Vestibulum sit amet diam maximus, luctus nulla mollis, vestibulum nisi.

Keynote Lecture

Beat Keller - Molecular analysis of wheat – fungal pathosystems and applications in resistance breeding

Department of Plant and Microbial Biology, University of Zürich, Switzerland

Several hundred resistance genes against fungal diseases have been genetically described in the gene pool of wheat and a few of them have been cloned. The molecular analysis of their origin, diversity and function has resulted in insight on their evolution and also suggested better ways for their use in classical as well



Figure 10: This image should really be one that nicely summarises your topic. Not Roger.

as molecular breeding. For example, the discovery of molecular suppressor activities of certain powdery mildew resistance genes has resulted in a better understanding of earlier observations in wheat breeding and allows to predict breeding outcomes. The current focus of our work is on two different aspects of disease resistance in wheat: first we study the molecular basis of durable disease resistance and we focus here on the *Lr34* gene which was originally described as a QTL for durable, quantitative disease resistance against the fungal pathogen leaf rust. Second, we are analyzing at the molecular level the interaction of wheat and the fungal pathogen powdery mildew. This work also includes the identification of pathogen determinants that are involved in resistance. The new developments in wheat genomics including the availability of a high-quality reference genome sequence allow us to develop more efficient ways to isolate resistance genes. There is a rapidly increasing number of innovative new approaches to clone genes from the wheat genome which I will discuss briefly, and we can expect many more resistance genes being isolated in the near future. The consequences of these developments for a better use of genetic diversity in wheat resistance breeding will be discussed. The molecular diversity that has been revealed by studies on agronomically important resistance genes can inspire a number of research directions to improve resistance breeding. For example, natural diversity provides important clues how to engineer immune receptors for broader recognition spectrum. Furthermore, modification of gene expression as well as combination of genes using transgenic approaches has revealed novel ways to improve disease resistance. Several of these approaches will be presented for the case of leaf rust and powdery mildew resistance in wheat.

About Beat Keller

Dr. Keller received his PhD from the University of Basel, Switzerland, in 1985, and then was postdoctoral fellow at the Salk Institute for Biological Studies in La Jolla, San Diego with a long-term fellowship of the European Molecular Biology Organization. After returning to Europe he started a research group in collaboration with the wheat breeding program in Switzerland and became a full professor for Plant Molecular Biology at the University of

Zurich in 1997. The group of Dr. Keller has focused on the molecular understanding of fungal disease resistance in the wheat, maize and barley crop plants. This has resulted in the molecular identification of a number of agronomically important resistance genes. More recently, the group has started a large project on the wheat powdery mildew pathogen to understand resistance at the molecular level. In addition, fungal pathogenomics is used to study the evolution of this highly host-specific pathogen. Dr. Keller was Vice-president of the Swiss Academy of Sciences from 2000-2006, has led several large research consortia in Switzerland and was an ERC Advanced Investigator grant holder from 2010 to 2015. He is a member of the Research Council of the Swiss National Science Foundation and currently Head of the Division of Biology at the University of Zurich.

Practical Session - Practical Session Title

Led by Matt Moscou

Aims and Objectives

1. Understand different methods for resistance loci
2. Introduce basic R packages for QTL analysis
3. Understand how to link genotype (genetic maps) with phenotype (continuous/discrete measurements) with packages.

Mendelian inheritance of resistance to plant pathogens was first established by Sir Roland Henry Biffen in 1907. By the end of the 20th century, the link of phenotype and genotype was established by map-based cloning of plant resistance genes to a range of plant pathogens. Although substantial advances have been made in cloning resistance genes from model species with small genomes, technical hurdles still exist for large complex genomes that may have little or no sequence information. This practical will consist of a general introduction of the different methodologies and approaches for identifying loci contributing to resistance in complex uncharacterized genomes and a tutorial on using R/qtl to link genotype (genetic maps) with phenotype (both quantitative and qualitative data).

General Information

Arriving into Norwich

Arriving by Air

Norwich International Airport

Norwich International Airport¹³ is served by KLM¹⁴ and the regional carriers Flybe¹⁵, BMI Regional¹⁶, and Eastern Airways¹⁷, with direct connections to Amsterdam, Manchester, Edinburgh, and Aberdeen. From the airport you can take a taxi or local bus¹⁸ (with transfer) to get to the Norwich Research Park. Note that if you fly out of Norwich airport you will need to pay a £10 Airport Development Fee¹⁹ before you can go to your gate. Norwich airport is, however, a very convenient way to reach Norwich from outside the UK.

London Airports

You can also arrive at any of the London airports and take a train or coach to Norwich. Stansted Airport is the closest and has direct rail connections to Norwich.

Arriving by Train

Another option, especially if you are based in the UK, or are arriving at another airport, it to take Britain's national train network to Norwich Train Station²⁰, located in downtown Norwich. From there you can take a local bus or a taxi to Norwich Research Park. National Rail²¹ and TheTrainLine²² are two comprehensive online resource for booking train travel within the UK.

¹³<http://www.norwichairport.co.uk/>

¹⁴<http://www.klm.com/>

¹⁵<http://www.flybe.com/>

¹⁶<http://www.bmiregional.com/>

¹⁷<http://www.easternairways.com/>

¹⁸http://www.travelineeastanglia.org.uk/ea/XSLT_TTB_REQUEST?language=en&command=direct&net=ea&line=21603&sup=%20&project=y08&outputFormat=o&itdLPxx_displayHeader=false&lineVer=1&itdLPxx_spTr=1

¹⁹<http://www.norwichairport.co.uk/content.asp?pid=92>

²⁰http://www.nationalrail.co.uk/stations_destinations/NRW.aspx

²¹<http://www.nationalrail.co.uk/>

²²<http://www.thetrainline.com/stations/norwich>

Arriving by Coach

You can also arrive in Norwich via coach. Norwich Bus Station²³ is downtown and is also served by local buses and taxis once you arrive.

Arriving by Car and Parking

See the Sainsbury Laboratory²⁴ and UEA²⁵ *getting here* pages.

Parking at the Conference Centre is “ample and free for all events²⁶”. If you require parking at the conference venue, please use the JIC Visitor’s car park (follow the signs we will put up). When you are at registration, please tell us your license plate number, make, and colour so we can register it with JIC security.

Those staying at UEA will be able to park in the Main car park on campus. Parking will be available in the Main car park on campus. UEA car park charges are listed on the UEA car parking for visitors²⁷ page

Getting from and into Central Norwich by Bus

First Group²⁸ is the major local bus provider in Norwich. This map shows the complete network²⁹, and these buses specifically serve the Norwich Research Park or the UEA campus:

- **11/12**³⁰: Can catch these routes by walking down Colney Lane, towards the Hospital, and at the first bus stop past the roundabout.
- **13/13A/13B/13C/X13**³¹: Can catch these routes by walking down Colney Lane, towards the Hospital, and at the first bus stop past the roundabout.
- **21/21A/22**³²: Can catch these right outside Norwich Research Park, on Colney Lane.
- **25**³³: Goes from the rail station through the UEA campus, all the way to the end of Chancellor’s Drive.
- **26/26A**³⁴: Goes from the rail station to University and nearby hospital. Can catch the 26 right out Norwich Research Park, on Colney Lane.
- These bus stops have been added to the Summer School Google Map³⁵.

²³http://www.norfolk.gov.uk/Travel_and_transport/TravelNorfolk/Buses/Bus_interchanges_and_stops/NCC155388

²⁴<http://www.tsl.ac.uk/contact/>

²⁵<https://www.uea.ac.uk/about/visiting-staying/getting-here>

²⁶<http://www.venue-norwich.info/FAQs.html>

²⁷<https://portal.uea.ac.uk/estates/travel-and-transport/by-car/parking-for-visitors>

²⁸http://www.firstgroup.com/ukbus/suffolk_norfolk/journey_planning/maps/

²⁹http://www.firstgroup.com/ukbus/suffolk_norfolk/assets/pdfs/journey_planning/maps/norwich_map.pdf

³⁰<https://www.firstgroup.com/norfolk-suffolk/plan-journey/timetables/?operator=22&service=11/12&page=1&redirect=no>

³¹<https://www.firstgroup.com/norfolk-suffolk/plan-journey/timetables/?operator=22&service=13/13A/13B/13C/X13&page=1&redirect=no>

³²<https://www.firstgroup.com/norfolk-suffolk/plan-journey/timetables/?operator=22&service=21&page=1&redirect=no>

³³<https://www.firstgroup.com/norfolk-suffolk/plan-journey/timetables/?operator=22&service=25&page=1&redirect=no>

³⁴http://www.firstgroup.com/ukbus/suffolk_norfolk/journey_planning/timetables/index.php?operator=22&service=26&page=1&redirect=no

³⁵<https://drive.google.com/open?id=1z7gP4EFxyaGBmp69A2woREZWNjo&usp=sharing>

Checking into Accommodation

Accommodation will be at the UEA, Britten House. Check in will be at the UEA Security Lodge at any time from 2pm on the day of your arrival.

Meals

Breakfast and Lunch

Breakfast in the Zest Restaurant is served each day from 8 am to 9 am. Lunches can be purchased from The Centrum building on site, a range of salads, sandwiches, soups and hot meals are available. Coffee, tea and other beverages will be available during breaks.

Evening Meals

Evening meals are self catered. The official conference dinner is on Friday 4th August.

Dining Out

Norwich is a compact, walkable city with abundant restaurants and cafes. Head to either Rampant Horse Street area, The Lanes, or the Tombland area.

UEA Restaurant facilities on campus provide everything from a simple coffee and sandwich to a full meal at eateries Blend, Zest, Vista, Café Direct, Cafe 57 and the Sainsbury Centre for Visual Arts Gallery Café, though these have restricted opening times, usually until 8pm only and especially outside of terms.

Dining In

The lodging buildings each have a shared kitchen and you will be able to prepare small meals there. There is a small supermarket on site in the main plaza, a short walk from the lodging. There are also three supermarkets just off campus and within walking distance. These are marked on the included map.

Getting Around

Lodging is at The University of East Anglia, in Britten House. Parking is available in the main campus car park. There is a custom Google Map with all venues and Norwich Airport and train and bus stations: Summer School Map³⁶

³⁶<https://drive.google.com/open?id=1z7gP4EFxyaGBmp69A2woREZWNjo&usp=sharing>

Site Registration

On the first morning of The Summer School, please go to the John Innes Centre Reception for 10 am. We can then sign you into the register on-site and show you to the training rooms.

Emergency Contacts

Internal Emergency First Aid

Dial **333**, ask operator for a First Aider or an ambulance. Or dial **9 999** for emergency services directly.

Hospital

The Norfolk and Norwich University Hospital is directly located on the Norwich Research Park: Colney Lane, Norwich, NR4 7UY. Tel: 01603 286286.

Transport

- Taxis – Goldstar Taxis 01603 700700 or ABC Taxis 01603 666333
- Bus - First Group is the major local bus provider in Norwich and these buses specifically serve the Norwich Research Park or the UEA campus:
- **11/12³⁷**: Can catch these routes by walking down Colney Lane, towards the Hospital, and at the first bus stop past the roundabout.
- **13/13A/13B/13C/X13³⁸**: Can catch these routes by walking down Colney Lane, towards the Hospital, and at the first bus stop past the roundabout.
- **21/21A/22³⁹**: Can catch these right outside Norwich Research Park, on Colney Lane.
- **25⁴⁰**: Goes from the rail station through the UEA campus, all the way to the end of Chancellor's Drive.
- **26/26A⁴¹**: Goes from the rail station to University and nearby hospital. Can catch the 26 right out Norwich Research Park, on Colney Lane.

These bus stops have been added to the Summer School Google Map⁴².

³⁷<https://www.firstgroup.com/norfolk-suffolk/plan-journey/timetables/?operator=22&service=11/12&page=1&redirect=no>

³⁸<https://www.firstgroup.com/norfolk-suffolk/plan-journey/timetables/?operator=22&service=13/13A/13B/13C/X13&page=1&redirect=no>

³⁹<https://www.firstgroup.com/norfolk-suffolk/plan-journey/timetables/?operator=22&service=21&page=1&redirect=no>

⁴⁰<https://www.firstgroup.com/norfolk-suffolk/plan-journey/timetables/?operator=22&service=25&page=1&redirect=no>

⁴¹http://www.firstgroup.com/ukbus/suffolk_norfolk/journey_planning/timetables/index.php?operator=22&service=26&page=1&redirect=no

⁴²<https://drive.google.com/open?id=1z7gP4EFxyaGBmp69A2woREZWNjo&usp=sharing>

Norwich and Norwich Research Park

At the heart of East Anglia Norwich is a vibrant inviting city. Steeped in historic charm; The Cathedral, The Castle, the most complete medieval street pattern in the UK, the largest collection of pre-reformation churches in Northern Europe and the oldest hotel in the UK are all situated in Norwich. In medieval times Norwich was the second largest city in England. Norwich has the largest open-air market in England plus its own mustard “Colmans mustard” and Norwich City Football Club, The Canaries. In 2012 Norwich became a UNESCO city of literature. Norwich has a fantastic surrounding countryside including the Broads and Norfolk coast.

The Norwich Research Park, with six independent partner institutions; UEA, The John Innes Centre, Earlham Institute, Quadram Institute, Norfolk and Norwich Hospital and The Sainsbury Laboratory. Norwich Research Park is ranked fourth in the UK for the number of internationally recognised scientists. UEA has the second highest graduate retention rate in the country with almost half of all graduates living and working locally.

Wifi Connections

Wifi is available throughout the site. If you have EDUROAM available, please use that. If you don't have EDUROAM, you can get guest wireless passes from The John Innes Centre Reception.

Social Media

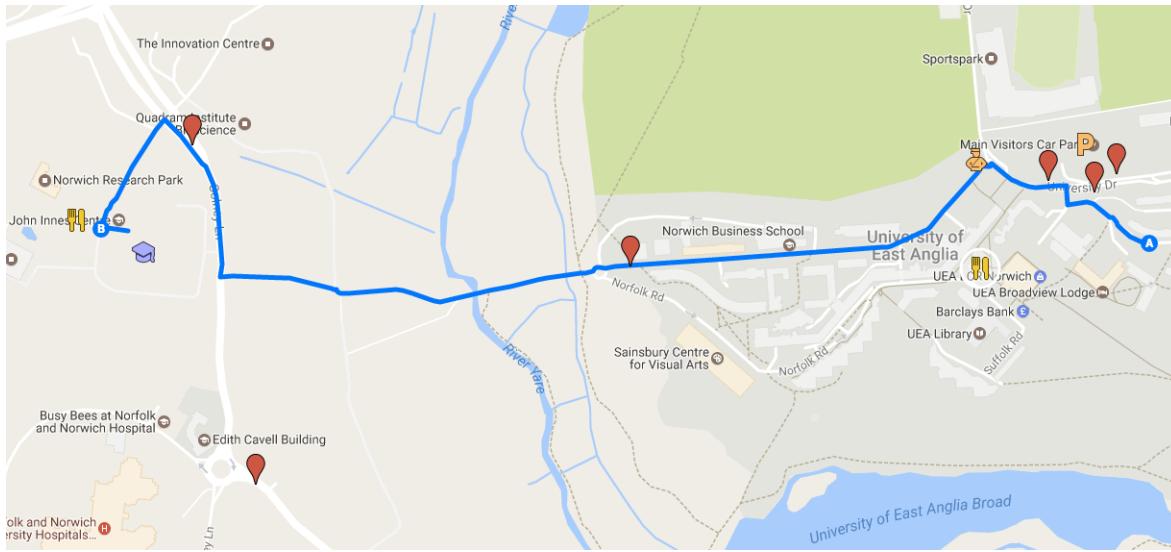
Tweeting and other social media activity are encouraged. #tslsummerschool, @TheSainsburyLab

Map

A live Google Map with useful sites marked is available here⁴³

A static version is presented below.

⁴³<https://drive.google.com/open?id=1z7gP4EFxyaGBmp69A2woREZWNjo&usp=sharing>



Excursion

Sunday is a day of relaxation and we have booked a trip to the north Norfolk coastal town of Cromer. Perched on the very edge of the north Norfolk coast, Cromer is famous for its tasty crabs, wide open beaches, a traditional pier complete with a theatre providing seaside special variety shows and is awash with small local independent shops. The town offers a wide choice of restaurants and cafes with not a single coffee shop chain or national eating or drinking venue to be found. Instead you have cafes, bars and restaurants owned and operated by local residents all eager to serve both local residents and visiting guests. The bus will depart at 1100 am from John Innes Reception.

Later in the day, we have booked a boat in the nearby village of Morston to take us for a 1 hour trip out on Blakeney Point to see the population of seals that live there. The boat will leave at 16:45 and the bus will drop off at Morston from Cromer.

Your hosts for this trip will be Karen Smith and Janina Tamborski, valuable members of TSL. They'll be around to co-ordinate logistics on the day.

Bibliography

- Aebersold, R. and Mann, M. (2016). Mass-spectrometric exploration of proteome structure and function. *Nature*, 537(7620):347–355.
- Anders, S. and Huber, W. (2010). Differential expression analysis for sequence count data. *Genome biology*, 11(10):R106.
- Andrews, S. (2017). *FastQC: A quality control tool for high throughput sequence data*.
- Bigeard, J., Rayapuram, N., Bonhomme, L., Hirt, H., and Pflieger, D. (2014a). Proteomic and phosphoproteomic analyses of chromatin-associated proteins from *Arabidopsis thaliana*. *Proteomics*, 14(19):2141–2155.
- Bigeard, J., Rayapuram, N., Pflieger, D., and Hirt, H. (2014b). Phosphorylation-dependent regulation of plant chromatin and chromatin-associated proteins. *Proteomics*, 14(19):2127–2140.
- Boch, J., Scholze, H., Schornack, S., Landgraf, A., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A., and Bonas, U. (2009). Breaking the code of DNA binding specificity of TAL-type III effectors. *Science (New York, N.Y.)*, 326(5959):1509–1512.
- Boller, T. and Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annual review of plant biology*, 60(1):379–406.
- Couto, D. and Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. *Nature reviews. Immunology*, 16(9):537–552.
- Etherington, G. J., Monaghan, J., Zipfel, C., and MacLean, D. (2014). Mapping mutations in plant genomes with the user-friendly web application CandiSNP. *Plant methods*, 10(1):41.
- Fields, S. and Song, O. (1989). A novel genetic system to detect protein-protein interactions. *Nature*, 340(6230):245–246.
- Goecks, J., Nekrutenko, A., Taylor, J., and Galaxy Team (2010). Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome biology*, 11(8):R86.
- Grau, J., Reschke, M., Erkes, A., Streubel, J., Morgan, R. D., Wilson, G. G., Koebnik, R., and Boch, J. (2016). AnnoTALE: bioinformatics tools for identification, annotation, and nomenclature of TALEs from *Xanthomonas* genomic sequences. *Scientific reports*, 6:21077.

- Hogenhout, S. A., Van der Hoorn, R. A. L., Terauchi, R., and Kamoun, S. (2009). Emerging concepts in effector biology of plant-associated organisms. *Molecular plant-microbe interactions : MPMI*, 22(2):115–122.
- Jones, J. D. G. and Dangl, J. L. (2006). The plant immune system. *Nature*, 444(7117):323–329.
- Kadota, Y., Sklenar, J., Derbyshire, P., Stransfeld, L., Asai, S., Ntoukakis, V., Jones, J. D., Shirasu, K., Menke, F., Jones, A., and Zipfel, C. (2014). Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. *Molecular cell*, 54(1):43–55.
- Kaipla, J., de Rycke, R., van Montagu, M., and Angenon, G. (1997). An Agrobacterium-mediated transient gene expression system for intact leaves. *Plant Science*, 122(1):101–108.
- Langmead, B. and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature methods*, 9(4):357–359.
- Li, H. and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)*, 25(14):1754–1760.
- Li, L., Li, M., Yu, L., Zhou, Z., Liang, X., Liu, Z., Cai, G., Gao, L., Zhang, X., Wang, Y., Chen, S., and Zhou, J.-M. (2014). The FLS2-associated kinase BIK1 directly phosphorylates the NADPH oxidase RbohD to control plant immunity. *Cell host & microbe*, 15(3):329–338.
- Lindbo, J. A., Silva-Rosales, L., Proebsting, W. M., and Dougherty, W. G. (1993). Induction of a Highly Specific Antiviral State in Transgenic Plants: Implications for Regulation of Gene Expression and Virus Resistance. *The Plant cell*, 5(12):1749–1759.
- Luo, R., Liu, B., Xie, Y., Li, Z., Huang, W., Yuan, J., He, G., Chen, Y., Pan, Q., Liu, Y., Tang, J., Wu, G., Zhang, H., Shi, Y., Liu, Y., Yu, C., Wang, B., Lu, Y., Han, C., Cheung, D. W., Yiu, S.-M., Peng, S., Xiaoqian, Z., Liu, G., Liao, X., Li, Y., Yang, H., Wang, J., Lam, T.-W., and Wang, J. (2012). SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *GigaScience*, 1(1):18.
- Ma, X., Xu, G., He, P., and Shan, L. (2016). SERKing Coreceptors for Receptors. *Trends in plant science*, 21(12):1017–1033.
- MacLean, D., Jones, J. D. G., and Studholme, D. J. (2009). Application of 'next-generation' sequencing technologies to microbial genetics. *Nature reviews. Microbiology*, 7(4):287–296.
- Page, D. R. and Grossniklaus, U. (2002). The art and design of genetic screens: *Arabidopsis thaliana*. *Nature reviews. Genetics*, 3(2):124–136.
- Picotti, P., Bodenmiller, B., and Aebersold, R. (2013). Proteomics meets the scientific method. *Nature methods*, 10(1):24–27.
- Pumplin, N. and Voinnet, O. (2013). RNA silencing suppression by plant pathogens: defence, counter-defence and counter-counter-defence. *Nature reviews. Microbiology*, 11(11):745–760.
- Ranf, S., Eschen-Lippold, L., Fröhlich, K., Westphal, L., Scheel, D., and Lee, J. (2014). Microbe-associated molecular pattern-induced calcium signaling requires the receptor-like cytoplasmic kinases, PBL1 and BIK1. *BMC plant biology*, 14(1):374.

- Rayapuram, N., Bonhomme, L., Bigeard, J., Haddadou, K., Przybylski, C., Hirt, H., and Pfleger, D. (2014). Identification of novel PAMP-triggered phosphorylation and dephosphorylation events in *Arabidopsis thaliana* by quantitative phosphoproteomic analysis. *Journal of proteome research*, 13(4):2137–2151.
- Richter, A., Streubel, J., Blücher, C., Szurek, B., Reschke, M., Grau, J., and Boch, J. (2014). A TAL effector repeat architecture for frameshift binding. *Nature communications*, 5:3447.
- Robinson, M. D., McCarthy, D. J., and Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics (Oxford, England)*, 26(1):139–140.
- Saunders, D. G. O., Win, J., Cano, L. M., Szabo, L. J., Kamoun, S., and Raffaele, S. (2012). Using hierarchical clustering of secreted protein families to classify and rank candidate effectors of rust fungi. *PloS one*, 7(1):e29847.
- Schwessinger, B., Roux, M., Kadota, Y., Ntoukakis, V., Sklenar, J., Jones, A., and Zipfel, C. (2011). Phosphorylation-dependent differential regulation of plant growth, cell death, and innate immunity by the regulatory receptor-like kinase BAK1. *PLoS genetics*, 7(4):e1002046.
- Seybold, H., Trempel, F., Ranf, S., Scheel, D., Romeis, T., and Lee, J. (2014). Ca²⁺ signalling in plant immune response: from pattern recognition receptors to Ca²⁺ decoding mechanisms. *The New phytologist*, 204(4):782–790.
- Trapnell, C., Pachter, L., and Salzberg, S. L. (2009). TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics (Oxford, England)*, 25(9):1105–1111.
- Win, J., Chaparro-Garcia, A., Belhaj, K., Saunders, D. G. O., Yoshida, K., Dong, S., Schornack, S., Zipfel, C., Robatzek, S., Hogenhout, S. A., and Kamoun, S. (2012). Effector biology of plant-associated organisms: concepts and perspectives. *Cold Spring Harbor symposia on quantitative biology*, 77:235–247.
- Win, J., Kamoun, S., and Jones, A. M. E. (2011). Purification of effector-target protein complexes via transient expression in *Nicotiana benthamiana*. *Methods in molecular biology (Clifton, N.J.)*, 712:181–194.
- Wu, C. H., Abd-El-Haliem, A., Bozkurt, T. O., Belhaj, K., Terauchi, R., Vossen, J. H., and Kamoun, S. (2016). NLR signaling network mediates immunity to diverse plant pathogens. <http://dx.doi.org/10.1101/090449>.