Project Ref	PI	Project Title	PI Contact
GFS01	John Armour	Understanding Variation and Evolution in Human Salivary Amylase genes	john.armour@nottingham.ac.uk
GFS02	Jonathan Aylott	A high-resolution cell biological approach to investigate root gravitropism using fluorescent nanosensors.	jon.aylott@nottingham.ac.uk
GFS03	Jonathan Ball	Emerging viruses in livestock	jonathan.ball@nottingham.ac.uk;
GFS04	Michael Birkett	Elucidating the Biosynthesis of the Aphid Sex Pheromone	mike.birkett@rothamsted.ac.uk
GFS05	Jan Bradley	The ecological and evolutionary consequences of immune variation in wild house mice*	jan.bradley@nottingham.ac.uk
GFS06	Toby Bruce	Host plant colonisation by the model pest aphid, *Myzus persicae*	toby.bruce@rothamsted.ac.uk;
GFS07	Alan Cockayne	Identification of factors affecting growth and neurotoxin production in the food pathogen Clostridium botulinum using forward genetics and Next Generation Sequencing"	alan.cockayne@nottingham.ac.uk
GFS08	Kin-Chow Chang	Exploiting calcineurin signalling as a potential pathway for anti-influenza targets identification	kin-chow.chang@nottingham.ac.uk
GFS09	Janet Daly	Development of a Venezuelan equine encephalitis recombinant vaccine	janet.daly@nottingham.ac.uk
GFS10	Cristina De Matteis	"Identifying inhibitors of the enzymes InhA and UGM from *Mycobacterium bovis,* the causative agent of bovine TB"	Cristina.De_matteis@nottingham.ac.uk
GFS11	Neil Foster	Investigation of the immunogenicity and efficacy of novel Salmonella vaccine candidates in pigs	n.foster@nottingham.ac.uk
GFS12	Zinnia González-Carranza	Investigating the role of abscission-related genes during plant development, and as a source to generate a degradation enzymatic cocktail	Zinnia.Gonzalez-carranza@nottingham.ac.uk
GFS37	Kevin Gough	The development of candidate therapeutic and diagnostic ligands for prion diseases	kevin.gough@nottingham.ac.uk
GFS38	Wayne Grant Carter	Detection and monitoring of environmental pesticides	wayne.carter@nottingham.ac.uk
GFS13	David Gray	Microalgae as Food Supplements and Functional Food Ingredients*	david.gray@nottingham.ac.uk
GFS14	Jonathan Hirst	Novel macrocyclic antimicrobials for food spoilage control and biofilm inhibition*	jonathan.hirst@nottingham.ac.uk

GFS15	Michael Holdsworth	Comparative evolution of the N-end rule pathway of targeted proteolysis*	michael.holdsworth@nottingham.ac.uk
GFS16	Ian Kerr	Understanding the actions of a plant hormone by analysis of auxin transporter proteins and modelling of cellular auxin flux.	ian.kerr@nottingham.ac.uk
GFS17	John King	Analysis of the mechanism regulating anther opening: a tool to control fertility for breeding hybrid crops with increased yield	john.king@nottingham.ac.uk
GFS18	Julie King	Genes controlling wheat grain bound phenolics	j.king@nottingham.ac.uk
GFS19	John King	Multi-scale modelling of a molecular mechanism controlling plant response to the environment*	john.king@nottingham.ac.uk
GFS20	Theodore Kypraios	Bayesian Inference for Dynamical Systems: From Parameter Estimation to Experimental Design.	theodore.kypraios@nottingham.ac.uk
GFS21	Andrew MacColl	The genetic and immunological basis of parasite resistance in fish: a model species approach.	andrew.maccoll@nottingham.ac.uk
GFS22	Ken Mellits	Intervention of Probiotic S.boulardii in stress related bowel dysfunction in the weaning pig.	ken.mellits@nottingham.ac.uk
GFS23	Ian Mellor	Characterizing the aphid voltage-gated sodium channel as an insecticide target	ian.mellor@nottingham.ac.uk
GFS24	Erik Murchie	Optimising photosynthetic productivity in novel vertical farming systems using LED technology and canopy imaging	erik.murchie@nottingham.ac.uk
GFS25	Kevin Pyke	Plastid Dynamics and Tracking During Gravitropic Responses in Roots	kevin.pyke@nottingham.ac.uk
GFS26	Andrew Salter	Impact of long chain omega-3 enriched GM plant oils on the development of atherosclerosis	andrew.salter@nottingham.ac.uk
GFS27	Sofie Sjögersten	The plant - soil - microbe continuum: does oxygen release by tree roots govern the biogeochemical functioning of tropical peatlands and their response to climate and land use change*	Sofie.Sjogersten@nottingham.ac.uk
GFS28	Liz Sockett	How does Bdellovibrio Gliding Motility Allow Predator-Spread Across Crop Surfaces, and How Can we Use this to Control Food Pathogens?*	liz.sockett@nottingham.ac.uk
GFS29	Reinhard Stöger	Environmental stresses: exploring molecular mechanisms conferring developmental robustness in agriculturally beneficial insects	reinhard.stoger@nottingham.ac.uk
GFS30	Ranjan Swarup	The role of long non protein coding RNA in lateral root development.	ranjan.swarup@nottingham.ac.uk
GFS31	Dylan Sweetman	Identifying the developmental basis of enhanced production characteristics in broiler chickens	dylan.sweetman@nottingham.ac.uk

GFS32	Frederica Theodoulou	Molecular characterisation of the N-end rule pathway- a protein degradation pathway of agricultural importance*	freddie.theodoulou@rothamsted.ac.uk
GFS33	Neil Thomas	Identifying inhibitors of the enzymes involved in arabinogalactan biosynthesis from Mycobacterium bovis, the causative agent of bovine TB.*	neil.thomas@nottingham.ac.uk
GFS34	Steve Thomas	Identification of alternative dwarfing genes for bread wheat improvement	steve.thomas@rothamsted.ac.uk
GFS35	Bill Wickstead	Characterisation of major antigen gene families in trypanosomes.	bill.wickstead@nottingham.ac.uk
GFS36	Zoe Wilson	Analysis of the mechanism regulating secondary thickening in the anther: a tool to control secondary thickening and fertility in crops	zoe.wilson@nottingham.ac.uk

Project title: Understanding Variation and Evolution in Human Salivary Amylase genes

Research theme: Molecules, Cells and Organisms

Location: Medical School

Rotation: 1,2 & 3

Contact: Prof. John Armour

Lab Rotation Project Description:

Measuring salivary amylase gene copy number in human DNA

The technical challenges of measuring gene copy number are most severe when copy number is high (above 4 copies) and when the gene of interest has highly similar paralogues. Both of these complications apply to the human *AMY1* gene, with stringent restrictions placed on measurement methods by the pancreatic amylase (*AMY2*) gene (>90% nucleotide identity), which is also variable in copy number, but independently of *AMY1*.

In this mini-project, students will use PCR-based methods (PRT/allele ratio determination) and capillary electrophoresis with fluorescence measurement to measure the copy number of *AMY1* in a series of human DNA samples for which gene copy number has already been measured, including samples for which read-depth from next-generation sequencing is used to calibrate gene dosage. Data will be analysed to investigate the accuracy of the different methods used, and to combine their outputs into a consensus gene copy number.

Gene copy number measurements from reference samples (including the HapMap samples) will be used to undertake further analyses, such as investigation of LD (linkage disequilibrium) with adjacent SNP (single nucleotide polymorphism) markers. Evidence of LD with local SNPs could be useful in deducing evolutionary histories of different copy number alleles, as well as in generating a simplified predicted test based on SNP genotypes, that could be useful in population surveys.

Students will receive training in advanced methods of DNA analysis, population genetic analysis of variation data, and statistical analysis (including implementation of a maximum-likelihood framework).

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	*Basic skills in Excel*	**Computer Skills**	*Use of GeneMapper analysis software*
	Basic handling of DNA sequences		*Use of genome databases*

			Collation and curation of large data sets
Numeracy Skills	*Basic descriptive statistics*	**Numeracy Skills**	*Analysis of ratio data from PRT*
	Simple laboratory arithmetic		*Introduction to likelihood methods*
General Lab. Skills	*Basic liquid-handling*	**General Lab. Skills**	*High-throughput processing of samples in 96-well format*
	Maintaining accurate laboratory records		*Capillary electrophoresis*
			Emulsion-fusion PCR
Communication Reading/Writing/ Presenting Skills	*Basic writing skills*	**Communication Reading/Writing/ Presenting Skills**	*Formal scientific writing*
	Investigation of the scientific literature		*Clear presentation of complex data*
	Communication with colleagues		

Linked PhD Project Outline:

Background: Although most human genes are present in exactly 2 copies per diploid cell – one inherited from each parent – many genes are now known to be variable in their copy number. In the case of the human salivary amylase (*AMY1*) genes, a very wide range of gene copy numbers (approximately 2 to 16) is compatible with apparent normality. There is some evidence that high copy numbers of *AMY1* are correlated with high-starch diets, and with levels of salivary amylase enzyme[1]. Nevertheless, this dramatic variation has not been firmly related to phenotype, nor have the processes leading to the evolution of this variation been properly defined. This is mainly due to the difficulty of accurate measurement of copy numbers (for example in distinguishing gene copy numbers of 9 and 10), and the poor structural definition of distinct amylase alleles in human populations.

We are applying our methods for accurate measurement of gene copy number[2-4] in a current BBSRC-funded project to investigate the relationship between copy number and gene expression, including work on the *AMY1* gene. This DTP project builds on results from that work, in which we are beginning to define the structures of variant gene clusters, in some cases spanning many hundreds of kilobases.

Project aims and plan: Understanding variation and evolution in salivary amylase gene copy number is limited both by the difficulty of accurate measurement, and by the lack of information on allele structure, leading to uncertainty about the relationships between variant alleles. This project will reconstruct the variation present in DNA samples from human populations by combining three approaches:

- Highly accurate measurement of gene copy number using PRT-based methods will define an accurate total diploid copy number for each DNA sample
- Emulsion-fusion PCR will be used to interrogate the spatial organisation of amylase variant structures
- Whole-genome methods, using either established (fosmid) methodology or methods currently under development, will include the amylase locus as a test-bed for evaluating the efficiency and accuracy of determining phased structural alleles.

Structures determined in this way will be used to understand the relationships between alleles, and their association with flanking SNP variation. The structural alleles and associated flanking variation will be tested for association with gene expression, and for signals of selection.

Training provided: Students will receive advanced training in DNA methodology, statistical aspects of quality control (see mini-project below), testing hypotheses about selection using analysis of variation, and the organisation of work involving human subjects.

Strategic fit: This work is relevant to both the Global Food Security and the Molecules, Cells and Organisms DTP themes. The work has strong relevance to the BBSRC strategic priority area "healthy and safe food" under the Global Food Security umbrella, and in particular with the "personalised nutrition" theme. There is also clear overlap with the "lifelong health and well-being" strand of the BBSRC strategic priority area Basic Biosciences Underpinning Health, and in particular the "diet, physical activity and health during ageing" theme.

[487 words]

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3. Abu Bakar, S., Hollox, E.J. and Armour, J.A.L. (2009). Allelic recombination between distinct genomic locations generates copy number diversity in human β-defensins. *Proc. Natl. Acad. Sci. USA* **106**, 853-858.

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Project title: A high-resolution cell biological approach to investigate root gravitropism using fluorescent nanosensors.

Research theme: Global Food Security

Location: School of Pharmacy, Boots Science Building

Rotation: 1**,** 2 & 3

Contact: Dr Jonathan Aylott

Lab Rotation Project Description:

The mini-project will follow the same topic as the main project; to use nanosensors to measure the biochemistry of the plant root.

The first two weeks of the mini project will be based in Pharmacy synthesising and characterising pH sensitive nanosensors. This is routine within the Aylott lab and will introduce the students to nanoparticle synthesis (and associated chemical handling and safety), fluorescence spectroscopy, and particle size characterisation techniques (dynamic light scattering, disc centrifuge and Nanosight).

Once nanosensors have been prepared and characterised the students will work in plant sciences and crop sciences at SB to deliver the nanosensors to the plant root using the gene gun.

The final two weeks of the project will be spent imaging the plant roots transfected with fluorescent nanosensors using confocal microscopy.

The training project will be multi-disciplinary and carried out within the labs of the applicants in two different Schools. The students will gain practical experience in several routine and specialised techniques in Biochemistry, Molecular Biology and Cell Biology as listed below

Aylott lab

Nanosensor / nanoparticle synthesis

Nanoparticle size characterisation (dynamic light scattering, centrifugal particle sizing, SEM, Nanosight)

Spectroscopy (fluorescence And UV-Vis)

Confocal microscopy

Swarup/Bennett/Wells lab

Gene gun transformation

In vitro plant culture

Root gravitropic assays

Automated image acquisition and imaging

Live fluorescent imaging

In situ immunolocalisation.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills		**Computer Skills**	Image analysis
			Image processing
Numeracy Skills		**Numeracy Skills**	Treatment of errors
			Statistical significance
General Lab. Skills		**General Lab. Skills**	Nanoparticle synthesis
			Fluorescent microscopy
			In vitro plant culture
Communication Reading/Writing/ Presenting Skills		**Communication Reading/Writing/ Presenting Skills**	As per all PhD studies

Linked PhD Project Outline:

With an ever increasing world population, *food security* and *sustainable agriculture* are two of the major challenges facing crop improvement programmes[1]. Root system architecture at both the macro-scale (root length, branching and growth angle) and micro-scale (root diameter and root hair production) determines a plant's capacity to capture nutrients, minerals and water (Fig. 1). Root angle, important in determining efficient capture of phosphates and micronutrients, is primarily regulated by the gravitropic response.

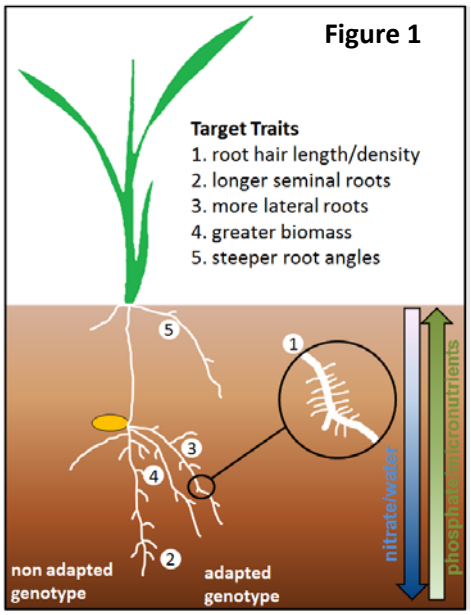

Figure 1

This project combines advances in optical nanosensors pioneered in the School of Pharmacy with expertise in plant physiology, genetics, and imaging in the School of Biosciences to develop and use new tools to deliver fundamental insights into the molecular regulation of root gravitropism with the aim of identifying key regulators to inform the design of strategies for future crop improvement programmes.

Root gravitropism involves generation of an auxin gradient at the root tip. Recent research has revealed this response to be detectable within 10 minutes of a gravity stimulus[2, 3], during which timeframe changes in the surface pH of elongation zone

cells have been detected[7]. Several studies have implicated changes in Ca^{2+} fluxes in root gravitropism[4-6]. For example, asymmetric Ca^{2+} gradients have been reported[5], and application of Ca^{2+}-chelators results in a loss of gravitropic sensitivity[4]. Reactive oxygen species (ROS) have also been implicated to play a key role in root gravitropism[7-8]. ROS have been shown to accumulate on the lower side of gravity-stimulated roots and appears to act downstream of auxin as application of hydrogen peroxide induces curvature even in roots treated with the auxin transport inhibitor NPA[8].

Despite the importance of pH, Ca^{2+} and ROS, their precise role in regulating root gravitropism is unknown, partly due to difficulties in their measurement. Fluorescent nanosensors[9,10], developed and applied to intracellular measurement in mammalian cells (Figure 2), are potential measurement tools that can be applied for measurement of the root biochemistry. We have previously demonstrated sensors for pH and Ca^{2+} and have delivered them to mammalian cells using gene gun transfection. In addition we have developed nanoscale devices capable of generating ROS by conjugating porphyrin molecules to polymeric nanoparticles[10]. In this project we will use fluorescent nanosensors to measure pH and Ca^{2+}, and ROS generating nanoparticles to produce localised ROS bursts, to allow the role of these factors in tropism to be investigated.

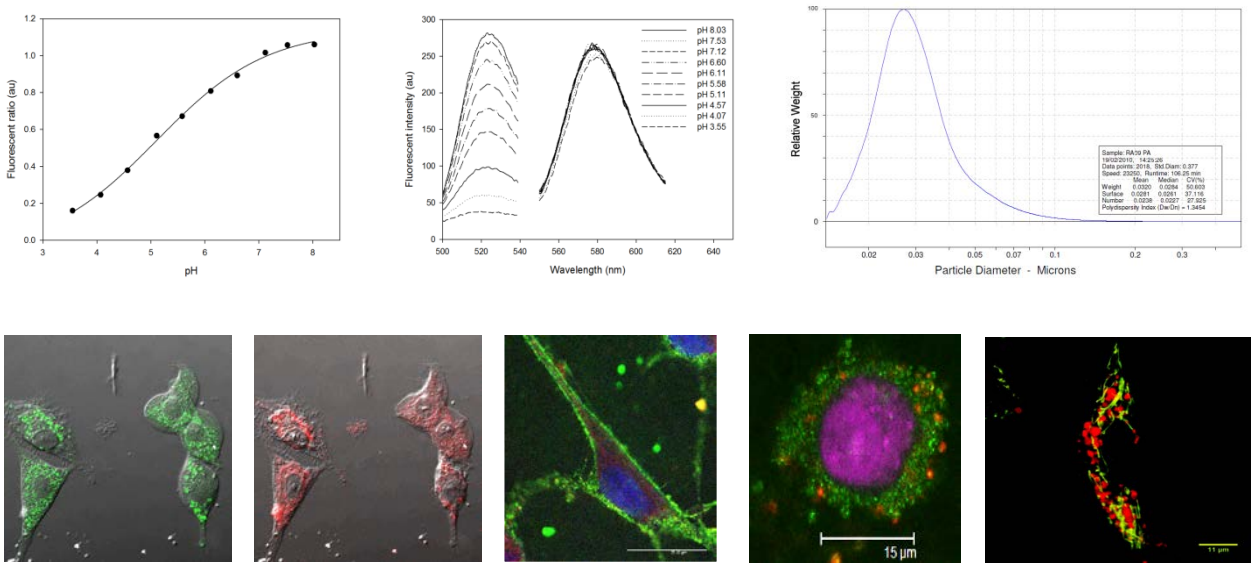

Figure 2: pH calibration of ratiometric fluorescent nanosensors containing entrapped Oregon Green, carboxyfluorescein (FAM) and tetramethyl rhodamine (TAMRA) (top left). Fluorescence spectra of pH nanosensors (top middle), disc centrifuge sizing data showing nanosensor size centred at 30 nm (top right). Confocal fluorescence images of nanosensors delivered to; C6 glioma cells, green channel (bottom, far left), red channel (bottom left); human mesenchymal stem cells (hMSC) showing nanosensors (red), cell membrane (green) and nuceus (blue) (bottom middle); 3T3 fibroblast showing nanosensors (green), endosomes (red) and nucleus (purple) (bottom right); hMSC showing ROS generating nanoparticles (red) and mitochondria (green) (bottom far right).

Work plan:

- Apply fluorescent nanosensor techniques to plant tissues
- Monitor pH and Ca^{2+} changes to pinpoint their role in root gravitropism
- Analyse root gravitropic phenotypes of mutants defective in either gravity perception, signal transmission and/or response[11, 12] in presence of varying concentration of ROS to place ROS in the genetic pathway.
- Investigate the relationship between ROS and auxin gradients using auxin response reporters DR5:VENUS and auxin sensor DII28:VENUS[13].

This research programme combines innovative chemical, cell biological and genetic approaches to investigate root gravitropism and will deliver fundamental new insights into the molecular regulation of root gravitropism for translation into crop breeding programmes.

Relevance:

This project is relevant to the following BBSRC Strategic Priorities: Technology Development for the Biosciences, Data Driven Biology, and the cross- council priority of Global Food Security.

References:

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Project title: Links between DNA replication and glycerol metabolism in Archaea

Research theme: Global food security

Location: School of Life Sciences

Contact: Prof JK Ball

Lab Rotation: Any

The mini project:

The mini-project will focus on development of a pseudotype and/or reverse genetics system for the study of Schmallenberg virus (SBV) entry, cell tropism and neutralising antibody response. A vaccine to protect vulnerable livestock has recently been licensed but its future effectiveness is unclear. Two recent papers suggest that the surface protein of SBV exhibits regions of hyper variability which might lead to antibody, including vaccine induced antibody, escape. We will develop PCR methods that will enable us to recover surface glycoprotein genes from field isolates and then to define their molecular epidemiology. The ability of convalescent immune or vaccine-induced sera will then be assessed for its ability to neutralise entry of pseudotypes or authentic chimeric viruses in a range of cell lines (animal / insect). This will define effects of genetic change on cell tropism and inform us if antibodies induced by vaccination or through natural infection will protect against future and emerging variants of SBV and therefore identify future vaccination strategies.

Techniques:

- Molecular biology - PCR, cloning, protein expression and analysis (e.g. Western blot, enzyme immunoassays)
- Sequencing and bioinformatics
- Cell and virus culture techniques

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Basic use of a PC	**Computer Skills**	Bioinformatics packages
Numeracy Skills	General lab calcualtions	**Numeracy Skills**	
General Lab. Skills	Handling pipettes, accurate measurement.	**General Lab. Skills**	Molecular techniques

			Protein work
			Cell culture
Communication Reading/Writing/ Presenting Skills	Good literacy. Use of scientific literature databases and able to use PowerPoint	**Communication Reading/Writing/ Presenting Skills**	Critical analysis of scientific papers and data presentation

Linked PhD Project Outline:

As the recent Bluetongue and Schmallenberg virus outbreaks demonstrate, emerging infections – especially those of viral aetiology – pose a significant threat to future animal welfare and agricultural productivity and, therefore future food security. Despite biosecurity measures, intensive agricultural practices, global food trade and land use pressures provide increased scope for farmed and wild animals to mix. Whilst it is impossible to predict with certainty where the next emerging virus will originated from, it is becoming increasingly clear that other mammal species, particularly those that are ubiquitous throughout the world, e.g. rodents, are an important potential source. Viruses cannot simply jump from one species to another. Instead, an initial jump occurs and then adaptation to the new host then onward transmission. This 'species barrier' exists at many stages of virus replication, but it is thought to be particularly important during host cell entry.

Through collaboration with veterinarians based at Twycross, we have access to a large number of wild-caught rodents and will screen these using metagenomics methods for the presence of viral sequences. Once we have determined the rodent virome we will then determine the ability of pseudotyped virus and, if appropriate chimeric viruses, for their ability to infect primary cells and cell lines representative of important agricultural species.

Where possible, we will also try to drive virus adaptation for entry into animal cell lines and then define the molecular determinants associated with adaptation. Definition of critical adaptive mutations can then be defined using a reverse genetics approach.

In essence this project will enable us to establish a platform for the screening and characterisation of novel viruses that have the potential for livestock infection and therefore, enable appropriate surveillance and development of preventative measures.

Project title: Elucidating the Biosynthesis of the Aphid Sex Pheromone

Research theme: Food security

Location: Rothamsted Research

Rotation: 1, 2 & 3

Contact: Dr Michael A. Birkett

Lab Rotation Project Description:

The aim of the mini-project at Rothamsted will be to provide training in the isolation, analysis and identification of aphid sex pheromones, which is essential to the success of the full PhD project. The following activities will be carried out over a six-week period:

1.Volatile collection of aphid sex pheromone. Purified air will pass through glass vessels containing bean plants, *Vicia faba*, heavily infested with sexual pea aphids, *Acyrthosiphon pisum*. Volatiles will be collected over 4 days onto a porous polymer (Porapak Q,50 mg), and after collection will be eluted with redistilled diethyl ether (750 µl). Samples will be stored in a freezer (−22°C) sealed in glass ampoules.

2. GC Analysis of aphid sex pheromone samples. Collected volatile samples will be analysed by high resolution gas chromatography (GC) using an Agilent 6890 GC fitted with both polar (DB-wax) and non-polar (HP-1) GC columns, cool-on-column injectors, deactivated retention gaps (1 m×0.53 mm inner diameter), and flame ionization detectors (FIDs).

3. Pheromone identification. Coupled gas chromatography-mass spectrometry (GC-MS) analysis of volatile samples will be performed on a VG Autospec Ultima magnetic sector mass spectrometer coupled to an Agilent 6890 GC fitted with a non-polar HP-1 column. Identifications of aphid sex pheromone components will be made by comparison of MS data with mass spectral databases (NIST) and literature spectra, and confirmed by GC peak enhancement with authentic standards. A multiple-point external standard method will be used to quantify the amount of identified chemical components present in volatile samples.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	*Knowledge of basic software*	**Computer Skills**	*Use of advanced data analysis software*
	Spreadsheet manipulation		*Use of mass spectrometry database/library searches*
Numeracy Skills	*Basic stats*	**Numeracy Skills**	

General Lab. Skills	*Understanding of the need for precision and accuracy*	**General Lab. Skills**	*Volatile collection from plants and insects*
	Basic equipment handling eg pipettes		*Gas chromatography analysis of collected samples*
			Coupled GC-mass spectrometry analysis
			Culturing of insects and plants
Communication Reading/Writing/ Presenting Skills	*Report-writing*	**Communication Reading/Writing/ Presenting Skills**	*Scientific paper-writing*
	Ability to carry out literature searching		*Ability to write a literature review*
	Ability to communicate to colleagues		*Ability to communicate to outside audiences*
			Oral and poster presentations

Linked PhD Project Outline:

Aphids (Homoptera: Aphididae) damage agricultural crops on a global scale, both directly and by transmitting plant viruses. The sex pheromones of many aphid species comprise a mixture of the iridoids (4a*S*,7*S*,7a*R*)-nepetalactone I and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol II with species integrity being maintained by species-specific ratios. Recently, the genome sequence of the pea aphid, *Acyrthosiphon pisum*, was published. The availability of the *A. pisum* genome provides an outstanding opportunity to characterise, for the first time, genes involved in the biosynthesis of the iridoids I and II by aphids, and to define the functional genes for the first full biosynthetic pathway to an animal secondary metabolite. This could in turn potentially provide new synthetic biology tools for the elaboration of molecules which are otherwise difficult to acquire through mainstream organic synthesis. To elucidate gene function, however, it is necessary to elucidate unequivocally the biosynthesis of I and II. In earlier studies at Rothamsted, it was proposed that biosynthesis involves oxidation of (7*S*)-citronellol to 8-hydroxy-(7*S*)-citronellol, which is then further oxidised to 8-oxo-(7*S*)-citronellal, followed by an intramolecular cyclization to an iridodial. This could then be cyclised further to the lactol II. Evidence for this pathway has been provided in our own lab, where volatile collections of sexual aphids show the presence of (7*S*)-citronellol. Studies elsewhere on Chrysomelid beetles suggest, however, that geraniol, rather than (7*S*)-citronellol could be the starting point for iridoid biosynthesis in aphids.

Overall project objective: To elucidate the biosynthesis of aphid sex pheromone components (4a*S*,7*S*,7a*R*)-nepetalactone I and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol II

Specific objectives:

1) Putative stable isotope precursors for the aphid sex pheromone components, i.e. deuterium (^{2}H) or carbon (^{13}C) labelled (7*S*)-citronellol or geraniol, along with intermediates in the proposed biosynthetic pathway (8-hydroxycitronellol, 8-oxocitronellal, 8-hydroxygeraniol, 8-oxogeranial, citronellal, 8-hydroxycitronellal, geranial, 8-hydroxygeranial, 8-oxocitronellol, 8-oxogeraniol), will be synthesized *de novo* using commercially available starting materials and novel synthetic chemistry routes. Structures will be confirmed by nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS).

2) Populations of *Acyrthosiphon pisum*, at the sexual stage, will be grown on host plants, and will be incubated in the presence or absence of different putative biosynthetic precursors. These will comprise labelled materials synthesized as above, as well as unlabelled materials.

3) The headspace from sexual aphid populations grown on host plants, incubated with labelled and unlabelled materials, will be collected using dynamic headspace collection (air entrainment) and other sampling techniques (e.g. SPME) where appropriate.

4) The collected volatile samples will be eluted from porous polymer (Porapak Q) and analysed by high resolution gas chromatography (GC) using non-polar and polar GC columns. The incorporation of deuterium (^{2}H) or carbon (^{13}C) labelling in volatile samples will be confirmed using coupled GC-mass spectrometry (GC-MS).

Training: The student will be provided with training in modern synthetic organic chemistry techniques for the synthesis of labelled putative biosynthetic substrates, incubation studies with aphids and synthesized labelled substrates, isolation of labelled pheromone components, and analysis of chromatographic and spectroscopic data (NMR, MS, GC, HPLC, FTIR).

Project title: Host plant colonisation by the model pest aphid, *Myzus persicae*

Research theme: Global Food security

Location: Rothamsted Research

Rotation: 1, 2 & 3

Contact: Prof Toby Bruce

Lab Rotation Project Description:

Students will learn key techniques used to study insect-plant interactions in a mini-project designed to compare the colonisation and performance of *Myzus persicae* aphids on cultivated and wild potatoes. We have wild potato *Solanum stoloniferum* lines at Rothamsted which are resistant to *M. persicae* and cultivated *Solanum tuberosum* which is susceptible. Aphid colonisation, growth rate and fecundity will be assessed on these (weeks 1-2). Having demonstrated differences in suitability of the two *Solanum* species as hosts, the students will be shown how to analyse volatile chemicals produced by the plants and how to extract RNA from the aphids. Plant volatiles will be collected by headspace sampling, analysed by GC-MS and insect responses observed in an olfactometer bioassay to test for attraction or repulsion (weeks 3-4). Samples of aphid RNA samples will be collected and analysed by QPCR to look at the expression of candidate genes by QPCR (weeks 5-6). At the end of the mini-project the students will have grounding in the specialised techniques used to investigate insect-plant interactions.

Lab Rotation Skills Matrix:

Skills you need	Skills you'll develop
Computer Skills	**Computer Skills**
Basic computer skills	• Excel and Genstat to analyse data • Literature searches • PowerPoint
Numeracy Skills	**Numeracy Skills**
To be numerate and have some idea of statistics	• Accurate recording of experimental data • Statistics for analysis of experimental data
General Lab. Skills	**General Lab. Skills**
To have had some experience in a laboratory	• Experimental design • Plant volatile collection • Aphid growth rate and fecundity bioassay • Olfactometer bioassay • Gas chromatography and GC-MS • Extraction of RNA • Introduction to quantitative PCR

Communication Reading/Writing/ Presenting Skills	Communication Reading/Writing/ Presenting Skills
Reasonable competence to allow progress	• Describing experiments • Effective use of science papers • Report writing (will have to prepare a report) • Presentation of research findings (will have to give a talk)

Linked PhD Project Outline:

Preventing crop losses to pests by host plant resistance plays an important role in food security and sustainable agriculture. The project will elucidate the molecular mechanisms influencing how a model pest species colonises host plants. It will focus on the interaction of peach-potato aphid, *Myzus persicae*, with potato and will investigate a wild potato species (*Solanum stoloniferum*) that is aphid resistant and the cultivated potato (*Solanum tuberosum*) which is susceptible. Cultivated potato went through a genetic bottleneck during domestication and much wider genetic diversity, including pest resistance traits, exists in wild potato species.

Objectives are:

1. Develop populations of potato lines by crossing *S. stoloniferum* x *S. tuberosum* and assess aphid performance on these and the parental lines. Carry out experiments to examine if different *M. persicae* clones (see below) are able to adapt over time to feeding on resistant or partially resistant potato lines.
2. Analyse secondary metabolites produced by the different *Solanum* lines and investigate changes induced by aphid infestation. Collect volatiles and conduct electrophysiological recordings and identify the compounds that are detected by *M. persicae.*
3. Test behavioural responses to these compounds to discover the key components and the blends of these compounds that elicit behavioural responses. Screen compounds with antibiotic effects.
4. Compare aphid global gene expression (including detoxification genes, and those encoding components of aphid saliva which could either elicit or supress plant defence) on different potato lines using microarrays.
5. Explore bioinformatics approaches to understand the interaction between plant secondary metabolite production and effects on aphid gene expression.

The project is timely because *M. persicae* is a notorious agricultural pest species of worldwide importance that directly damages crops and acts as a disease vector. It has evolved resistance to multiple insecticides (Puinean et al. 2010). Its genome is due to be fully sequenced soon (before or during the project) and is very similar to the already sequenced pea aphid. In addition at Rothamsted we have a unique collection of *M. persicae* clones from around the world, many of which

overexpress enzymes that detoxify insecticides and may also be able to break down plant defense chemicals (for example the P450 CYP6CY3). Because of these resources the *M. persicae* potato interaction could be an important model system for the study of insect-plant interactions. Furthermore, genomic resources are available for potato and therefore this system is strategically important for developing future research. The project will exploit the opportunities provided by aphid genomics and will investigate changes in secondary metabolism (and the molecular mechanisms which underlie this) after exposure to the aphid pest in compatible and incompatible interactions.

The project has a very good fit to the BBSRC priority and DTP project area of Food Security because *M. persicae* is a key crop pest and new sustainable control options are needed to safeguard against the losses it causes. A deeper understanding of the aphid-host interaction is expected to generate new control options. The project will identify molecules playing important roles at the whole organism level that determine the outcome of insect-plant interactions and therefore fits with the “Molecules, Cells and Organisms” DTP area. Both plant and aphid genomics will be used in the project thus addressing the BBSRC “Exploiting new ways of working” priority

Project title: Identification of factors affecting growth and neurotoxin production in the food pathogen *Clostridium botulinum* using forward genetics and Next Generation Sequencing

Research theme: Industrial Biotechnology/Bioenergy

Location: Centre for Biomolecular Sciences

Rotation: 1**,** 2 & 3

Contact: Dr Alan Cockayne

Lab Rotation Project Description:

The mini project will serve as an introduction to the available gene tools to be deployed in *Clostridium botulinum*. The student will:-

- receive a safety induction to cover safe laboratory practice within the confines of CRG laboratories and equipment within the CBS
- be trained in the use of anaerobic cabinets and the cultivation of *Clostridium botulinum*.
- be given a predesigned, retargeted ClosTron plasmid prepared by DNA2.0, and use it to generate a specific chromosomal mutant.
- become trained in the application of ACE technology, by integrative correction of a mutant *pyrE* allele in the chromosome
- master the procedures needed to create a transposon library, through the introduction of our newly developed conditional vector system and comparing cfu/ml on antibiotic supplemented plates in the presence or absence of IPTG.

Through all these procedures, the student will learn the basics techniques required to undertake the project. This will include, how to electro-transform and conjugate from *E.coli* into *Clostridium botulinum*, how to screen putative mutants for intron/ transposon integration by colony PCR and agarose gel electrophoresis, and how to derive nucleotide sequence data covering the intron/transposon insertion site. They will also be training in the use of the required DNA analysis software, including DNASTAR, GeNtle and CRGs Intron Design Tool at www.clostron.com.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Standard knowledge of computer software: office (word, excel, power-point) or similar	**Computer Skills**	Use of: DNA analysis tools, *in vitro* cloning, graph pad
	internet searches		Next Generation sequencing analysis software (CLC Bio WorkBench, DNASTAR NGen)

			Bioinformatics as required
Numeracy Skills	Basic concentration/dilution skills	**Numeracy Skills**	Basic stats as required
General Lab. Skills	Accurate record keeping	**General Lab. Skills**	Molecular skills: DNA handling, cloning, mutagenesis, PCR, nucleotide sequencing
	Basic equipment handling eg pipettes		Microbiology skills: working with aerobic (*E. coli*) and anaerobic (*Clostridium*) bacteria
			Phenotypic assays: sporulation, lichenase production
Communication Reading/Writing/ Presenting Skills	Literature search	**Communication Reading/Writing/ Presenting Skills**	Writing abstracts
	Communicate to colleagues		Writing literature reviews
			Scientific paper-writing
			Presenting work in seminars and at conferences, communicating to the lay person
			Protecting Intellectual Property

Linked PhD Project Outline:

Background: Botulism remains one of the most emotive of diseases due to its severity and relatively high fatality rate[1]. Even small outbreaks of food-borne botulism can precipitate a national emergency and inundate public health and acute care provision. The cost (per incident) of botulism is several orders of magnitude higher than any other form of foodborne illness and the damage to a food company's reputation can result in dramatic losses in sales, factory closures and liquidation. Accordingly, cases of botulism command a high public/media profile and, as a consequence, the organism has been the principal target in food processing for almost a century. To prevent food-borne botulism, it is imperative both to understand those environmental factors that affect the ability of the organism to grow and/or elaborate toxin when present in food. The requisite studies are impeded by the highly toxic nature of the organism. Using our patent-filed gene technologies[2], we have created strains in which the genes encoding the neurotoxin complex have been precisely deleted from the *Clostridium botulinum* chromosome and replaced with a *licB* reporter gene encoding a lichenase. The expression of *licB* (and by inference the neurotoxin gene) may now be monitored both qualitatively (by measuring zones of hydrolysis around colonies on Congo red stained lichenan plates) and quantitatively through enzyme assay of cell lysates. This safe, reporter strain provides the ideal system for studies aimed at understanding the molecular mechanisms that regulate neurotoxin production.

Aim: It is the overall objective of this project to both identify the regulatory systems that control neurotoxin expression and to determine those genes that are essential for growth of the organism in food stuffs through a combination of forward and reverse genetics.

Strategy: We have adapted our *Clostridium difficile mariner* transposon system[3] such that it now functions in *C. botulinum.* It will be exploited in our *licB* reporter strain to identify and thereafter characterise (i) positive (diminished zones of hydrolysis) and negative (enhanced zones of hydrolysis) regulators of toxin production, and; (ii) genes and their products essential for growth using "Transposon Directed Insertion-site Sequencing" (TraDIS) and Next Generation Sequencing (NGS). Genes and their expression profiles will be further characterised using a combination of phenotypic and biochemical tests, ClosTron technology (www.clostron.com) and transcription profiling using RNA.seq.

Training: Nottingham's Clostridia Research Group (CRG) are renowned experts in the genetic modification of Clostridium, and with 30+ (http://www.clostron.com/people.php) postdoctoral/ graduate researchers represent the largest clostridial molecular biology group in the world. The project offers training in anaerobic microbiology, advanced genetics, Next Generation Sequencing, bioinformatics, microbial physiology and transcriptomics (RNA.seq). In-keeping with CRGs aggressive strategy for protecting Intellectual Property, the student will also become conversant with the commercial aspects of research.

1. Minton NP (1994) Molecular genetics and phylogenesis of clostridial neurotoxins. *Curr Top Microbiol Immunol* **195:** 143-194**.**
2. Heap JT, **Ehsaan M**, Cooksley CM, Ng Y-K, Cartman ST, Winzer K, Minton NP (2012). Integration of DNA into bacterial chromosomes from plasmids without a counter-selection marker. *Nucl Acids Res* **40(8):**e59

Cartman ST, Minton NP (2010) A *mariner*-based transposon system for *in vivo* random mutagenesis of *Clostridium difficile*. *Appl Environ Microbiol,* **76:** 1103-1109.

Project title: Exploiting calcineurin signalling as a potential pathway for anti-influenza targets identification

Research theme: Global Food Security

Location: Vet School

Rotation: 1**,** 2 & 3

Contact: Prof. Kin-Chow Chang

Lab Rotation Project Description:

Our mini-project plan is to first provide the 2 prospective students a comprehensive overview of our substantial research programme on host innate immunity to influenza virus infection. Our strategic approach is to compare host response to virulent influenza virus infection (such as highly pathogenic avian influenza H5N1 virus) between resistant (e.g. pig and duck) and susceptible (human and chicken) species to identify targets for the development of intervention therapy to reduce disease severity. In the first week, the 2 students will shadow by rotation our PhD students and post-doctoral assistant in their ongoing laboratory work which could range from primary cells isolation to *in vitro* infection experiments. Such an exposure will provide prospective students a good overview of the range of laboratory techniques that they can expect to perform in due course. In the same week, they will be set a bioinformatics task of making biological connections of a small list of differentially expressed genes previously identified in our challenge experiments. This exercise, which could last for the next few weeks or more, will provide them the opportunity to use pathway analysis tools (such Pathway Studio available in our group), and to examine the relevant scientific literature on the broad subject of host innate resistance. They could work together on the bioinformatics assignment and present their findings to the research group during a regular laboratory meeting.

Concurrent to their bioinformatics assignment, they will be given a relatively simple but interesting and relevant cell-based project which is to examine the effects of selected chemical compounds on the pro-inflammatory response of influenza virus infected mammalian (pig and human) respiratory epithelial cells. These cells have been previously validated as stably transfected with either a firefly or renilla luciferase reporter gene driven by specific pro-inflammatory cytokine and chemokine promoters. We believe that this mini-project plan will provide a representative and exciting taster of what could be expected of a PhD project in our group.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Common PC skills	**Computer Skills**	Bioinformatics skills wrt NGS analytical work

Numeracy Skills	GSCE / "AS" level Maths	**Numeracy Skills**	As above
General Lab. Skills	Some basic experience helpful	**General Lab. Skills**	Extensive cutting-edged molecular and cellular biological laboratory skills
Communication Reading/Writing/ Presenting Skills	Good proficiency in English (communication and writing)	**Communication Reading/Writing/ Presenting Skills**	Excellent training opportunities in public speaking, scientific writing, and poster and conference presentations

Linked PhD Project Outline:

Exploiting calcineurin signalling as a potential pathway for anti-influenza targets identification

Rationale:

Calcineurin is a Ca^{+2}- and calmodulin-dependent protein serine/threonine phosphatase that plays a major role in Ca^{+2} dependent eukaryotic signal transduction pathways (Rusnak & Mertz 2000). Previous research suggests that calcineurin plays an important role in activating host innate immune responses. For example, calcineurin strongly activates NF-κB in muscle C2C12 cells (Alzuherri & Chang 2003), is required for effective T-cell development in mice (Bueno et al. 2002), and regulates anti-fungal immunity in neutrophils (Greenblatt et al. 2010). Despite being the principal target for inhibition to prevent organ rejection in transplant patients, in cases of lymphocytic choriomeningitis virus (LCMV) infection, the inhibition of calcineurin with drugs like cyclosporine a or FK506, could paradoxically trigger a fatal cytokine storm characterised by high levels of tumour necrosis factor-α (TNFα) and interleukin-6 (IL6), through the activation of pathogenic T-lymphocytes (Araki et al. 2010). It appears that the specific effects of calcineurin in host immune regulation are cell type specific. For instance, calcineurin in macrophages, but not in other cell types, negatively regulates toll-like receptor (TLR) mediated antimicrobial and pro-inflammatory gene activation (Kang et al. 2007).

Studies in our laboratory showed that over-expression of a constitutively active form of calcineurin (CnA*) in skeletal muscle cells readily activates a host of genes, including IRF-7 and Mda5, that are

involved in innate immune signalling. Consistent with the up-regulation of innate immune genes, our infection studies with influenza A virus (low pathogenicity avian influenza H2N3 virus) in calcineurin over-expressing C2C12 murine muscle cells showed significantly reduced infective virus output compared with infected control cells. Skeletal muscle constitute up to 50% of body mass, making it a significant tissue type in the mediation of innate resistance to infection.

We recently proposed that early rapid apoptotic death of influenza infected host cells from resistant ducks is a host defence mechanism against highly pathogenic avian influenza (HPAI) viruses contributing to host survival (Kuchipudi et al. 2012). Calcineurin is also known to play an important role in mitochondria mediated apoptosis through its interaction with the members of bcl-2 family (Kantrow et al. 2000). **We propose that activated calcineurin confers an anti-viral state through the activation of innate immune genes. Furthermore, it is tantalising to speculate that activated calcineurin also enhances apoptosis thereby promoting rapid cell death ahead of virus build-up in infected cells. The ability to enhance innate resistance to influenza infections in mammalian and avian farm animal species will greatly improve productivity and welfare to the benefit of both farmers and animals.**

Hypothesis:

Calcineurin promotes an anti-viral and a pro-apoptotic state in the host innate defence against influenza A viruses

Objectives

1. To demonstrate the anti-influenza activity of CnA* in muscle and non-muscle cells types.
2. To ascertain the significance of CnA* in mediating apoptosis in influenza infected host cells
3. To determine the contribution of CnA* to the inflammatory cytokine response during influenza infection

This project will harness the use of an extensive array of molecular tools (such as deep sequencing, and gene over-expression and knock-down studies) to identify host targets that could interfere with influenza virus replication.

References

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Project title: Development of a Venezuelan equine encephalitis recombinant vaccine

Research theme: Global Food Security

Location: Pirbright Institute

Rotation: 2 & 3

Contact: Dr Janet Daly

Lab Rotation Project Description:

Schmallenberg virus (SBV), which recently emerged in Europe, is an orthobunyavirus. Few studies on the pathogenic mechanisms of the virus have yet been reported. The ability to generate recombinant viruses *in vitro* from the constituent genes (reverse genetics) is an invaluable tool for studying viral pathogenesis as it enables individual genes to be manipulated in a common genetic background in order. This allows, for example, the effect of particular changes to a viral protein on the ability of the virus to infect different host cells. Reverse genetics technology has been successfully applied to viruses related to SBV. The two students involved in this mini project will each clone one or two of the three gene segments of SBV into a suitable plasmid system and, if cloning is successful, attempt to 'rescue' recombinant SBV by transfecting these plasmids into an appropriate cell line. Training will be provided in the culture of mammalian cell lines and molecular biology techniques including the design of primers, PCR, cloning and transfection.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Basic computing skils	**Computer Skills**	
Numeracy Skills		**Numeracy Skills**	Cell counts / seeding rates
General Lab. Skills		**General Lab. Skills**	Cell culture
			PCR
			Cloning
			Transfection
Communication Reading/Writing/ Presenting Skills		**Communication Reading/Writing/ Presenting Skills**	Students will be expected to participate in Infection and Immunity theme meetings and journal club

Linked PhD Project Outline:

Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne virus that normally infects small mammals. Horses and people can become infected incidentally if bitten by an infected mosquito. Usually these infections are asymptomatic, but so-called epizootic strains cause significant disease in both humans and equines throughout Southern and Central America and the southern United States. An epizootic of VEE in Colombia and Venezuela affected an estimated 75,000 humans and 50,000 equines in 1995 with 300 human fatalities and more than ten times that number of equines dying from the disease. In many countries, the horse is regarded primarily as a companion animal. However, horses are important to the economy of several of the countries where VEEV circulates: Brazil was reported to produce around 78,000 tonnes of horse meat per year in 2009, in some regions, equidae are used in rounding up livestock; the equine industry was estimated to be worth $112.1 billion to the US economy in 1995. Effective vaccines against VEEV are lacking. An inactivated virus vaccine based on a strain attenuated by passaging 83 times in guinea pig heart cells (TC-83) is licensed for use in horses in the US, but immunity is short-lived and the vaccine is highly labile.

The aim of this project is to generate a recombinant viral vector vaccine. It has already been shown that the structural proteins of the TrD vaccine strain of VEEV, when delivered in a recombinant poxvirus or adenovirus, elicits solid protection against a lethal virus challenge in mice. Candidate recombinant vaccines will be generated and tested for their ability to raise an immune response in horses. Antibody and cell-mediated immune responses will be measured using conventional and novel assays. Constructs expressing a marker protein will be developed to allow a 'differentiation of infected from vaccinated animals (DIVA)' strategy to be applied.

Training will be provided in molecular techniques required to generate recombinant vaccines, cell culture, serological methods and assays to measure cell-mediated immunity including ELIspot and FACS analysis of interferon-gamma-producing cells.

Project title: Identifying inhibitors of the enzymes InhA and UGM from *Mycobacterium bovis,* the causative agent of bovine TB.

Research theme: Global Food Security

Location: University of Nottingham/School of Chemistry/ CBS

Contact: Dr Cristina De Matteis

Lab Rotation 1 and 2 only

Mini Project plan:

Structure-based design of novel InhA inhibitors: Virtual screening of compound libraries

The availability of high quality 3D structures of InhA allows structure-based drug design approaches to be used in this project to identify new molecules that will inhibit this enzyme. In-silico screening of libraries of drug-like compounds will be carried out, to identify molecules that will bind effectively to InhA. In this project students will learn how to use 3D protein structures from the Brookhaven database, to carry out virtual screening of compound libraries. This will involve experience using the GOLD virtual screening software, and exposure to a variety of compound libraries. Inhibitor molecules identified in this way, will subsequently be tested experimentally – but it is unlikely that sufficient time would be available to carry out this experimental work also within the 6 week period.

Activity Assay on InhA and evaluation of a proposed inhibitor (Prof. Neil R. Thomas)

A student will be trained in how to express and purify wild-type InhA from M. tuberculosis that has been cloned into an E. coli expression vector. The student will then determine the activity of the enzyme using a spectrophotometric assay involving monitoring the consumption of NADH. The student will be provided with a compound identified as a potential inhibitor of InhA (this could be from the mini project above) and will determine its IC50 value. Finally if there is sufficient time, the student will be trained to synthesize the CoA substrate required for the enzyme activity assay.

Lab Rotation Skills Matrix:

Linked PhD Project Outline:

DEFRA has reported that tuberculosis (TB) is a serious animal health problem. Nearly 25,000 cattle were slaughtered in England in 2010/11, at a cost to the taxpayer of £91 million and a further £30 million loss to farmers. During 2010, 22.8% of herds in South West England were under travel restrictions. The number of cattle infected in GB is doubling every 5-10 years [1]. Tuberculosis in cattle is caused by *Mycobacterium bovis*, a bacterium that is closely related to *M. tuberculosis* that normally causes TB in humans. In the developing world, where milk is not pasteurized, *M. bovis* is the cause of ≈6% of all tuberculosis deaths in humans. Before pasteurization (1940's), *M. bovis* caused up to 2000 TB human deaths in England and Wales pa [2]. As incidences of TB in cattle increase, culling is not a sustainable option

and therefore new drugs that can be used in cattle will be required making this a **Global Food Security** issue, whilst the underpinning science fits into the **Molecules Cells and Organisms** theme.

M. bovis is innately resistant to pyrazinamide: therefore the standard treatment is isoniazid and rifampicin for 9 month. This is expensive and hence only used with high value/rare breed cattle. Resistance to isoniazid in *M. tuberculosis/bovis* is increasing through mutations in the enoyl reductase InhA, to which the NADH-isoniazid adduct binds. It is therefore important to design and evaluate new inhibitors as the starting point for anti-tuberculosis agents. If a fast acting, low cost anti-tuberculosis agent can be discovered, this could be used to treat cattle and remove the need to cull them. In the longer term, these could be introduced into the food of the main feral reservoirs of *M. bovis*; badgers in UK; white tailed deer in North America.

Research Plan: This project will build on our research to develop inhibitors of *Mycobacterial* cell wall biosynthesis enzymes that are essential to *Mycobacterium* survival (InhA and UGM).

1. Expression of InhA and UGM enzymes, including the expression of *M. bovis* InhA mutants known to be resistant to isoniazid.
2. *In silico* screening of compound libraries, including the Nottingham University Compound Collection, against InhA and UGM.
3. Small drug-like fragments, that can be used to form **dynamic combinatorial libraries** (DCLs) (equilibrating disulfide/imine bonds), will be probed with InhA and UGM acting as templates (Fig1) [3], to stabilise and select the molecules that they bind most tightly. Initially, previously identified inhibitors (*Mol. Inform.*, 2011, 30, 873 or Loyin Olotu-Umoren, PhD thesis) will be used to identify suitable fragments for the initial (DCL) experiments.
4. Evaluation against isolated InhA and its mutants or UGM, using colorimetric and HPLC-based assays, to identify suitable hit compounds for SAR studies.
5. Evaluation against *M. bovis* and *M. tuberculosis* cells: Dr S. Bhaktra (Birkbeck College).
6. X-ray crystallography of InhA and UGM in complex with their best inhibitors to guide further structural optimisation.

References

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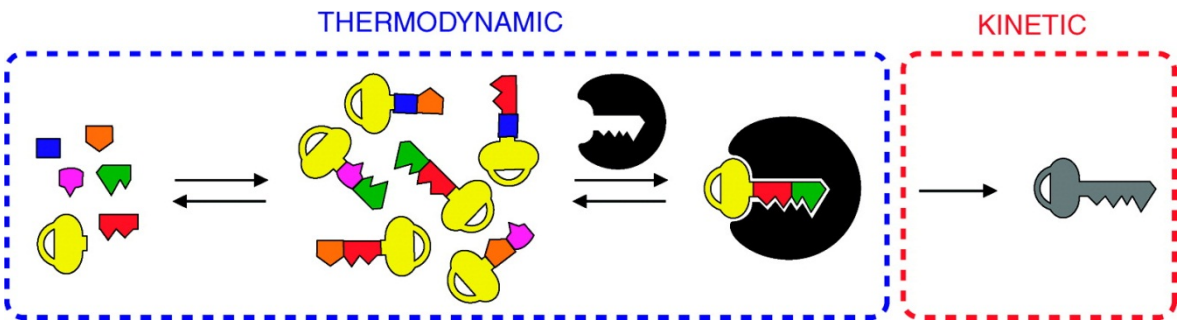
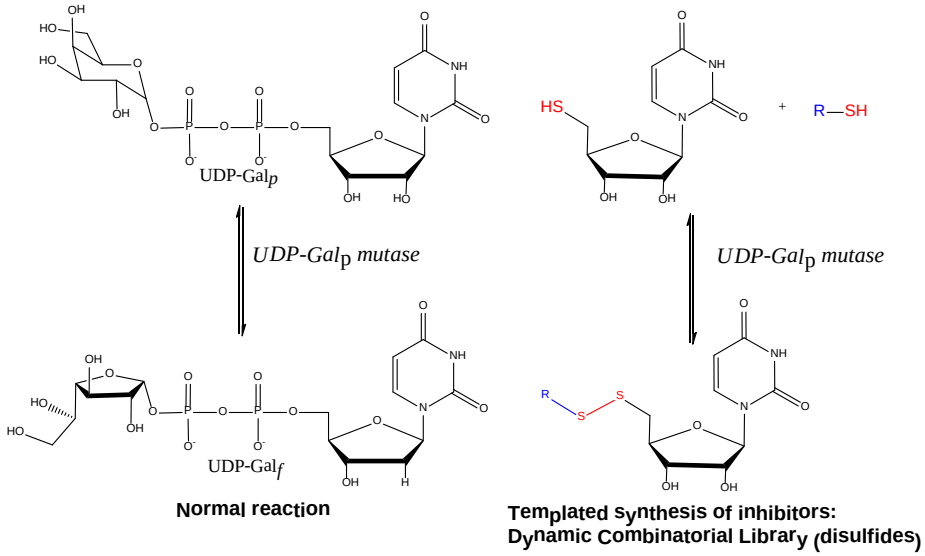
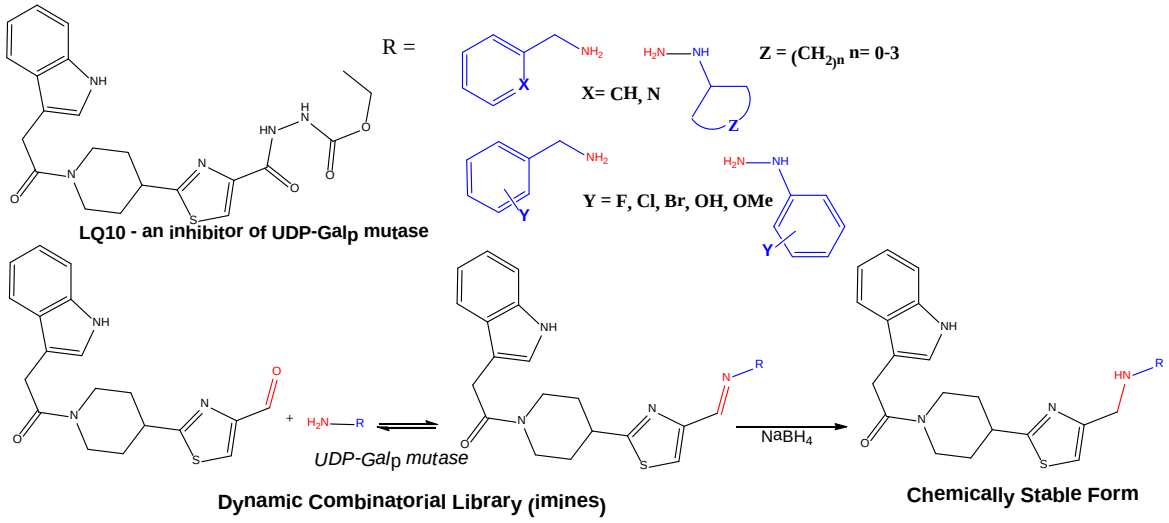


Fig1. **Dynamic Combinatorial Chemistry: A series of ligands that possess freely reversible chemical bonds and therefore in equilibrium with their precursor fragments. When exposed to an enzyme template, the composition of the library will redistribute to reflect those molecules that are stabilised (bind tightest) to the enzyme template. A chemically stable version of this molecule can then be generated as a drug lead compound.**





Project title: Investigation of the immunogenicity and efficacy of novel Salmonella vaccine candidates in pigs

Research theme: Global Food Security

Location: School of Veterinary Medicine and Science

Rotation: 1, 2 & 3

Contact: Dr Neil Foster

Lab Rotation Project Description:

Background: Salmonellosis remains a significant zoonotic disease but is also associated with economic loss (reduced weight gain) in the pig industry. Currently there are no licensed vaccines against *S.* Typhimurium in the UK and commercially available (*S*. Choleraesuis-specific) vaccines have little protective effect. There is, therefore, a great need for the development of *S*. Typhimurium vaccines in pigs.

Study plan: This mini-project aims to demonstrate the use of immunohistochemistry and FACS analysis to show changes in relevant cell populations within the intestine and mesenteric lymph nodes of pigs inoculated with vaccine strain and wild type *Salmonella*. Tissues from inoculated pigs would be available from similar, current studies within our group.

Training provided: Laboratory techniques: Tissue preparation for immunocytochemical analyses and analysis of these tissues using fluorescent and/or confocal microscopy. Training in tissue digestion of unfixed tissues and FACS analysis using relevant phenotypic antibodies would also be given. Other: general laboratory induction, containment level 2 laboratory working practices.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Knowledge of basic software	**Computer Skills**	Use of imaging software
	Spreadsheet manipulation		
Numeracy Skills	Basic stats	**Numeracy Skills**	Use of established statistical analysis of results and bacterial counts
	Basic concentration/dilution skills		
General Lab. Skills	Understanding of the need for precision and accuracy	**General Lab. Skills**	Laser capture microscopy
	Basic equipment		ELISA/ western blotting

	handling eg pipettes		
	Record keeping		FACS analysis
			PCR (including q-PCR)
			Immunohistochemistry
			Working at containment level 2
Communication Reading/Writing/ Presenting Skills	Report-writing	**Communication Reading/Writing/ Presenting Skills**	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Ability to represent a research group
			Interacting within a larger group

Linked PhD Project Outline:

Background: Between 1996-2000 over 73 000 cases of salmonellosis were reported in the UK and *Salmonella* was attributed to most fatalities which resulted from food-borne infection. In one study, *Salmonella* was isolated from the caeca of 23% of all pigs slaughtered in UK abattoirs, representing significant zoonotic risk. In pigs, clinical disease may occur in the post-weaning period but is usually sub-clinical. However, sub-clinical infections have been shown to have economic impact, by reducing weight gain. The three commercial vaccines available for use in pigs are all specific for *S*. Choleraesuis and currently there are no licensed vaccines against *S*. Typhimurium in the UK. Studies have shown that administration of these vaccines may confer some cross-protection against *S*. Typhimurium but some have been associated with serious adverse events and none have been shown to induce *S*. Typhimurium-specific antibodies, thus indicating that these vaccines do not elicit full immunological response to *S*. Typhimurium. Therefore, there is a great need to develop efficacious vaccines against *S*. Typhimurium in pigs as a means of reducing spread into the human food chain and of increasing livestock production.

Study plan: Novel *Salmonella* vaccine candidates will be obtained from Professor Paulo Pasquali (Rome), who currently collaborates with us on an EMIDA project. These will be tested for their ability to protect pigs against the three most important *Salmonella* serovars in the UK (Typhimurium, Derby and Enteritidis). Training in microbiology animal handling/infection and immunology will be given. Using small volumes of blood obtained at set time points throughput the experiments, XMAP/Luminex technology will be used to ascertain the concentration of cytokines relevant to the

development of a protective Th1 response followed by an antibody-inducing Th2 response. At the end point, the student will use ELISA and Western blot analyses to determine the concentration of *Salmonella*-specific IgG and IgA in blood and secretions. Histology and FACS analyses will determine the cell populations found in the intestine, mesenteric lymph nodes and spleen. Laser capture microscopy will be used to isolate in situ populations of lymphocytes and CD172a+ APCS in the T and B cell areas of the mesenteric lymph nodes and spleen. Expression of relevant cytokine genes in these cells and, in the case of APCs, MHCII and co-stimulatory molecules will then be analysed using qPCR. qPCR will also be used to compare inflammatory cytokine activity in the intestines, spleen and livers of vaccinated and non-vaccinated/challenged pigs.

Facilities/training: The study would take place in a highly active research group currently investigating other aspects of *Salmonella* vaccines in pig and poultry in designated facilities on Sutton Bonington campus. The student would be trained in appropriate animal and laboratory techniques, project management, scientific writing/presentation and would attend University led courses.

How the study fits with the BBSRC remit: The study fits within the “Food security” remit of the BBSRC research strategy addressing “Animal health” and “Healthy and safe food” priorities. The study also fits into the themes ‘Global Food Security’ and ‘Molecules, Cells and Organisms’ as DTP remits.

Project title: Investigating the role of abscission-related genes during plant development, and as a source to generate a degradation enzymatic cocktail

Research theme: Global Food Security

Location: Biosciences

Rotation: 1 & 2

Contact: Dr. Zinnia H. González-Carranza

Lab Rotation Project Description:

Among the Arabidopsis identified genes from our previous studies are: *At2g41850*, a polygalacturonase, and the previously unknown genes *At1g64405* and *At3g14380*. We have demonstrated that these three genes are expressed during abscission and other cell separation processes. Over-expressing plasmids from *At2g41850,* and homozygous over-expressing plants from *At1g64405* and *At3g14380* are available. Primary RNAi transgenic seeds from the *At1g64405* gene are also available.

Objectives:

1) To contribute to the identification of RNAi transgenic plants from the *At1g64405* gene. *The student will become familiar with growing Arabidopsis plants in vitro and in soil. He/She will be able to identify and transplant primary transformants.*

2) To extract genomic DNA from Arabidopsis wild type plants. *The student will be able to extract genomic DNA.*

3) To amplify the coding sequences from the genes: β-1,4 glucanase (*At4g24260*), Pectyn Methyl Esterase (*At4g02330*), and Xylanase (*At1g09610*) from Arabidopsis Columbia-0 wild type plants using PCR techniques. *The student will become familiar with the use of online biological resources, including The Arabidopsis Information Resource. The student will be able to perform PCR reactions and understand the principles from it.*

Outcome:

At the end of the six week placement, primary RNAi plants from the *At1g64405* gene will be growing in the growth rooms, and coding sequences for the genes β-1,4 glucanase (*At4g24260*), Pectyn Methyl Esterase (*At4g02330*), and Xylanase (*At1g09610*) will be available to produce over-expressing lines and $SAG12_{pro}$ driven constructs.

Time scale:

Objective:	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1						
2						
3						

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Use of Microsoft Office, Word, Powerpoint and Excel.	**Computer Skills**	To understand and manipulate data obtained from biological websites.
			use software to analyse DNA and RNA sequences and generation of primers.
			To use Genevestigator, TAIR and other biological resources.
			Use of Photoshop to generate images to publication standards.
Numeracy Skills	The ability to handle numbers, including the basic skills of addition, subtraction, multiplication, division and percentages.	**Numeracy Skills**	You will be able to gather and compile statistical data in various formats, and analyse, interpret and present the data in a clear way.
General Lab. Skills	Use of Gilson pipettes	**General Lab. Skills**	DNA and RNA extraction.

	Ability to record data in an organised and clear way.		Growth, crossing and caring of Arabidopsis plants.
	Conducting lab work in a professional manner.		Ability to record data in an organised and clear way.
Desirable	Some experience in the PCR techniques		*In vitro* culture of Arabidopsis seeds.
			Enzymatic extraction and assays, including HPLC.
			Use of microscopes.
			Generation of plasmids.
			Analysis of sequences.
			Primer design.
Communication Reading/Writing/ Presenting Skills	Use of Powerpoint	**Communication Reading/Writing/ Presenting Skills**	To communicate and present your results in a professional way.
	Reading of scientific literature.		To read and develop skills to research and read literature relevant to the project.
			To write a report in a professional way.

Linked PhD Project Outline:

Abscission occurs when a specialized layer of cells undergoes an intricate series of molecular and biochemical events. This process involves organ shedding, protection against water loss, and protection against pathogen invasion (Roberts and Gonzalez, 2007). Untimely abscission of commercial crops results in important yield losses and extreme yield variability. The PI and Co-I have extensive experience in the field and a recent breakthrough in this area has been the identification of 1,200 abscission-related genes from Arabidopsis, many of which are of unknown function (Gonzalez-Carranza et al., 2012).

Hypotheses:

1) **Previously unknown abscission-related genes *At1g64405* and *At3g14380,* may be involved in cutin biosynthesis and cell wall modifying processes respectively.**

   Experiments:

   1) Identifying RNAi lines and T-DNA knockout plants from the *At1g64405* gene.
   2) Quantifying cutin monomers by HPLC in knockout, wild type and over-expressing plants from the *At1g64405* gene.
   3) Analysing permeability and ultrastructure of the cuticular membrane knockout, wild type and over-expressing plants from the *At1g64405* gene.
   4) Analysing and comparing cell wall composition: pectic acid, pectin, cellulose, starch and hemicelluloses from knockout, wild type and over-expressing lines from the gene *At3g14380*; using microtiter plate assays, enzymatic assays and gas chromatographic methods.

Outcome:

The experiments proposed will provide information about the role of *At1g64405* and *At3g14380* during abscission and other plant development events. On completion of this doctoral project investigation of orthologue genes from *At1g64405* and *At3g14380* in rice will be pursued, aiming to generate rice plants resistant to water loss, chemical and biotical aggressors (*Global Food Security Theme*).

2) **An enzymatic cocktail obtained from *Arabidopsis* abscission-related genes can be used to degrade different biological materials, including crop residues, cardboard and vegetable house waste.**

   Experiments:

   1) Generating over-expressing plants from genes encoding cell wall degrading enzymes: β-1,4 glucanase (*At4g24260*), Pectyn Methyl Esterase (*At4g02330*); and Xylanase (*At1g09610*) driven by the constitutive Cauliflower mosaic virus-

CaMV *35S*$_{pro}$ and *SAG12*$_{pro}$ (*Senescence Associated Gene12*) promoters in Arabidopsis plants.

2) Analysing enzymatic activity and cell wall composition of pectic acid, pectin, cellulose, starch and hemicelluloses; in wild type and over-expressing plants from the genes: Polygalacturonase (*At2g41850*), β-1,4 glucanase (*At4g24260*), Pectyn Methyl Esterase (*At4g02330*), Xylanase (*At1g09610*) and *At3G14380*; using microtiter plate assays, enzymatic assays and gas chromatographic methods.

3) Generating and purifying enzymatic cocktail using fungal expression hosts.

4) Investigating the efficiency of the created enzymatic cocktail to degrade crop residues, cardboard and vegetable house waste.

5) Exploring the use of the biomass generated using the enzymatic cocktail to produce bioenergy using fermentation techniques.

6) Recycling of enzymatic cocktail by ultrafiltration.

Outcome:

The experiments proposed will generate a degrading enzymatic cocktail, which may represent an efficient and cost effective way of producing bioenergy generated from sustainable resources (*Industrial Biotechnology/Bioenergy, Molecules, Cells and Organisms Themes*).

Time scale:

Hypothesis/ Experiment:	Year 1	Year 2	Year 3	Year 3.5
1/1				
1/2				
1/3				
1/4				
2/1				
2/2				
2/3				
2/4				
2/5				
2/6				

References:

González -Carranza ZH et al., (2012) Plant Physiol **160:** 1342-1356.

Roberts & González-Carranza (2007) Encyclopedia of Life Sciences. John Wiley & Sons, New York, pp 1–8

Project title: The development of candidate therapeutic and diagnostic ligands for prion diseases
Research theme:

Contact: Dr Kevin Gough

Lab Rotation: Rotation 1

Mini Project plan

Background: Prion diseases are fatal neurological disorders that have no effective cure or treatment. They affect humans and food production animals. BSE in cattle arose in the mid 1980s and was then found to cause disease in humans. To date, over 200 people have died from BSE infections and a recent study of archived human lymphatic tissues estimates that 1 in 2000 of the UK population may be carrying the BSE agent.

The causal agent of these diseases is a misfolded version of the cellular prion protein (PrPC), termed PrPSc. The most logical strategy to identify therapeutic agents is to find ligands that bind specifically to either PrPSc or PrPC and which disrupt the autocatalytic replication of PrPSc from PrPC substrate.

Study plan: This mini-project aims to demonstrate the use of phage-display to isolate ligands that bind the prion proteins. Phage-display antibody libraries would be bound to both PrPC and PrPSc. Identification of the bound antibodies would be carried out by amplification of the antibody genes and analysis by next generation sequencing (NGS). Coupling the huge diversity of phage-display libraries with the power of NGS will facilitate the isolation of even very rare binding events and afford an unprecedented level of screening for ligands to these proteins. The identification of antibodies that bind specifically to prion proteins would be carried out by bioinformatic analysis.

Training provided: Laboratory techniques: prion misfolding assay, phage-display technology, bioinformatic analysis of deep sequencing data. Other: general laboratory induction, cat3 containment laboratory working practices.

Mini Project Skills Matrix

Skills you need		**Skills you'll develop**	
Computer Skills	Knowledge of basic software	**Computer Skills**	Use of imaging software
	Spreadsheet manipulation		Bioinformatic analysis of datasets
Numeracy Skills	Basic stats	**Numeracy Skills**	Use of established statistical analysis of bioinformatics data
	Basic concentration/dilution		

	skills		
General Lab. Skills	Understanding of the need for precision and accuracy	**General Lab. Skills**	Protein misfolding assays
	Basic equipment handling eg pipettes		Electrophoresis/western blotting
	Record keeping		Phage display
			PCR (including q-PCR)
			NGS sequencing
			Working at containment level 3
Communication Reading/Writing/ Presenting Skills	Report-writing	**Communication Reading/Writing/ Presenting Skills**	Scientific paper-writing
	Ability to carry out literature-searching		Ability to write a literature review
	Ability to communicate to colleagues		Ability to communicate to outside audiences
			Ability to represent a research group
			Understanding of QA processes, project management and financial/project risk assessment (all training on these topics given by an Industry partner)

Linked PhD Project Outline:

Background: Prion diseases are fatal neurological disorders that have no effective cure or treatment. They affect humans and food production animals. Bovine spongiform encephalopathy (BSE), or “mad cow disease”, arose in the mid 1980s and caused an epidemic in UK cattle; BSE contaminated food was then found to cause disease in humans. The BSE epidemic had a devastating effect at a cost of over £4 billion to the UK alone. To date, over 200 people have died from BSE infections and a recent study of archived human lymphatic tissues estimates that 1 in 2000 of the UK population may be carrying the BSE agent.

The causal agent of these diseases is a misfolded version of the cellular prion protein (PrPC), termed PrPSc. Within an infected individual PrPSc molecules interact with PrPC and induce a conformational change in this bound PrPC to form more PrPSc; in this autocatalytic manner PrPSc slowly accumulates over long incubation periods, particularly in the central nervous system causing neuronal damage and clinical symptoms. The search for an effective treatment for prion diseases is a pressing research objective. The most logical strategy is to find ligands that bind specifically to either PrPSc or PrPC to disrupt the autocatalytic prion propagation process.

Study plan: We propose to use the very latest techniques in ligand screening to isolate a wide range of antibodies and peptides that bind specifically to PrPC and PrPSc. The strategy involves binding phage-display ligand libraries (containing billions of individual peptides or antibodies linked to their corresponding genes) to both PrPC and PrPSc. Identification of the bound ligands would be carried out by amplification of the ligand genes and analysis by next generation sequencing (NGS). The coupling of the huge diversity of phage-display libraries with the power of NGS will facilitate the isolation of even very rare binding events and afford an unprecedented level of screening for ligands to these proteins. The selected ligands would then be screened for their ability to disrupt PrPSc propagation using established in vitro prion replication systems as well as within a cell culture model. As well as their therapeutic potential, isolated ligands would also have considerable application in disease diagnosis. The study would therefore also look to develop novel diagnostic platforms using PrPSc-specific ligands.

Facilities/training: The study would take place in a highly active research group including 6 bench scientists. The study would use a dedicated prion research laboratory within SVMS. As well as attending University training courses, the student would be trained in appropriate laboratory techniques and containment laboratory processes and would also gain training from industry (ADAS UK) in topics such as QA processes, project management and financial/project risk assessment.

How the study fits with the BBSRC remit: The study fits within the “Food security” priority area of the BBSRC research strategy addressing both the “Animal health” and “Healthy and safe food” priorities. In terms of the DTP themes it fits into the themes ‘Global Food Security’ and ‘Molecules, Cells and Organisms’.

Project title: Detection and monitoring of environmental pesticides.

Research theme: Global food security

Location: School of Graduate Entry Medicine & Health

Rotation: 1, 2 & 3

Contact: Dr Wayne Grant Carter

Lab Rotation Project Description:

Animal tissues, or cells grown in culture, will be homogenised to give a preparation of cells. These cells will be incubated with pesticides and pesticide target proteins tagged. These target proteins will be purified, to enable their identification. Once identified an analysis of target protein function can be instigated.

The students will be trained in general laboratory techniques including pipetting, use of weighing balances, use of centrifuges etc. The students will then be trained in animal tissue homogenisation, and also cell culturing. The students will be trained in protein quantitation by spectrophotometric analysis. The students will then undertake training in protein separation techniques such as polyacrylamide gel electrophoresis, and protein identification using antibodies (Western blotting).

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	General competency	**Computer Skills**	Powerpoint, word, excel
			Graph plotting. Searching databases online.
Numeracy Skills	General competency	**Numeracy Skills**	Excel, statistics, graph plotting.
General Lab. Skills	General competency	**General Lab. Skills**	Pipette usage, general lab equipment such as balances, centrifuges.
			Gel electrophoresis
			Western blotting
			CCD image capture
Communication Reading/Writing/		**Communication Reading/Writing/**	

Presenting Skills		Presenting Skills	
	General competency		Maintaining a lab book, preparing a poster/abstract/ instruction in manuscript writing

Linked PhD Project Outline:

Exposure to pesticides is an inevitability of food consumption in our Western diet. Pesticides enter our bodies through several routes that include food ingestion, inhalation, and skin contact. Once inside our systems, pesticides bind to a number of target proteins, and some of this protein binding is thought to contribute to ill-health. To understand the molecular basis for ill-health arising from pesticide exposure, we need to identify all the targets of pesticides, and monitor how our body copes with their removal.

This project will utilise a range of biological techniques to identify pesticide targets, consider how we can better monitor exposure to pesticides, and treat their unwanted biological actions.

Project title: Understanding the actions of a plant hormone by analysis of auxin transporter proteins and modelling of cellular auxin flux.

Research theme: Global Food Security

Location: University Park and SB campuses

Rotation: 1**,** 2 & 3

Contact: Dr Ian Kerr

Lab Rotation Project Description:

The student would spend the 6 weeks experiencing both the major facets of the program. For 3-4 weeks the student would work in the Kerr lab in the School of Biomedical Sciences, receiving training in membrane protein expression in heterologous systems, and the analysis of membrane transport. Some of this work may be use other transporters to enable the student to get some first-hand experience of the techniques in systems that are "tried and tested". We would also discuss and plan the research strategy so that the student felt that they "owned" the project from as early a time as possible. The student would then spend another 2-3 weeks in the CPIB (Centre for Plant Integrative Biology) at Sutton Bonington campus, seeing how data from plant physiology, and plant genetics, is combined to produce mathematical and computational models of plant development. Again, this would involve a degree of "shadowing" as the student would likely have no experience of working at the biochemistry/mathematics interface.

The mini-project would leave the student perfectly clear about the direction and the scope of the main project, and will have given them insights in to the laboratory and non-laboratory skills that they would receive training in as part of that full project.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	MS Office skills, reference manager software experience useful	**Computer Skills**	Individual-based simulation and model reduction methods
Numeracy Skills	Good grasp of basic laboratory maths (i.e.	**Numeracy Skills**	Dose-response curve analysis of kinetics and

	molarities) and statistics (i.e. means, s.d.)		pharmacology. Advanced statistical analysis of data sets.
General Lab. Skills	1st degree in a related subject. Biochemistry, molecular biology etc.	**General Lab. Skills**	Membrane protein expression and characterization
	Final year lab project a must		Quantitative pharmacology and kinetics by radioligand binding/transport
	Experience of protein expression useful.		
Communication Reading/Writing/ Presenting Skills	Effective communication skills, data presentation experience	**Communication Reading/Writing/ Presenting Skills**	The ability to communicate complex ideas at the interface of biochemistry and maths

Linked PhD Project Outline:

Introduction

The plant hormone auxin (indole 3-acetic acid; IAA) controls an astonishing array of processes in plants including embryogenesis, lateral root development and root gravitropism. These effects require auxin gradients, which are established through a combination of membrane diffusion and carrier-mediated transport. At least three membrane protein families contribute to this: the AUX-LAX family of proteins are auxin importers, PIN proteins are auxin efflux pumps, and members of the ATP binding cassette (ABC) family have been implicated in both export and import of IAA (1).

To understand the role of auxin concentration gradients requires a concerted effort to assemble evidence from auxin transporter localization, analysis of plants carrying mutations in transporters, *in vitro* characterization of transporters, and mathematical modelling of auxin fluxes. The University's Centre for Plant Integrative Biology (www.cpib.ac.uk) has made massive strides towards this goal, and the current studentship would enable the student to be a part of the bigger research picture of Global Food Security.

Aims of the project

The project will pick up two of the four strands of research mentioned above and will:

- Characterize AUX-LAX, PIN and ABC transporter proteins for their affinity to transport IAA across cell membranes (2)
- Use these data to determine parameters that will enable the more accurate modelling of auxin fluxes and refine the model in the light of these data (3)

Research programme

Goal 1 (year 1): establish the experimental system. The student will express auxin transporters in two non-plant systems (4). These afford a low "background" (i.e. there are no specific auxin transporters present), whilst retaining eukaryotic membrane protein processing machinery. Firstly, mammalian cell lines would be established use in auxin *transport* experiments (*Goal 2*). Secondly, an insect cell expression system would be used to express the transporters for characterization of auxin *binding* studies *(Goal 3)*.

Goal 2 (year 2-3) *Studies of transport kinetics*. Both auxin uptake and efflux transporters would be studied in whole-cell transport studies using radiolabelled auxin. Michaelis-Menten analysis of the data would provide Km and Vmax values. Determination of transporter density would enable estimations of the transport rate (flux) of auxin transporters.

Goal 3 (year 2-3) Studies of substrate and inhibitor binding. Auxin transport inhibitors are important agriculturally and the insect cells expression system will enable characterization of the affinities of auxin transporters for auxin analogues. Goals 2 and 3 are independent so offering the student multiple possibilities to acquire important data sets.

Goal 4 (year 3-4) Modelling of auxin fluxes in roots.

The existing multicellular model for auxin transport (developed in CPIB) will be revisited in the light of these new data, being refined where necessary and simulated extensively to shed light on the implications of the new quantitative information for a variety of the key developmental processes alluded to above. Model simplification that exploits asymptotic methods will also be enabled by the experimental results and will be pursued to tease out the dominant transport mechanisms in such contexts.

Training and supervision

The student who graduates from this project will have a unique skill-set that will equip them well for a post-doctoral career either in plant biology or membrane protein biochemistry generally. They will have a broad appreciation of multidisciplinary science and an understanding of how biological processes can be modelled mathematically, both of which are essential in any "translational" research programme. Finally, outside of science, the student would be well equipped for any career in which data interpretation and predictive modelling is important.

The three supervisors on this project have a perfect blend of expertise to guide this project. Dr Kerr has expertise in membrane protein biochemistry, Professor Bennett in plant systems biology, and Professor King in mathematical biology. All three supervisors have collaborated in the past, and successfully trained numerous PhD students and other early career researchers.

Fit with BBSRC Remit

This is a systems biology project, closely linked with an existing systems biology initiative at the University. Understanding of how root architecture is determined by auxin fluxes is at the heart of crop improvement efforts, and so is firmly within the global food security strand of the DTP.

Project title: Analysis of the mechanism regulating anther opening: a tool to control fertility for breeding hybrid crops with increased yield**.**

Research theme: Global Food Security

Location: University Park (Maths) and Sutton Bonington Campuses (Plant and Crop Sciences).

Rotation: 1, 2 & 3

Contact: Prof John King

Lab Rotation Project Description:

The miniproject will combine biological lab work and mathematical modelling. The former will involve the analysis of aquaporin expression in the anther during dehiscence. Aquaporins are a class of proteins that are found in cellular membranes and mediate the passive movement of water. The aquaporin family is large, however global transcriptomic information is available about the expression patterns of the family members in anther tissues, suggesting selective expression of certain family members. Bioinformatic analysis will be used to identify and confirm the key players during Arabidopsis anther dehiscence. This expression pattern will then be confirmed by RT-PCR analysis using staged anther tissues. This will then be expanded to include a detailed time-series analysis of anther development. Subsequently localisation of aquaporin-fluorescent protein fusions will be conducted by confocal microscopy to provide the spatial resolution. Insertional mutants containing mutations in the relevant aquaporins will be analysed for alterations in dehiscence. These data will then be used in guiding the development of a preliminary mathematical model describing water transport during dehiscence (building on recent modelling work of the role of aquaporins in laterial root emergence). This will incorporate the data generated from the aquaporins and tissue dehydration analysis and set the stage for its inclusion in the anther dehiscence opening model

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	XXX	**Computer Skills**	XXX
Numeracy Skills	XXX	**Numeracy Skills**	XXX
General Lab. Skills	None, but an interest in wet lab work is essential	**General Lab. Skills**	RNA extraction
			Expression analysis
			Confocal microscopy

Communication Reading/Writing/ Presenting Skills	*Report writing*	**Communication Reading/Writing/ Presenting Skills**	*Scientific paper writing*
	Ability to carry out literature searches		*Ability to write a literature review*
	Ability to communicate to colleagues		*Ability to communicate to internal and outside audiences*

Linked PhD Project Outline:

Understanding the processes that underlie pollen release is a prime target for controlling fertility to enable selective breeding and the efficient production of hybrid crops, which typically produce 20-30 times more yield than inbreeding alternatives. These are targets that would aid strategies for increasing crop yield with the goal of sustainable global food security. Pollen release requires anther opening, which involves changes in the biomechanical properties of the anther wall (Wilson et al, 2011): this process requires selective dehydration, combined with changes in the biochemical properties of the anther cell walls.

We have recently developed a mathematical model describing the biomechanics of anther opening (Nelson et al, 2012) which incorporates the bilayer structure of the mature anther wall: this comprises the outer epidermal cell layer, whose turgor pressure is related to its hydration, and the endothecial layer, whose walls contain helical secondary thickening, which resists stretching and bending. The model describes how epidermal dehydration, in association with the thickened endothecial layer, creates forces within the anther wall causing it to bend outwards, resulting in anther opening and pollen release. This work has generated a number of significant open questions relating to the processes of selective dehydration, the role of key factors involved in water transport, e.g. aquaporins, and the biochemical changes that are occurring in the anther during the dehiscence process.

This project will combine a lab-based analysis of the processes of dehiscence (using a range of Arabidopsis mutants that have alterations in the secondary thickening properties of the anther wall or in the dehydration process/targeted removal of water from the anther) with partial-differential-equation-based mathematical modelling, which will need to encompass a variety of complex and multiscale biomechanical, biochemical and transport processes, over scales ranging from gene regulatory networks to the anther. Together, this systems-biology approach will enable a greater understanding of the dehiscence process combined with providing the opportunities for manipulating the dehiscence process for controlling plant fertility.

Project title: Genes controlling wheat grain bound phenolics

Research theme: Global Food security

Location: Rothamsted Research

Rotation: 2 & 3

Contact: Julie King

Lab Rotation Project Description:

Students will make DNA constructs designed to suppress or overexpress candidate genes (within the glycosyl transferase 61 and BAHD gene families) which we believe encode the enzymes for the final steps of synthesis of bound ferulic acid and coumaric acid in wheat aleurone . This will introduce them to many of the key skills required for molecular biology: cloning strategy, use of bioinformatics software, primer design, isolation of PCR products, cloning, restriction digest, ligation etc. Final sequencing of their constructs will demonstrate their degree of success.

Successfully completed constructs will then be used to transform wheat (using Rothamsted "Designing Seeds" ISP resources) after the rotation, so that when a student rejoins the project, transgenic material will be available as early as possible within the studentship for analysis.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	General computer literacy including experience of word, excel, power point etc	**Computer Skills**	Bioinformatics Statistical analysis of data
Numeracy Skills	High level of general numeracy skills	**Numeracy Skills**	Data analysis using statistical methods/packages
General Lab. Skills	Good laboratory practice	**General Lab. Skills**	Molecular biology techniques Analytical biochemistry techniques

Communication Reading/Writing/ Presenting Skills	Sound knowledge of English, both spoken and written Experience of data interpretation	**Communication Reading/Writing/ Presenting Skills**	Ability to interact effectively with, and present research to, colleagues Sound interpretation of scientific literature Writing scientific reports and manuscripts Presentation of results at national and international conferences

Linked PhD Project Outline:

Wheat grain constitutes a large component of the human diet. Efforts are currently underway to develop novel wheat germplasm with increased genetic diversity and improved properties to meet Global Food Security goals e.g. Ian and Julie King's current projects at Nottingham will produce lines of wheat carrying small chromosome segments from related species which carry genetic variation for key agronomic traits, (BBSRC/partner – BB/H0112834/1, BB/I00260X/1, SCPRID BB/J011827). At the same time it is important to maintain or enhance the levels of beneficial dietary components in wheat foods. Recently there has been much interest in the health benefits associated with phenolic compounds present in wheat grain, the majority of which is ferulic acid and coumaric acid ester-linked within cell walls in the grain outer layers, with lesser amounts of free and conjugated forms. The phenolic compounds are powerful antioxidants and also have anti-inflammatory and anti-proliferative activity and there is increasing evidence that they confer health benefits by reducing incidence of cardiovascular disease and possibly bowel cancer. A large diversity screen carried out in the European HEALTHGRAIN project showed only very limited genetic variation in bound phenolics between elite varieties (Fernandez-Orozco et al., 2010), so novel sources of variation may be required. The Rothamsted cell wall group have identified candidate genes for the last steps in the synthesis of these bound phenolics (Mitchell et al., 2007 Plant Physiology 144: 43-53). The studentship will seek to identify the major genes controlling the amount of bound phenolics in wheat grain by (1) manipulating bound phenolic content by altering expression of candidate genes in aleurone of transgenic wheat (2) screening wheat lines with alien introgressions for those exhibiting large variation in bound phenolics and comparing expression of genes in grains of these with lines lacking the introgressions. During the 6-week mini project RNAi suppression or overexpression cassettes for candidate genes driven by the 7s globulin aleurone-specific promoter will be constructed providing an introduction to molecular biological techniques. The constructs will be used to transform wheat using other resources so that the resultant transgenic lines are available for the student to analyse as soon as possible during the studentship. While these transgenic lines are being generated, grain from

selected wheat lines developed by the Nottingham group will be screened for bound phenolic content of milled mature grain, as previously done in the HEALTHGRAIN diversity screen. This will involve training in biochemistry, statistics and data analysis. If large variation is discovered which can be attributed to an introgression , expression of genes in developing grain tissues will be studied in comparable lines with and without the introgression. The student will therefore learn gene expression methods such as qRT-PCR and analyses of these. They will also analyse the phenolic composition of the transgenic lines in grain from homozygous plants. This combination of approaches should provide strong evidence for the identity of genes controlling phenolic acid content in wheat grain. The student will gain excellent training in molecular biology and analytical biochemistry and a good chance of high-impact publications.

Project title: Bayesian Inference for Dynamical Systems: From Parameter Estimation to Experimental Design.

Research theme: Global Food Security

Location: School of Mathematical Sciences but also with regular visits to the School of Biosciences.

Rotation: 1, 2 & 3

Contact: Dr Theodore Kypraios

Lab Rotation Project Description:

The main purpose of this mini project is to introduce the students the concepts of 1) mathematical modelling and 2) Bayesian parameter estimation using both analytical and computational techniques. Essentially, the students will learn how to generate synthetic data from simple mathematical models used in Biology (such as the logistic growth model) and learn how to fit such models to synthetic and experimental data. Below is a plan for this six week.

- Week 1: Introduction to mathematical modelling and programming in R/Matlab.
- Week 2: Introduction to generating synthetic data from ODE models and fitting ODE models using conventional methods such as least squares minimization.
- Week 3: Introduction to Bayesian inference and relevant methodology.
- Week 4-5: Introduction to computational statistics methods, such as Markov Chain Monte Carlo. This will involve learning about methods such as the Metropolis-Hastings algorithm, and apply it to estimate the parameters of a simple ODE model using synthetic data.
- Week 6: Write up a report illustrating the above concepts and present the results of fitting a simple ODE model to some experimental data.

Lab Rotation Skills Matrix:

Skills you need	**Skills you'll develop**		
Computer Skills		**Computer Skills**	
A-levels		Programming	
Numeracy Skills		**Numeracy Skills**	
A-levels		Advanced mathematical and statistical techniques.	Analytic thinking,

General Lab. Skills		**General Lab. Skills**	
Communication Reading/Writing/ Presenting Skills		**Communication Reading/Writing/ Presenting Skills**	
A-levels		independent working	time management
		and project organisation	

Linked PhD Project Outline:

Mathematical modelling has long played an important role in describing and predicting the behaviour of Biological processes. For example, deterministic non-linear ordinary differential equation models (ODEs) have been used from describing the average concentration of a protein within a population of cells or for describing the metabolic activity of enzymes in a population of cells in varying concentrations of substrates. The proposed project is concerned with the development of robust, and computationally efficient statistical methods 1) to infer the parameters of dynamical systems (system of non-linear ODEs) given experimental data, and 2) novel approaches for optimum experimental design within a Bayesian framework.

Inferring the parameters of a dynamical system given limited experimental data can be a challenging task. In more than three or four dimensions deterministic approaches are no longer feasible and we must resort to simulation-based Monte Carlo methods such as Markov Chain Monte Carlo (MCMC) by constructing a Markov chain that converges to the distribution of interest, e.g. the posterior distribution in a Bayesian setting. Even though such methods have successfully been used, their implementation in practice is far from being straightforward. This is due to two main issues: i) the posterior distribution is often highly multi-modal making it difficult to find the global optimum and this can cause *convergence* and *"mixing"* problems of standard MCMC algorithms and ii) exploring the posterior distribution requires repeated numerical solution of the ODEs (via numerical integration) and that makes such algorithms being computationally very expensive. We will develop novel algorithms to enable efficient exploration of the parameter space and build upon methods which are computationally less demanding (e.g. using spline regression techniques) that will reduce the need to solve the ODEs numerically.

Biological experiments need, in general, considerable resources in terms of scientists' time, expensive reagents and expensive laboratory equipment. Thus, optimal experimental design is extremely important not only to save resources, but also to ensure which or how to do the experiment is going to provide us with the most "suitable" data from which model parameters can be best inferred. Unlike parameter estimation, methods for experimental design have not attracted

much attention in the literature. The second aim is to develop novel approaches to experimental design within Bayesian framework by utilizing the developed methods for parameter inference.

Although the project is concerned with the development of generic novel statistical methodology, it is relevant to all three strands of the BBSRC DTP. The project will specifically focus on models of transcription regulation and models of metabolic activity, that typically include hyperbolic (e.g. Hill and/or Michaelis Menten) functions for which parameter estimation is difficult. Specifically, we will use models for: (i) the regulation of anti-microbial metal efflux proteins in pathogenic *E. coli* strains in relation to their use in animal feeds; and (ii) models for the activity of bioluminescent Lux proteins in the presence of varying substrates, that are used as molecular reporters in a wide range of experimental settings. These modelling activities have been chosen because they are supported by data generated on BBSRC grants currently held at UoN.

Project title: The genetic and immunological basis of parasite resistance in fish: a model species approach.

Research theme: Global Food Security

Location: Biology

Rotation: 3

Contact: Dr Andrew MacColl

Lab Rotation Project Description:

Comparison of immune gene expression in infected and uninfected sticklebacks.

The students will investigate differences in the immune profiles of naturally or artificially infected sticklebacks using quantitative PCR on existing samples. They will receive training in basic immunology and parasitology, carry out molecular genetic lab work (primer design, RNA extraction, qPCR) and analyse the data.

We already have tissue samples frozen in RNAlater, from both (i) wild sticklebacks with naturally high and low parasite burdens (ii) lab-raised artificially infected and control fish. One student will work with each of these sets of samples. Both students will have tutorials with the supervisors on the parasitological and immunological background to their projects. They will then receive training in (a) extracting RNA from their samples, (b) designing primers for one key immune gene, using the stickleback genome sequence (c) running qPCR reactions for their samples and (d) analysing their data. At the end they will be able to compare differences in the expression of their immune gene between naturally (wild) and artificially (lab) infected fish.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills		**Computer Skills**	
Numeracy Skills		**Numeracy Skills**	Database handling
			Statistical modelling
General Lab. Skills	Basic lab skills	**General Lab. Skills**	PCR methods
	DNA extraction		
Communication Reading/Writing/		**Communication Reading/Writing/**	Scientific presentations and writing, effective

Presenting Skills		**Presenting Skills**	exploration and reading of scientific literature

Linked PhD Project Outline:

The genetic and immunological basis of parasite resistance in fish: a model species approach.

Aquaculture and fisheries are a major source of protein for humans [1], but macroparasitic disease causes substantial economic loss to these industries [2]. The study of how hosts resist parasites is a central plank of research into the biology of molecules, cells and organisms, yet understanding of the genetic and immunological basis of resistance to macroparasites has lagged behind that for bacterial, viral and non-infectious disease. *This project provides an exceptional opportunity for a student to draw on the complementary expertise of three supervisors, and build on existing data and methodologies to examine the mechanistic basis of parasite resistance in an outstanding emerging model fish, the three-spined stickleback* [3-8]. The student will combine whole genome re-sequencing data for nine stickleback populations, currently being generated by David Kingsley's lab (Stanford), and quantitative PCR assays of immune gene expression, with artificial infection experiments and existing knowledge on variation in resistance in natural populations. These populations show strong contrasts in macroparasite infection rates [9], and in resistance to artificial infections [6]. Line-cross analyses in these populations and their hybrids shows that resistance has a largely additive genetic basis (Fig 1., [10]), suggesting polymorphism in resistance genes. Using RNAseq (Fig 1) we have begun to identify immune genes that contribute to this variation [10]. This system is ripe for exploration of immune gene variation using qPCR that is well established in JEB's lab [7]. The student will concentrate on key genes identified from our RNAseq experiment, and from work on parasite resistance in other fishes.

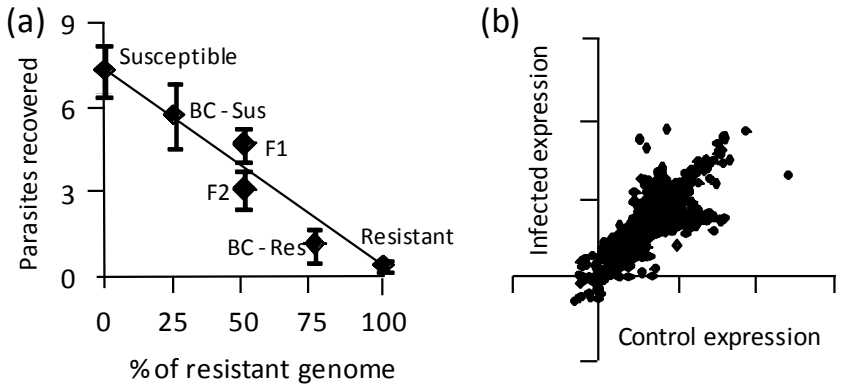


Figure 1. (a) Line-cross analysis of parasite resistance in sticklebacks. Number of parasites recovered from fish after a dose of 20 infective stages, plotted against proportion of the resistant genome present in fish, for susceptible and resistant parentals, F1s, F2s and back-crosses. (b) Relative expression of 22456 genes ($75x10^6$ reads) from pooled samples of infected and control fish from a resistant population, using an RNAseq approach.

The student will receive training in molecular genetics and immunology, parasitology and statistical and bioinformatic analysis, to address the following questions:

(1) How do immunological profiles vary between individuals and populations? *The student will use qPCR methods to estimate variation in the expression of key immune genes, within and between populations.*

(2) Are candidate immune loci polymorphic within or across populations? *The student will examine evidence for the evolution of immune genes across populations by analysing genomic re-sequencing data. S/he will use targeted re-sequencing of polymorphic genes to determine whether there is variation within populations. S/he will examine the data for signatures of divergent and balancing selection.*

(3) Can the immunological and genetic differences identified in (1) and (2) account for variation in resistance? *The student will: (a) examine the association between immunological profiles and parasite burdens of wild-caught sticklebacks, mirroring the approach used by JEB with small mammals [8], (b) expose lab bred individuals with known immunological profiles to infection, with methodology already used by ADCM [4].*

References: [1]. Frid, C.L.J. & O.A.L. Paramor. *ICES J. Mar. Sci.* 69: 145-150. **[2]**. Costello, M.J., 2009. *J. Fish Dis.* 32: 115-118. **[3]**. MacColl, A.D.C., 2009. *Ecography* 32: 153-160. **[4]**. MacColl, A.D.C. & S.M. Chapman, 2010. *Func. Ecol.* 24: 847-856. **[5]**. Friberg, I.M., et al., 2010. *Trends Parasitol.* 26: 540-549. **[6]**. de Roij, J., et al., 2011. *Func. Ecol.* 25: 217-226. **[7]**. Jackson, J.A., et al., 2011. *Mol. Ecol.* 20: 893-909. **[8]**. Turner, A.K., et al., 2011. *PLoS Genet.* 7. **[9]**. de Roij, J. & A.D.C. MacColl, in review. *Parasitology*. **[10]**. El Nagar, A. & A.D.C. MacColl, *Unpublished data*.

Project title: Intervention of Probiotic *S.boulardii* in stress related bowel dysfunction in the weaning pig.

Research theme: Global Food Security

Location: University of Nottingham, School of Biosciences, Division of Food Sciences.

Contact: Dr K H Mellits

Lab Rotation: 1, 2 or 3

Mini Project plan

(All successful applicants will be required to provide a six week training programme/mini project that will be used to introduce prospective students to the main project as part of a laboratory rotation to be carried out during the first six months of the studentship. There will normally be two students assigned to the mini-project. Please provide, in no more than 250 words, your plan for such a mini project and the training that it will provide.)

A 6 week training project includes a structured programme starting with discussion of the mini-project, a literature review and presentation in week one, so as to insure understanding, followed by practical work in weeks 2-5, followed by a presentation to the group. The programme will be determined during week one with discussion between student and supervisor but will always involve some basic techniques used in the supervisors laboratory, such as extraction of DNA from intestinal contents, analysis of diversity by pcr 16srRNA DGGE and similarity profiles determined FP-quest followed by construction of phonetic trees for similarity. In addition, it is likely that previously generated microflora pyrosequencing data from a previous trial will be analysed. In this way, students will be exposed group philosophy, including emphasis on understanding of the scientific literature (literature review), formulation of a hypothesis (hypothesis driven science), and emphasis on controlled, carefully performed experiments. Students will be exposed to common laboratory techniques, bioinformatics, analysis and interpretation of data. The student will be present at weekly informal group meeting on Friday mornings, as well as formal lab meetings, of which the student will present in week 6. The supervisor is well accustomed to rotations having experienced the system as a PhD student at Cold Spring Harbor Laboratory, USA. During rotation the supervisor and student must interact and both determine together if there is a “good fit.”

Linked PhD Project Outline:

Project Outline

Please provide an outline of the proposed project and training provided in no more than 500 words. This should clearly demonstrate the fit with BBSRC remit and the DTP theme(s) identified above: Less than 500 words including legends

Proposed Project: We have previously used a 4 pig weaning model (figure 1) to show evidence of stress related bowel dysfunction in pigs including loss of adsorption, increase in the stress glucocorticoid cortisol and degranulation of mast cells in weaned pigs compared to unweaned controls (figure 2). We have already performed several sets of weaning experiments using our verified 4 pig weaning model. Currently we are investigating the role of the host response to weaning stress and the effect of the probiotic *S. boulardii* using illumina sequencing of RNA extracted from colonic tissue of 4 replicate pigs from unweaned and weaned pigs at +1, +4, +14days relative to weaning, we have samples from control and also S. boulardii supplemented pigs (48 samples in total).

To better understand the role of changes in the microbiome on stress related bowel dysfunction and to correlate with host response, we plan on analysing microflora present in intestinal contents (which we have frozen) from these colonic tissues described above. To do this we first ensure diversity by analysis of 16SrRNA and Density Gradient Gel Electrophoresis (DGGE). We then sequence by 454 roche pyrosequencing and analyse changes in species. In this way we can determine the microflora changes that occur in a stressed animal suffering from bowel dysfunction, correlate those changes with transcriptional changes in host, and finally determine the effect of the probiotic *S. boulardii* on these effects.

Training: Individual training courses are assessed by formal student feedback at the end of each course and modifications made as appropriate. These training courses are designed to enhance the professional and personal skills of the student. Some of these courses are compulsory including the effective utilisation of library and IT resources, presentation skills and a laboratory based module, Basic Laboratory Techniques. Students are required to accumulate a minimum of 20 credits worth of training (equivalent to 2 weeks contact time) from such courses during their Ph.D programme. In addition students produce a presentation at the end of year one and a scientific paper at the end of year two as part of training.

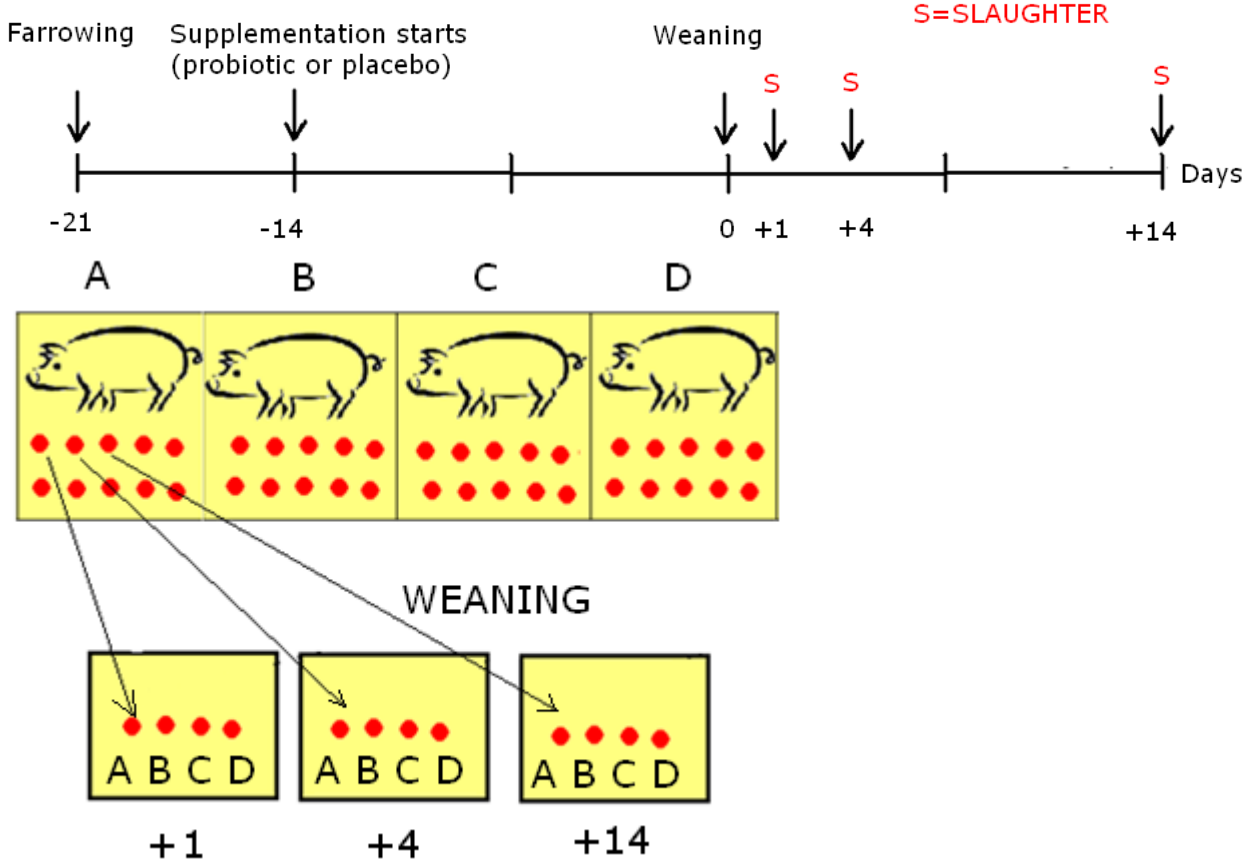


Figure 1. Four pig weaning model timeline. Piglets are supplemented individually and daily with probiotic (3e9CFU) *S.boulardii* or control paste from -14 d relative to weaning. At weaning one piglet is mixed with 3 non litter mates on same treatment, into each of 3 pens for slaughter (+1,+4,+14), unweaned control piglets remain on sow. At +1, +4 and +14d relative to weaning pens of 4 are slaughtered and analysed together with one unweaned pig on same treatment from each sow. 8 sows are used 4 with litters supplemented with control paste and 4 supplemented with the probiotic *S. boulardii*

Project title: Characterizing the aphid voltage-gated sodium channel as an insecticide target

Research theme: Global Food security

Location: School of Biology

Rotation: 1, 2 & 3

Contact: Ian Mellor

Lab Rotation Project Description:

The mini project will be closely related to the main project but will focus more on the electrophysiological techniques that will be employed. The students will express drosophila voltage-gated sodium channels that are known to function in Xenopus oocytes and measure some of their basic electrophysiological properties using two-electrode voltage-clamp. On successful completion of this part of the project they will investigate the actions of some known modulators of insect voltage-gated sodium channels including tetrodotoxin and pyrethroid insecticides.

The mini project will provide training in preparation of Xenopus oocytes and their injection with RNA; two-electrode voltage-clamp recording; measurement, analysis and interpretation of electrophysiological and pharmacological data. It will also provide training in basic laboratory practice and techniques.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Experience of basic software	**Computer Skills**	Electrophysiological data recording and analysis
	Spread sheets		Use of bioinformatics software
Numeracy Skills	Basic statistics	**Numeracy Skills**	Curve fitting and parameter estimation
	Calculating concentrations and dilutions		
General Lab. Skills	Precision and accuracy	**General Lab. Skills**	Dissection
	Handling basic equipment, e.g. pipettes		Cell culture
	Recording experimental details and results		Molecular biology

			Electrophysiology
Communication Reading/Writing/ Presenting Skills	Report writing	**Communication Reading/Writing/ Presenting Skills**	Literature reviews
	Literature searching		Poster presentation
			Oral presentation

Linked PhD Project Outline:

A significant proportion of the world crop productivity is threatened by insect pests. These may consume or damage plant crops directly, cause the spread of plant pathogens or both as in the case of aphids. Effective control by the use of chemical pesticides is now threatened by the development of resistance to most insecticides, and in addition there is significant public concern over the toxicity and environmental impact of pesticides. In the face of the need to ensure long-term food security there is a clear need to replace these pesticides with alternatives which overcome current resistance problems and satisfy public concern.

Following the completion of the aphid genome project, the co-supervisors based at Rothamsted Research identified an aphid voltage-gated sodium channel (VGSC) that has some unique properties compared to those of all others known in insects. Most strikingly, whereas all other known eukaryotic VGSCs are single protein subunits with four repeat domains expressed from a single gene, the aphid channel appears to be the product of two distinct genes and the protein assembled from two corresponding subunits, each containing two repeat domains. Aside from this, despite very good overall sequence homology with other insects, there are some important amino acid substitutions that impact on the pharmacology of the aphid VGSC. Preliminary attempts to express the aphid VGSC have failed and the characterization performed so far has come from the transfer of important substitutions to the well characterized *Drosophila* VGSC. However, the attempts to express the aphid VGSC have been far from exhaustive.

In this project we propose to pursue alternative methods successfully to express the aphid VGSC or chimeras with the *Drosophila* VGSC in a system suitable for electrophysiological characterization. RNA transcripts will be injected into *Xenopus* oocytes and then subjected to voltage-clamp recording to analyse ionic currents mediated by the VGSCs. This has been a standard test-bed for a wealth of other work performed in our laboratories and those of others to characterize insecticide actions on insect VGSCs. We will also attempt to dissect neurons from larger species of aphid (Nguyen et al 2012) and perform patch-clamp studies on these as a means of characterizing their VGSCs in situ. Characterization of both expressed and in situ aphid VGSCs will involve examination of their activation and inactivation properties, and their pharmacological profile with known VGSC modulators including insecticides known to target VGSCs.

The project will provide the student with training in a wide range of biological techniques including molecular biology techniques to produce RNA transcripts, introduce mutations and generate chimeric constructs; protein expression in *Xenopus* oocytes; electrophysiological techniques including voltage-clamp and patch-clamp; computer based techniques for analysis of nucleotide and amino acid sequences and for the measurement and analysis of electrophysiological/pharmacological data.

With the emergence of resistance amongst aphids to existing insecticidal compounds, including pyrethroids and more recently neonicotinoids, this work will present us with a new, unique target that may be exploited by the insecticide industry, and will provide a means to selectively target aphids while sparing non-pest insects.

Project title: Optimising photosynthetic productivity in novel vertical farming systems using LED technology and canopy imaging

Research theme: Global food Security **Location:** Biosciences

Rotation: 1,2 & 3

Contact: Dr Erik Murchie

Lab Rotation Project Description:

The student will actively set up growing systems and take measurements. Techniques acquired : (1) light interception and distribution in canopies, (2) LED technology (construction and control), hydroponic and horticultural cultivation. (3) Rapid and non-invasive measurement of photosynthesis , (4) Imaging plant canopies and light tracing.

The lab of Erik Murchie provides expertise in techniques for measuring photosynthesis, light responses and resource use efficiency. Dr Andrew French and the Centre for Plant Integrative Biology provides expertise in canopy imaging and light tracing.

Week 1: Light measurement in plant canopies using various PAR sensors, spectroradiometers and ceptometers and the rapid assessment of important parameters such as extinction coefficients.

Week 2-3: The student will set up a vertical hydroponic cultivation system and understand the principles by which the major resources are provided and controlled. This may be carried out at an industry partner vertical farming site e.g. Saturn Bioponics, Birmingham with whom the PI has an ongoing collaboration. LED technology will be explored via School of Electrical engineering.

Week 4-5: Photosynthesis measurement: infra red gas analysis and how it is the 'industry standard' used in field and lab to measure water loss and carbon gain in crop plants. The Licor 6400 XT system will be the focus. Modulated chlorophyll fluorescence techniques will be used to assess photosynthetic efficiency and photoprotection.

Week 6: Imaging techniques for plant canopies focussing on IR and stereo cameras. The use of Light tracing software and models of plant and canopy photosynthesis.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Word processing	**Computer Skills**	Basic processing for 3D image analysis
	Reference searches (e.g. web of science)		
Numeracy Skills	Use of spreadsheets, Basic statistical analysis for biologists	**Numeracy Skills**	Analysis of photosynthetic response curves, Light tracing in canopies,

			possibly mathematical models of photosynthesis
General Lab. Skills	Basic experimental design	**General Lab. Skills**	Gas exchange analysis, fluorescence analysis, growth analysis, horticultural skills, hydroponics
	Some basic experience with bench work or use of scientific equipment		Basic biochemistry.
Communication Reading/Writing/ Presenting Skills	Experience of dissertation writing. Preferably some experience of project presentations	**Communication Reading/Writing/ Presenting Skills**	Scientific paper presentation, writing report in the form of a paper, interaction with other post docs and PhD students

Linked PhD Project Outline:

Urban farming and 'vertical' growing systems have the potential to make a substantial contribution to global food security by providing local food with low food miles. Whilst arable cropping remains essential, growing conditions are rarely optimal. Vertical systems can impose unprecedented levels of control over resource application effectively pushing water, nutrient and radiation use efficiency close to their theoretical maxima. It is now essential to combine agricultural and plant science expertise with technical advances in horticulture.

Plants respond to spectral quality by altering morphology to create canopies that permit the plant to intercept light at high efficiency and drive photosynthesis to produce adequate yield. A key goal of all plant production is to maximize yield per unit energy input. Of the photosynthetically active regions of the spectrum, red and blue are energetically most efficient at driving photosynthesis while photoreceptors that include phytochrome and cryptochrome activate discrete developmental pathways to change leaf area, thickness and stem length. Light emitting diodes (LEDs) now offer cheap, cool, controllable sources of light that can selectively and quantitatively provide different wavelengths. This provides us with a new opportunity to manipulate the quality and quantity of produce for markets and meet demands of retailers, even to time deadlines. In the natural environment this has a financial cost to the industry (http://www.bbc.co.uk/news/uk-scotland-tayside-central-17413495). A recent workshop at Nottingham on vertical farming showed that light 'recipes' are required to optimize yield and quality.

Another key aspect is *architecture* of canopies: this determines light availability in complex vertical systems. It is necessary to understand how variation in light within complex 3-D environments limits and determines the level of photosynthesis i.e. photosynthesis and photoprotection should be

maximised but not saturated. Nottingham has a major BBSRC project developing techniques for rapid canopy imaging and calculating light distribution in arable crop canopies.

This project will seek new ways of improving photosynthetic productivity in vertical systems (glasshouse and growth room) by combining plant science with advances in light emitting diode technology (LEDs) and new techniques for canopy imaging being developed at Nottingham. Existing industry links in vertical farming will form a part of the programme.

Techniques will be physiological and biochemical supplemented with those from imaging and mathematical projects to predict optimal photosynthesis. Theories of photosynthetic and photoprotective optimisation will be tested. (1) Selected model plants (rice and Arabidopsis) and valuable horticultural species (lettuce and herbs) will be grown in existing LED based rooms (variable spectra) and glasshouses. (2) Using physiological techniques, optimal spectra ('recipes') identified for development and photosynthesis. Selected photosynthetic mutants will be used to test canopy optimisation theory. (3) Canopy imaging techniques developed in a parallel BBSRC project at Nottingham will be used to 'map' photosynthesis and predict productivity. (4) Partners developing vertical farming techniques will be involved in developing test systems combining glasshouse and supplementary LED lighting.

This project clearly falls within the Global Food Security remit of the DTP and also the BBSRC strategic priorities for crop science, agri systems approaches and living with environmental change.

Project title: PLASTID DYNAMICS AND TRACKING DURING GRAVITROPIC RESPONSES IN ROOTS

Research theme: Global Food Security

Location: SCHOOL OF BIOSCIENCES, SUTTON BONINGTON

Rotation: 1**,** 2 & 3

Contact: KEVIN PYKE

Lab Rotation Project Description:

During the six week rotation, the student carry out a detailed analysis of plastid dynamics and morphology in different cells within the root tip using confocal microscopy and the GFP plastid targeted lines available in Arabidopsis and wheat. Such a study will be a valuable underpinning exercise for commencement of the PhD project. Little is known of the variation in root plastid morphologies, numbers or dynamics in the different cells of the root tip including the columella and especially in crop species such as wheat. Learning state of the art confocal imaging techniques from the cohort of supervisors on this project will be a valuable skill. In addition, the student can initiate a crossing programme to building suitable genetic lines carrying fluorescent markers and mutations which will be key to the PhD programme.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Basic computer skills, ideally some experience in imaging and image analysis	**Computer Skills**	Confocal microscopy, inaging and image analysis, some aspects of modelling
Numeracy Skills	*Basic statistics*	**Numeracy Skills**	Modelling
General Lab. Skills	Record keeping, attention to detail,	**General Lab. Skills**	Plant cell biology, developmental biology of multicellular systems, use of fluorescence markers to track organelles, plant genetics

Communication Reading/Writing/ Presenting Skills	*Report-writing, communication skills with colleagues*	**Communication Reading/Writing/ Presenting Skills**	*Scientific paper-writing, Communicaion results to internal and outside audiences*

Linked PhD Project Outline:

Despite the importance of root gravitropism in plant growth, development and for the interaction with the soil environment, early events in gravity perception are still not well understood. A central part of the process of root gravitropism is the movement of specialised starch containing plastids, statoliths, in a distinct gravisensing cell type in the root tip; the columella cells. It is generally accepted that the sedimentation of these plastids triggers the signal transduction pathway that facilitates a change in the directionality of root growth in response to the gravitational field facilitated by differential auxin gradient across the root (Band et al 2012, Brunoud et al 2012). Recent analysis of statolith's behaviour during root bending in response to a gravistimulus gave rise to the tip-point hypothesis where statoliths fall back to the bottom of the cell only when the root reaches a threshold angle (Band et al. 2012), there by preventing over growth of the root in its graviresponse. The movement of statoliths may well be affected by the geometry of the basal cell wall of the columella cells, a feature which changes with different files and layers of columella cells.

This project will exploit transgenic lines of Arabidopsis, tomato and wheat with plastids containing GFP in order to visualise their real time movement and tracking of plastids in columella cells during graviresponse, and to explore how plastid morphology may function in this process, in particular the behaviour of stromules emanating from plastids in these cells. By crossing such lines with different Arabidopsis mutants with altered statolith morphology and starch content, the precise behaviour of these plastids can be explored during gravistimulation and during sedimentation dynamics.

A variety of Arabidopsis mutants are available in which either plastid division and/or morphology are altered (*arc3, arc5, arc6, arc12*). For example, Arabidopsis *arc6* or tomato suffulta3 mutants contain only a very few large plastids but it is not very clear how they affect gravi-sensing.

In addition, we intend to exploit Arabidopsis mutants (such as *chup1, cav1, jac1)* which perturb interaction between the plastid and the cytoskeletal elements which facilitate controlled dynamic movement.

Recently, a role for plastid TOC (translocon of outer membrane of chloroplasts) complexes is proposed for ARG1 mediated gravity signal transduction (Stanga et al 2009, *Plant Phys* **149**, 1896). In a screen for enhancers of arg1, mar1 and mar2

mutations were identified. MAR1 and MAR2 encode different components of the TOC complex. What the plastid import machinery has to do with gravitropism is not clear. There is some evidence that the pea homolog of MAR2 can bind to actin (Jouhet and Gray, 2009, *J Biol Chem* **284**, 19132). Thus mar mutants provide a useful tool to test a direct link between statolith sedimentation and the actin cytoskeleton.

Besides, auxin sensor DII Venus (Brunoud et al, 2012) will also be incorporated in all the above mutant background to test the effect of those mutations on the output of the gravity signal transduction pathway.

With the advancement of vertical confocal imaging, we are in a unique position to investigate to investigate the role of plastid morphology and number during gravi sensing in real time.

In addition, we will also investigate the mechanistic aspect of columella cell development. In Arabidopsis, columella cell layers in the primary root develop from columella initial. Using these novel plastid targeted GFP lines, we will investigate how plastid development occurs precisely between the potential columella cells leaving the root meristem and then become part of a standing wave of columella development. It is expected that the student will be able to extend these studies in tomato and wheat as well.

The student on this project will be able to use state of the art confocal imaging technology developed by members of CPIB over the last few years and associated with this project, alongside Kevin Pyke's long standing expertise in plastid biology. The student will also have the opportunity to work closely with modelers in adapting and using mathematical models to further analyse their experimental observations. As such this project will be part of a world leading research group, worthy of plant science at Nottingham.

Project title: Impact of long chain omega-3 enriched GM plant oils on the development of atherosclerosis

Research theme: Global Food Security

Location: SCHOOL OF BIOSCIENCES, SUTTON BONINGTON

Rotation: 1**,** 2 & 3

Contact: Professor Andrew Salter

Lab Rotation Project Description:

We currently have an archive of samples and tissue taken from mice fed a variety of oils, including different doses of fish oil. Using these samples students will be given specific training on the quantification of atherosclerotic lesions in aortic tissue. This will include basic histological techniques and computer-aided imaging methods.

They will be introduced to a range of techniques associated with measurements of lipids and lipoproteins including lipoprotein separation and the analysis of fatty acid composition by gas chromatography.

Previous work has also include qPCR array analysis of hepatic genes specifically associated with cholesterol and lipoprotein metabolism. Students will confirm results from these arrays by selecting genes for further analysis by qPCR and/or protein measurements by Western Blotting.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Microsoft Office	**Computer Skills**	Image Analysis
Numeracy Skills	Basic Numeracy/Statistics	**Numeracy Skills**	Statistical analysis of data (e.g. ANOVA, regression analysis)
General Lab. Skills	Basic pipetting, weighing etc	**General Lab. Skills**	Animal handling/diet prearation/designing nutritional studies
			Lipid and lipoprotein analysis

			Histology
			Molecular Biologu
Communication Reading/Writing/ Presenting Skills	Basic Literature searching	**Communication Reading/Writing/ Presenting Skills**	Interpretation of data,
	Scientific report writing		Presentation of data in both oral and written reports both within the University and at appropriate conferences

Linked PhD Project Outline:

Diets rich in n-3 long chain-polyunsaturated fatty acids (LC-PUFA), have the potential to protect against atherosclerotic cardiovascular disease (CVD). However, the only significant source at the moment is marine fish, and while the planet is currently producing around 1 million tonnes of fish oil/year, for every person on the planet to consume his or her recommended level of n-3 L-PUFA it would require 2.5 million tonnes/year. Therefore, there is an urgent need for a completely new source of n3 LC-PUFA, which is produced *de novo* by a more sustainable system whose output can increase as required to meet future demands. **The main hypothesis of this proposal is that novel plant oils produced from the transgenic oilseed crop, Camelina sativa, can directly replace marine fish oils as sources of n-3 LC-PUFA .**

Work at Rothamsted Research, funded by BBSRC has developed transgenic oil crops able to synthesize 15-20% of their fatty acid composition as EPA. Most recently plant oils containing EPA and DHA (14% & 10% respectively) have also been produced. Furthermore, while these oils are specifically enriched in n-3 LC PUFA, this is set against a very different background fatty acid composition to that of fish oil. Professor Johnathan Napier (Rothamsted Research) has agreed to make these oils available to us for this project. The aim is to assess the efficacy of, these novel sustainable plant sources of n-3 LC-PUFA in prevention of atherosclerotic vascular disease. These studies will be performed in a transgenic mouse model which is specifically vulnerable to the development of atherosclerosis.

Groups of mice will be fed atherogenic diets (rich in cholesterol and saturated fatty acids) supplemented with a variety of oils including native Camelina oil, n-3 LC-PUFA- plant oil and fish oil. At the end of the study they will be sacrificed and blood and tissues harvested. Plasma lipids, lipoproteins and other potential CVD risk factors will be measured. Tissue and red blood cell membrane fatty acid composition will be measured. Atherosclerosis will be determined in frozen sections of aorta by techniques well established in our laboratory. In addition, the impact of different oils on expression of a range of hepatic genes, for proteins known to regulate lipid and lipoprotein metabolism will be determined using qPCR techniques.

Over all the project will yield information on the efficacy of this novel, sustainable source of n-3 LC-PUFAs, which if incorporated into the human food chain may promote long term public health, and will broaden our understanding of the role n-3 LC-PUFA play in the prevention of CVD. As such the

proposal directly addresses 2 of the 3 major Strategic Priorities of the BBSRC, namely Food Security and Basic Bioscience Underpinning Health. In developing an alternative, sustainable source of n-3 LC-PUFA it specifically addresses the BBSRC remit of, 'minimising negative environmental impacts and preserving biodiversity and other ecosystem services' by protecting the marine environment from unsustainable exploitation. It also specifically advances our understanding of the role of these fatty acids in sustaining 'lifelong health and wellbeing'.

Project title: Environmental stresses: exploring molecular mechanisms conferring developmental robustness in agriculturally beneficial insects

Research theme: Global Food Security

Location: Biosciences

Rotation: 1 and 2 only

Contact: Dr Reinhard Stöger

Lab Rotation Project Description:

Validation of RNA-Seq data by quantitative RT-PCR / data analysis
The Mini-project will mainly be carried out in the Molecular Biology Suite / South Laboratory / Sutton Bonington Campus. The student will be able to choose from a list of candidate genes that have been identified to be stress-responsive in our previous work. The student will read background papers and provide reasons, why he/she wants to further analyse a particular gene. DNA sequence analysis programs will be used to design primers for q-RT-PCR.

Depending on the season of the year, worker bee larvae will be either directly collected from bee-hives located close to the Sutton Bonington Campus, or samples will be processed that have been stored in 4% paraformaldehyde. Nucleic acids (RNA / DNA) will be isolated from larval samples and subsequently quantified. The RNA samples will be used to measure the relative abundance of a given RNA species. Depending on the progress of the project and research interests of the student, additional experiments can and will be performed. For example, DNA methylation levels for the gene of interest will be measured, using bisulfite-sequencing techniques.

The Mini-project will provide training in the most common molecular biology techniques: nucleic acid isolation and manipulation – core lab skills. Further, data analysis and bioinformatics techniques will be applied to curated data sets.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills		**Computer Skills**	
X		X	
Numeracy Skills		**Numeracy Skills**	
X		X	
General Lab. Skills		**General Lab. Skills**	

		X	
Communication **Reading/Writing/** **Presenting Skills**		**Communication** **Reading/Writing/** **Presenting Skills**	X X X

Linked PhD Project Outline:

How do animals respond to recurrent and novel environmental stressors? This question addresses a fundamental problem in Biology and will increase our knowledge of aging processes affecting virtually all multicellular organisms. Yet, the question also addresses a pressing practical issue: global food security.

Global declines of pollinating insects, including the economically important honeybee (*Apis mellifera*) have been recorded in recent years. These declines have serious implications for agriculture and food security. A number of different environmental stressors, such as parasites, viruses, agricultural chemicals/intensification and limited food sources, are thought to be contributing to impaired health and fitness of pollinators.

We have recently generated large data sets derived from whole transcriptome sequencing (RNA-Seq) and LC/MS-based lipid profiling of honeybee larvae that were transiently exposed – in the field – to low levels of an insecticide widely used for crop protection. Our analysis shows a multifaceted, physiological response of larvae to stress early in life. We detected subtle differences in microRNA and mRNA signatures, along with altered lipid profiles. Gene enrichment analysis indicates utilisation of existing, partly Myc-regulated pathways, in response to this stressor. Among those affected are genes functioning in a lipid-carbohydrate-mitochondrial network. RNA levels for a cluster of genes encoding detoxifying P450 enzymes are elevated, with coordinated down-regulation of sugar-metabolising genes. Expression of an environmentally responsive, developmental gene is also reduced, suggesting diminished buffering and stability (canalisation) of the developmental program [*Manuscript submitted to PNAS (October 2012) / under review*].

The PhD project will be based on the findings of our field/laboratory experiments performed in honeybees. Developmental plasticity and susceptibility of larval development to environmental stressors appear to induce subtle physiological changes that – if they persist - may influence health later in life and thus, affect an entire bee colony. The PhD student will explore molecular mechanisms that lead to the multifaceted, physiological stress responses that we observed in honeybee larvae. Most of the genes and lipids with altered levels of abundance in stressed bee larvae are evolutionarily conserved. Thus, experiments and research design will not be restricted to honeybees (e.g. parasitoid wasps which are beneficial to agriculture), but may include other insect species as well as mammalian cells tissues and organisms.

The student will gain a wide variety of skills, necessary to succeed in academia and/or life sciences industry. The skill-set will include current molecular biology / epigenetic-detection techniques, combined

with training in bioinformatics – handling of large data sets, statistical analysis and gene annotation. That is, the student will benefit from bioinformatics-collaborations established through the on-going project (i.e. Alessandro Guffanti, Head, Bioinformatics, Genomnia; Dan Lawson at the European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton Coordinator Ensembl Genomes, insect genome initiative) and from training in insect biology and experimental design and data analysis from Dr Hardy.

This project covers multiple strategic research priorities established by the BBSRC:

- Global food security
- Animal health
- Basic bioscience underpinning health
- Lifelong health and wellbeing (Ageing research)

Exploiting new ways of working:

- Analysis and interrogation of next generation sequencing datasets
- Capturing variation and linking biological processes through to phenotypic traits

Project title: Identifying the developmental basis of enhanced production characteristics in broiler chickens

Research theme: Global Food Security

Location: Biosciences

Rotation: 1**,** 2 & 3

Contact: Dylan Sweetman

Lab Rotation Project Description:

- This project will identify differences in muscle and skeletal formation between broiler and layer strains of chicken during early embryo development. A key approach will be *in situ* hybridisation which allows spatio-temporal localisation of mRNA expression *in vivo*.
- The mini project will compare the expression patterns of selected muscle and skeletal marker genes in broiler and layer embryos. This will include probes already available in the laboratory, such as MyoD, as well as cloning other markers, such as Pax1. This will provide an initial characterisation of important determination genes in different lines of chicken.
- This will provide experience of a combination of molecular and embryological techniques including PCR, cloning plasmid constructs, in vitro transcription, embryo dissection, *in situ* hybridisation and sectioning. These approaches are widely used in many different areas and the training in basic techniques will be applicable in many different labs.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills		**Computer Skills**	Imaging
Numeracy Skills		**Numeracy Skills**	Molecular calculations
General Lab. Skills	Record keeping	**General Lab. Skills**	PCR / cloning
			In situ hybridisation
			Embryo dissection
Communication		**Communication**	Presenting at lab meetings

Reading/Writing/ Presenting Skills		**Reading/Writing/ Presenting Skills**	
			Journal club presentations

Linked PhD Project Outline:

Selective breeding of chickens for meat production has led to the development of the modern broiler chicken[1], characterised by greatly increased growth of muscle tissue to maximise production, particularly of breast meat (pectoralis major muscle)[2, 3]. However this enhanced meat production leads to severe welfare consequences for these animals as they develop skeletal abnormalities[4, 5] and the parent generation birds (broiler breeders) are fed a restricted diet[6]. According the UN Food and Agriculture Organisation, in 2009 over 18 billion chickens were bred for meat worldwide[7]. As the world population increases the question of how to achieve global food security becomes ever more important and it is inevitable that chickens, a critical part of the global food chain, will continue to be selected and bred for maximum efficiency in meat production. Therefore it is important to develop methods to facilitate muscle growth while minimising the negative effects on welfare apparent with current broiler strains. This will require a detailed understanding of the development of the affected organs in these animals. To work towards this aim we will investigate in detail the embryological events which contribute to enhanced muscle growth in these animals.

The genetic programme leading to formation of muscle cells is well characterised. Myoblasts, proliferative precursors of mature muscle fibres, express the Pax3 and Pax7 transcription factors[8]. Signals from surrounding tissues then induce the expression of muscle specific genes in these cells such as members of the MRF family of transcription factors including Myf5 and MyoD[9]. MRFs act as master regulators of muscle cell development and initiate the expression of a suite of genes that commit the cell to myogenesis[10, 11]. In contrast precursors of the skeletal system express genes such as Pax1 and Sox9, both of which drive cells towards a skeletal fate.

Objectives:
The overall aim of the project is to analyse the early development of the muscular and skeletal systems of broiler chickens using a variety of techniques and molecular markers. The specific objectives are:
Objective 1: Mapping early musculoskeletal development in broiler chickens.
Objective 2: Production of a detailed developmental map of pectoralis major muscle formation.
Objective 3: Comparison of intrinsic and extrinsic differences between muscle and skeletal cells derived from layer and broiler strains.
Objective 4: Identifying differentially regulated genes in broiler pectoral muscle development.

The strains of chicken used in this project fall into two classes. The broiler strains used will be the Ross 308 and Ross 708 broilers generated and supplied by Aviagen (http://en.aviagen.com/) while the layer strains will be Dekalb white, a hybrid layer strain obtained from Henry Stewart & Co

(www.medeggs.com) and a strain with ubiquitous GFP expression generated at the Roslin institute[12]. The Ross 308 and Ross 708 strains are widely used internationally for meat production and have distinct growth characteristics with Ross 708 chickens producing significantly more breast muscle than Ross 308 chickens[13]. Using both of these strains will provide critical insights into how embryonic changes relate to different production characteristics.

Project title: The role of long non protein coding RNA in lateral root development

Research theme: Global Food Security

Location: Biosciences

Rotation: 1**,** 2 & 3

Contact: DR RANJAN SWARUP

Lab Rotation Project Description:

Student choosing to do the six week rotation will learn basic bioinformatics techniques to identify targets for long npcRNAs. They will also learn how to use publically available resources to investigate gene expression. Besides bioinformatics, the students will gain practical experience in several routine and specialised techniques in Molecular Biology and Cell Biology as listed below-

- In vitro plant culture
- Root gravitropic assays and lateral root development
- Automated image acquisition and imaging
- Live fluorescent imaging
- *In situ* immunolocalisation

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	Basic computer skills	**Computer Skills**	Student will be able to use specialised softwares and web based resources for data mining, analysis and interpretation.
Numeracy Skills	Basic numeracy Skills with some knowledge of statistics	**Numeracy Skills**	They will learn several statistical techniques to analyse and validate date.

General Lab. Skills	Basic lab skills including good record keeping.	**General Lab. Skills**	Generic training for several routine and specialised lab techniques
Communication Reading/Writing/ Presenting Skills	Basic communication and reading skills will be important whereas basic writing skills will be desirable.	**Communication Reading/Writing/ Presenting Skills**	It is expected that the students will improve all these skills.

Linked PhD Project Outline:

Regulatory npcRNAs range from small RNAs to very large transcripts (or long npcRNAs) and play diverse functions in development and/or environmental stress responses (1-3). Small RNAs (smRNAs) of 20-40nt are capable to mediate transcriptional (TGS) and post-transcriptional (PTGS) gene silencing. They act by degrading mRNA targets, repressing their translation or modifying chromatin through homologous interaction with target loci and include microRNA (miRNA) and short-interfering RNA (siRNA) but also tasiRNAs (trans-acting siRNAs) and nat-siRNAs (natural antisense mediated siRNAs).

In contrast to small RNAs, much less is known about the large population of long npcRNAs. In plants, they have been implicated in the regulation of mRNA transcription, localisation and translation during different processes as nodulation, flowering time and flower development, abiotic stress responses and sex chromosome-specific expression. Certain long npcRNAs inhibit the action of small RNAs, may act as cargo molecules required for subcellular protein localization or for recruitment of polycomb repressive complexes (4-5).

As an ongoing collaboration between French and UK laboratories, we are investigating the role of npcRNA during lateral root development. Root architecture is crucial for efficient uptake of resources from the soil particularly under conditions of biotic and abiotic stresses and low resource availability (6). A greater understanding of npcRNAs and their role in regulating root architectural traits like lateral root development (7) are likely to identify key regulators that may provide the tools to design novel strategies for future crop improvement programmes.

Using high throughput RNAseq approach, we have recently done a detailed expression profiling during different stages (initiation, meristem formation, pre-emergence and emergence) of lateral root development. Bioinformatic studies have identified over 160 long npcRNA in this unique dataset that cluster into 8 different groups.

This studentship proposal will focus specifically on the role of long npcRNA in lateral root development.

The key objectives of the current proposal are-

a) Bioinformatic analysis

Based on their sequence, potential target genes will be defined based on complementary prediction programs (potential antisense RNA) or in their co-regulated expression with flanking mRNAs (chromatin-related action of regulatory long npcRNAs)

b) *Expression patterns of long npcRNAs during LR formation*

Several long npcRNA candidates will be selected for detailed expression profiling during lateral root development using qPCR based approaches.

c) *Molecular Studies*: Detailed investigation including expression and sub cellular localisation of selected target genes and long npcRNAs will then be done to further investigate their role in lateral root development. Their transcriptional response upon environmental stresses, nutrition deficiencies or hormone treatment (mainly auxins, cytokinins and ABA) will be tested to identify potential regulation and cross talks with known signalling pathways linked to lateral root development.

This research programme combines innovative genomic, bioinformatics, cell biological and genetic approaches to investigate the role of long npcRNA in lateral root development and will deliver fundamental new insights into the molecular regulation of lateral root development for translation into crop breeding programmes.

Relevance:

With the ever increasing world population, food security and sustainable agriculture are two of the major challenges for the future crop improvement programmes (8-10). This project is relevant to the BBSRC Strategic Priorities on Global Food Security.

References:

[1]Wirth & Crespi (2009) *RNA Biol* **6:2**, 161-164. [2]Sunkar *et al* (2007) *Trends Plant Sci,* **12***,* 301-309.[3]Urano *et al* (2010) *Curr Opin Plant Biol,***13,** 132-138. [4]Wierzbicki (2012) *Curr Opin Plant Biol* **15**, 1–6. [5]Jouannet and Crespi (2010) *In: "Long non-coding RNAs" Series in Progress of Mol Cell Biol.* D Ugarkovic (Ed). Springer Verlag. [6]Lynch (2007) *Aust. J. Bot.* **55**, 493–512. [7]Swarup et al 2008 *Nature Cell Biology,* **10**:946-954. [8]Royal Society (2009) ISBN: 978-0-85403-784-1. [9]IAASTD report (2009): Agriculture at a crossroads. [10]BIS, Foresight. The future of food and farming (2011).

Project title: Identification of alternative dwarfing genes for bread wheat improvement

Research theme: Global Food security

Location: Rothamsted Research

Rotation: 1, 2 & 3

Contact: Steve Thomas

Lab Rotation Project Description:

The main aim of a mini-project will be to prepare and test by inoculating onto wheat plants a set of VIGS constructs, which will be used as appropriate controls in the main project on silencing and identification of alternative height-reducing genes in wheat. One of the genes that can be used as a control is *Rht-1*, a well-known dwarfing gene utilised in wheat breeding worldwide. The second control gene is *GA20ox1*, encoding a key oxidase enzyme involved in GA biosynthesis. We have mutant lines containing loss-of-function alleles for all three *GA20ox1* homeoeologues for comparison.

This mini project should give the true flavour of the main project, and the student will have an opportunity to learn a range of useful lab techniques including:

- ✓ Predicting target specificity and silencing efficiency for each plant gene fragment selected for cloning into a VIGS vector using specialist software;
- ✓ Design and execution of TILLING assays for mutation scanning.
- ✓ PCR primer design using specialist software;
- ✓ Purification of RNA from plants;
- ✓ Basics of RT-PCR;
- ✓ Analysing DNA samples by gel-electrophoresis;
- ✓ Cloning of DNA using a ligation-independent cloning procedure;
- ✓ Transformation *E. coli*;
- ✓ Identification of recombinant *E. coli* colonies using colony-PCR;
- ✓ Purification of plasmid DNA;
- ✓ Transformation of *Agrobacterium tumefaciens*;
- ✓ Inoculation of plants with cloned viruses using Agro-infiltration procedure;
- ✓ Inoculation of plants with viruses using mechanical rub-inoculation onto the leaves;
- ✓ Assessing plant phenotypes resulting from VIGS and TILLING.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills		**Computer Skills**	
How to use Microsoft Word		How to use Si-Fi software for off-target silencing prediction	How to use Primer3 software for primer design
		How to do BLAST similarity search	

Numeracy Skills		**Numeracy Skills**	
Comprehending fundamental mathematics	How to use the Microsoft EXCEL spreadsheet	Presentation of data in graphs, charts, etc.	
How to calculate molarities, dilution factors, etc.			
General Lab. Skills		**General Lab. Skills**	
Pipetting	Reagent and buffer preparation	RNA Extraction, RT-PCR and cloning	Biosafety in a laboratory
Weighing	Instruments handling (centrifuge, spectrophotometer)	Horizontal gel electrophoresis, and gel documentation	Basic bioinformatics
Sterile technique and media production	Use of digital camera for documentation	Transformations, selective media and plasmid minipreps	Basic plant virology techniques and experience of VIGS gene silencing procedures
Communication Reading/Writing/ Presenting Skills		**Communication Reading/Writing/ Presenting Skills**	
How to give a talk using PowerPoint presentation	Basic elements of writing and spelling	Accurate recording and record keeping	Use of images, tables, and graphs in reports

Linked PhD Project Outline:

BACKGROUND: Modification of shoot architecture has been a major factor in increasing wheat yields, particularly since the late 1950s when the introduction of the reduced height (*Rht*) genes was critical to the success of the Green Revolution. However, although over 80% of wheat cultivars currently contain *Rht* dwarfing alleles, their presence can have adverse effects, such as compromising seedling establishment under dry conditions and grain set at high temperatures. Consequently, there is scope for broadening the genetic base for plant height and for further improvements in yield potential by exploring alternative dwarfing genes.

Rht-1 encodes DELLA domain protein involved in gibberellin (GA) signalling. Furthermore, GA biosynthesis is targeted by most commonly-used plant growth retardants. There is thus substantial evidence that GAs are important hormones in determining plant height and can be manipulated without affecting other characteristics. The GA biosynthetic and signalling pathways have been well characterised in Arabidopsis, rice and maize. Several recessive GA- dependent semi-dwarf mutants with improved grain yield have been identified in rice, and the corresponding genes have been isolated. The recently released draft genome assembly and private RNA-seq data for various wheat tissues and developmental stages have allowed identification of wheat genes involved in GA biosynthesis and signalling. The function of

most of the wheat GA-biosynthetic genes has been confirmed by heterologous expression. Based on the current knowledge and own unpublished data we have shortlisted approximately 6 gene candidates for testing their effectiveness in height control.

WORK PROGRAMME*:* The aim of this project is to use reverse genetic approaches to establish whether these 6 candidate GA biosynthesis and signalling genes are potential targets for identifying novel semi-dwarfing alleles that can improve wheat grain yields. To rapidly establish which the most promising genes are, the project will take advantage of VIGS (Virus-induced gene silencing), a novel but proven reverse genetic technology that has recently been established at Rothamsted (Lee, Hammond-Kosack, Kanyuka 2012). The phenotype of plants infected with virus containing short fragments of the target gene often resembles that of a null or reduced function mutant. The student will use VIGS to test the influence of each gene on stem architecture and grain set. Phenotypes of the silenced plants will be characterised in detail and plant height and various fertility and yield associated parameters will be measured. Once the most promising height-reducing genes have been identified, their potential as novel targets for wheat improvement will be further explored through identification of EMS-induced mutants using TILLING (Targeting Induced Local Lesions in Genomes).

TRAINING: The student will receive training in key molecular biology and plant virology techniques, as well as TILLING, plant handling and the use of biological containment. There will also be scope for the student to learn basics of bioinformatics and to propose additional genes for functional analyses. The student will gain experience of wheat anatomy and development, including reproductive development through the collaboration with Nottingham.

STRATEGIC RELEVANCE: The project fits well within the DTP theme of Global Food Security, which is also a BBSRC strategic priority. It also fits firmly in the 2020 wheat® ISP.

Project title: Analysis of the mechanism regulating secondary thickening in the anther: a tool to control secondary thickening and fertility in crops

Research theme: Global Food Security

Location: Sutton Bonington Campuses (Plant and Crop Sciences) and University Park (Maths).

Rotation: 1**,** 2 & 3

Contact: Prof Zoe Wilson

Lab Rotation Project Description:

The miniproject will combine biological lab work and mathematical modelling. The former will involve the analysis of a number of transcription factors, which are expressed in the anther during dehiscence. Available array data about the expression patterns of these genes will be used to select key factors for analysis. Bioinformatic analysis will be used to identify and confirm these new (currently uncharacterised) players during Arabidopsis anther dehiscence. We know that there are uncharacterised factors from our current model, however are not sure of their identity, although a number of possible targets have been identified.

Their expression pattern will then be confirmed by RT-PCR analysis using staged anther tissues and inducible expression in floral tissues. This will then be expanded to include a detailed time-series analysis of anther development and ChIP-PCR analysis. Subsequently localisation of key protein fusions will be conducted by confocal microscopy to provide the spatial resolution (time-permitting). Insertional mutants containing mutations in the relevant genes will be analysed for alterations in dehiscence. These data will then be used in guiding the development of a preliminary mathematical model describing induction of secondary thickening in the anther. This will build on our recent modelling work of dehiscence and anther opening. This will incorporate the data generated from our previous analyses and set the stage for its inclusion in the anther dehiscence opening model.

Lab Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills	*Yes*	**Computer Skills**	*Yes*
Numeracy Skills	*Yes*	**Numeracy Skills**	*Yes*
General Lab. Skills	*None, but an interest in wet lab work is essential*	**General Lab. Skills**	
	None, but an interest in	*RNA extraction and*	

	mathematical modelling is essential	*analysis*	
		Expression analysis	
		Confocal microscopy	
		Mathematical modelling	
Communication Reading/Writing/ Presenting Skills	*Report writing*	**Communication Reading/Writing/ Presenting Skills**	*Scientific paper writing*
	Ability to carry out literature searches		*Ability to write a literature review*
	Ability to communicate to colleagues		*Ability to communicate to internal and outside audiences*

Linked PhD Project Outline:

Understanding the processes that underlie pollen release is a prime target for controlling fertility to enable selective breeding and the efficient production of hybrid crops, which typically produce 20-30 times more yield than inbreeding alternatives. These are targets that would aid strategies for increasing crop yield with the goal of sustainable global food security. Pollen release requires anther opening, which involves changes in the biomechanical properties of the anther wall (Wilson et al, 2011): this process requires selective dehydration, combined with changes in the biochemical properties of the anther cell walls.

Anther opening, and therefore pollen release and male fertility, is controlled by the presence of secondary thickening in the anther endothecium. The production of this thickening is regulated by a number of transcription factors, which show tissue specific and temporal expression. We have identified a number of factors that are involved in the process of induction of thickening, however there are also other uncharacterised factors involved in the network. The later steps of the pathway seem to be conserved in secondary thickening pathways in different tissues and in other plant species. However the initial stages of induction show tissue and stage specific regulation. These are therefore valuable tools to both understand and subsequently manipulate thickening for control of anther opening, but also secondary thickening pathways in different tissues and plant species.

The regulatory network for induction of thickening appears to be non-linear and also to involve a number of regulatory loops. This network will be modelled mathematically using our currently available information on key regulatory genes in this pathway. This will be used to make predictions about the expected profiles of the unknown factors in the pathway. The possible identity of these factors will then be identified using network inference and available expression data. The model and the identity of these factors will be subsequently confirmed experimentally using up and down regulation of the various components in the network and gene expression analysis.

This project will combine a lab-based analysis of the processes of the gene expression pathways associated with dehiscence (using a range of Arabidopsis mutants that have alterations in the secondary thickening properties of the anther wall) with partial-differential-equation-based mathematical modelling, which will need to encompass a variety of complex and multiscale processes. Together, this systems-biology approach will enable a greater understanding of the dehiscence process combined with providing the opportunities for manipulating the dehiscence process for controlling plant fertility.

Project title: **Characterisation of major antigen gene families in trypanosomes.**

Research theme: Global Food Security

Location: QMC

Rotation: 1**,** 2 & 3

Contact: Dr Bill Wickstead

Lab Rotation Project Description:

In the mini-project, students will undertake the first steps in isolation of metacyclic antigenic proteins from single strains of species causing AAT. Students will grow insect-form cells of *T. congolense* and *T. vivax* in axenic culture alongside *T. brucei* control lines. They will induce differentiation and purify metacyclic parasites by column chromatography. Subsequently, hypotonic lysis and GPI-phospholipase C-induced cleavage will be used to isolate semi-pure fractions of major surface proteins, which will be analysed by gel electrophoresis and lectin binding. Depending on time constraints, students will also isolate RNA from metacyclic and bloodstream forms and make cDNA, which will be a useful resource later on in the project for assessing expression levels of various surface proteins.

As well as introducing the student to some of the scientific concepts behind the work, this mini-project will provide training in aseptic cell culture and handling, parasite differentiation, basic biochemical purification methods, analysis of proteins on SDS-PAGE gels and RNA isolation. If successful, the student will have material which they can use productively in the main project, for instance moving on to protein identification by mass spectrometry. They will also have experienced several molecular techniques which would be useful in a large number of laboratories.

Rotation Skills Matrix:

Skills you need		**Skills you'll develop**	
Computer Skills		**Computer Skills**	
Basics in word processing, data analysis, etc.		Bioinformatic analysis of protein structure	
		Understanding of parasite genomic databases and sequence datasets	
Numeracy Skills		**Numeracy Skills**	
Maths for basic biology		Quantitative skills for molecular biology	
Ability to work quantitatively			
General Lab. Skills		**General Lab. Skills**	
Ability to work with precision and accuracy		Axenic parasite culture	
Good record keeping		Protein and RNA isolation and analysis techniques	
		Biochemical purification of proteins	

		Production of cDNA	
Communication Reading/Writing/ Presenting Skills		**Communication Reading/Writing/ Presenting Skills**	
		Presentation of scientific data to specialist audience	
		Mining and understanding scientific literature	

Linked PhD Project Outline:

African trypanosomes are protozoan parasites of the blood that cause the human disease of "sleeping sickness" and a severe wasting disease of domestic livestock in sub-Saharan regions. Animal African Trypanosomiasis (AAT) is widespread across 40 of the world's poorest countries. It is estimated that 50 million cattle and 70 million small ruminants are at risk, costing the continent ~$3 billion per annum.

AAT is caused by several trypanosome species. All parasitize the bloodstream in an exclusively extracellular form, evading the immune system through a system of antigenic variation of their major surface proteins. The human parasite *Trypanosoma brucei* has been widely studied as a model for this. In *T. brucei*, antigenic variation depends on a specialised genome architecture including ~100 small linear chromosomes and numerous expression sites. However, the greatest burden of AAT disease is caused by *T. congolense* and *T. vivax* and for these species little is known about the major antigenic protein families or genome architecture.

This project will use recent advances in the manipulation of AAT parasites alongside established techniques to analyse the major antigenic protein families in these species and their genomic context. It will also identify the subset of genes expressed early in infection that might have potential for parasite control by immunisation.

Analysis of antigen gene family: To understand antigenic variation in *T. congolense* and *T. vivax* it is necessary to identify the repertoire and the structure of the gene family. Using the recent draft genome sequences, major antigen genes will be analysed for family structure and conserved DNA features such as promoters, which will be tested for activity by marker gene integration. "Next-gen" sequencing of isolated minichromosomal DNA will identify the gene repertoire on these chromosomes and features specific to this set. Representative surface proteins will be expressed in bacteria for structural determination.

Identification of metacyclic major surface antigens: African trypanosomes are transmitted by fly bite. The final stage of development in the fly is a metacyclic form that is pre-adapted to survival in the mammalian host. Which surface proteins are displayed on the metacyclics of *T. congolense* and *T. vivax*, is unknown. Utilising the ability to differentiate these organisms in culture, major metacyclic antigen proteins will be identified by biochemical purification. Blotting of pulsed-field gels will be used to determine the genomic location of metacyclic expression sites, which will be analysed to identify conserved sequence features as above. By testing a number of different strains it will be possible to identify commonality in gene sets. Should parasites express a limited set of proteins at this stage then these have potential for the production of mixed-antigen vaccines against a protein family that is otherwise extremely unpromising for immunisation.

The work in this project is within the BBSRC remit for funding of basic biological research into animal disease and is closely aligned to the current strategic priorities on Animal health research and Livestock production. For these reasons, it addresses the DTP themes on Global Food Security and also Molecules, Cells and Organisms.