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Presentation and Poster Titles and Abstracts

BioSS

Katie Emelianova

Mining *Begonia* genomic resources to find candidate genes for ecological diversification

[Talk 6]

Begonia is one of the most diverse genera on the planet, with a species count approaching 2000 and a distribution across tropics in South America, Africa and South East Asia.

The genus has occupied a vast range of niches; many highly variable growth forms can be found across the distribution, and species exhibit very diverse morphologies, even in closely related species.

Much work has been done in the past to try to elucidate the source of *Begonia*'s diversity, including population genetic, cytological and phylogenetic approaches.

The generation of next generation sequencing (NGS) resources has revealed a putative whole genome duplication (WGD) event in the evolutionary history of *Begonia*, which has prompted an interest in investigating the impact gene and genome duplication has had on the diversification of *Begonia*.

Two closely related but morphologically and ecologically divergent species, *B. conchifolia* and *B. plebeja* have been chosen to use in a candidate gene study to identify genes which support a hypothesis of diversification via duplication.

Using multi-tissue RNA-seq data from *B. conchifolia* and *B. plebeja,* and the draft genome of *B. conchifolia*, a bioinformatics pipeline was used to mine the sequence data for candidate genes which may have been important in the rapid adaptation of different *Begonia* species to different environments and the resulting ecological diversity seen in the genus today.

The results of the candidate gene study will be presented, and the results and future perspectives discussed.

JHI

Ashley Gorman

The role of seedbank composition and biotic function in vulnerable erosive agroecosystems

[Talk 4]

Accelerated soil erosion degrades the physical and biogeochemical functioning of arable soils threatening sustainable food production. The impacts of soil erosion on the state and vitality of the seedbank of arable weeds is largely overlooked. This is despite the expected increase in frequency of extreme storm events resulting from climate change increasing the severity of soil erosion. As with mineral particles, there is selective erosion of seeds with detachment, transport and deposition depending on seed size, shape, presence of appendages and the ability to secrete mucilage. Differential mobility capacity therefore depends on seed morphology. The potential functions and ecological roles of myxospermous seeds have been reviewed recently (Yang et al. 2012), however quantitative studies are rare and limited to a single or a few model species (Deng et al. 2012, 2015, Engelbrecht et al. 2014, Garcia-Fayos et al. 2013). Due to the adhesive nature of mucilage, physical alterations could occur stabilising soil structure and enhancing water retention but the existence of a mucilage threshold beyond which soil properties are altered is unknown. Therefore, this study aims to investigate the extent to which specific species and community assemblages are capable of preventing their removal and soil particle transport following erosion events.

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Garcia-Fayos, P, M Engelbrecht, E Bochet (2013) Post-dispersal seed anchorage to soil in semiarid plant communities, a test of the hypothesis of Ellner and Shmida. Plant Ecol 214:941–952

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Jennifer Slater

Do plants have internet? Interplant communication via common mycorrhizal networks

[Talk 10]

When insect herbivores attack plants, they can signal to neighbouring plants via newly discovered belowground common mycorrhizal networks (CMNs). These networks are formed when the hyphae of arbuscular mycorrhizal fungi, associated symbiotically with plant roots, fuse together and form an underground connection between the root systems of different plants. This interplant signalling route can allow defence signals from an infested plant to be communicated to an uninfested plant, inducing a defence response in the uninfested plant prior to being attacked. However, can this mechanism of interplant signalling occur between any types of plant or only between plants of the same species? The hypothesis that interplant signalling is more likely to occur between closely related plant species was tested using legumes belonging to different cultivars, species, genera and families. By growing these plant types together in the presence of a CMN, attractiveness of plants to pea aphids (*Acyrthosiphon pisum*) and their natural enemies (the parasitoid wasp *Aphidius ervi*) could be quantified, and the composition of VOCs emitted by each plant type characterised. These data are used to assess the prediction that VOC blends are more similar between closely related plants, and that uninfested plants connected to infested plants by underground CMN are more likely to repel aphids and attract natural enemies if the plants are closely related. This research could be important when considering plant defence in mixed-cultivar and intercropped systems and reducing the need for pesticides.

Joseph Palmer

Pathogenic Clostridia in the Environment

[Talk 1]

This project aims to understand the abundance and behaviour of pathogenic Clostridia in soils and sediments. A new, high-throughput molecular protocol has been designed to allow the differential detection of multiple target pathogens from environmental samples. This protocol will initially be used to screen agricultural soils for Clostridial contamination; with pathogen abundance quantified using quantitative (q) PCR. Differences in pathogen abundance can be linked to soil, environmental or land-management factors. Key growth factors will be manipulated using soil microcosm studies. The resulting data will allow a better understanding of Clostridial pathogens in soil, and the underlying mechanisms that influence their survival and growth. In turn, this will help land-owners adopt risk-aversion strategies to minimise risk to human and livestock health. Additionally, a thorough taxonomic review of the *Clostridium* genus has been conducted using a rigorous phylogenetic approach, utilising newly-available genomic sequences. Our results support the reassignment of many former *Clostridium* spp., including the pathogenic *C. difficile* and *C. sordellii,* to alternate genera. Interestingly, our results also justify further reassignment of other species, such as *C. aceticum,* to existing, or potentially new genera.

Shona Strachan
The Search for Nematode Resistance: Mapping the *H2* Gene
[Talk 7]
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Plant parasitic nematodes (PPNs) of the genera *Meloidogyne*, *Heterodera*, and *Globodera* are the most economically important PPNs. The potato cyst nematodes *Globodera rostochiensis* and *G. pallida* cause economic losses in excess of £45 million per annum in Britain alone through crop losses and implementation of nematicidal pest control strategies.

The *H1* gene from *Solanum tuberosum* ssp. *andigena* CPC 1673 confers almost complete resistance to *G. rostochiensis* pathtotype Ro1 and Ro4, and its integration into commercial cultivars has been effective in reducing the threat from *G. rostochiensis* in Britain. This however has led to a shift in species prevalence, making *G. pallida* the UK’s main PCN problem. *G. pallida* populations are more genetically diverse than *G. rostochiensis*, and as a result no single resistance gene is likely to be both effective and durable against all British populations. The major resistance gene *H2* from *S. multidissectum* confers a high level of resistance against the Pa1 pathotype of *G. pallida* and partial resistance to the Pa2/3 pathotypes.

Using material containing the *H2* gene, isolated from a cross between a susceptible Picasso and resistant P55/7 cultivar, GenSeq gene enrichment was carried out. SNP (single nucleotide polymorphism) analysis of resistance and susceptible samples was performed, revealing that the largest number of informative SNPs is on chromosome 5. For each of the SNPs on chromosome 5 a set of KASP (Kompetitive Allele Specific PCR) markers were synthesised and used to genotypically analyse the samples. Using the KASP markers, the *H2* gene has been mapped to 4.7Mb on chromosome 5. Outlined here are the experiments and methods used to map the *H2* resistance gene using the potato reference genome.

Moredun

Rebecca McLean
Interactions between ovine lentiviral vaccine vectors and primary cells *in vitro*
[Talk 2 & Poster 4]
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There are many diseases of livestock for which we do not yet have effective vaccines. This is partly due to the challenges of novel antigen discovery and suitable delivery. Recently, viral vectors have risen to prominence as candidates for vaccine delivery to generate cellular and humoral immune responses. Of these viral candidates, lentiviruses are attractive as they have the ability to elicit sustained antigen expression in non-dividing antigen presenting cells1. Here, we describe the development of vaccine vectors derived from ovine lentivirus and their interactions with primary cells *in vitro*.

To increase the safety profile of the vector, viral enhancers in the 3'-long terminal repeat (LTR) were removed to produce self-inactivating vectors, decreasing the probability of the generation of replication-competent virus2. In addition, point mutations were introduced into the integrase protein to minimise the possibility of insertional mutagenesis3.

These self-inactivating, integration deficient vectors efficiently infect ovine dendritic cells cultured *in vitro.* Once infected, these dendritic cells undergo apoptosis, with further analysis suggesting this is triggered by the sensing of viral DNA. Furthermore, immature dendritic cells can phagocytose these apoptotic bodies, potentially leading to cross-presentation of peptides derived from the transgene to CD4+ and CD8+ T-cells.

We have created a novel, self-inactivating, integration deficient ovine lentiviral vector which retains the ability to express transgenes *in vitro*. Infected cells sensing viral DNA undergo apoptosis; this effect can be seen in commonly used vaccine adjuvants such as alum4, suggesting this mechanism could be beneficial for our vaccine delivery system. Functional analysis of this system is currently underway.

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Lynsey Melville

Benzimidazole resistance in *Nematodirus battus*

[Talk 11]

Benzimidazole (BZ) resistance has been identified in almost all of the economically important ovine gastrointestinal nematode (GIN) species and is now widespread in the UK and worldwide. *Nematodirus battus* was believed to be refractory to developing resistance to BZ compounds as efficacy has remained high in this species globally for over 50 years. Due to the high safety index and efficacy of BZ compounds, these drugs are commonly administered early in the UK grazing season to control *N. battus* infections in young lambs. The first case of BZ-resistance in this species was identified in sheep from a farm near Kendal in Northern England in 2010. Samples were submitted to Animal and Plant Health Agency following suspected treatment failure and anthelmintic resistance was later confirmed by controlled dose and slaughter test. Further investigation of the isolate identified that the single nucleotide polymorphism (F200Y) in the β-tubulin gene, commonly associated with BZ resistance in other GIN, was highly important in conferring resistance in this species. The aim of this project is to assess the prevalence of the BZ-resistant allele in *N. battus* populations in the UK and to investigate potential risk factors which may have attributed to the development and dissemination of anthelmintic resistance in this species.

A *N. battus*-specific pyrosequencing assay for genotyping of β-tubulin F200Y SNP has been developed to investigate the prevalence of this mutation in *N. battus* populations. Faecal samples were obtained from 146 flocks from across mainland UK. 79 populations were analysed from Scotland and 67 populations from England and Wales, 30 individual eggs and/or larvae were genotyped per population. Results show an overall low prevalence (±SEM) of the resistant allele (r-allele) in all regions; 3%±1. The r-allele was identified in 37 of the 146 populations tested, ranging from 2-97% allele frequency. Scottish farms were found to have a lower r-allele frequency than English and Welsh farms tested; 1%±0.4 and 5%±2 respectively. Several focal regions of increased r-allele frequency were also identified including the area surrounding the farm from which the primary BZ-resistant population was isolated.

Unlike other GIN species, where most development to infective larvae occurs on pasture, *N. battus* completes its development to an infective stage within the egg; historically, *N. battus* was believed to require a period of chilling followed by a mean day/night temperature above 10^{o}C in order to hatch. Recent reports suggest that *N. battus* is now being observed for longer periods throughout the grazing season with a secondary increase of infective larvae on pasture in autumn. Alteration of the life history of *N. battus* was identified as a possible risk factor in the development of resistance due to increased selection pressure as anthelmintic treatments are administered throughout the year to control other GIN species. The in vitro ability of *N. battus* eggs to hatch with and without a chill stimulus was assessed using 68 populations, possessing varying BZ-resistant allele frequencies, collected during 2015. Aliquots of developed eggs (i.e. eggs containing a formed third stage larvae within the shell) were subjected to different hatching conditions. One sub-population received a chill before incubation at the optimal hatching temperature, the other was placed directly at the hatching temperature and hatch rates were calculated. Results indicated that a significant proportion of *N. battus* eggs were able to hatch in the absence of a cold stimulus; mean 13%, ranging from 0-81%. Further analysis is required to investigate a possible link between hatching profile and BZ-resistance and to investigate the mechanism which controls hatching in this species.

N. battus is a changing parasite which requires further characterisation. BZ-resistance in this species currently appears to be at a low frequency however, the presence of the mutation in a significant proportion of populations tested may indicate the potential for resistant allele frequency to expand in the future. The variability in hatching profiles observed suggests that the epidemiology of this nematode species may also be changing which could impact upon the development and dissemination of resistance. Further characterisation of *N. battus* will allow for a better understanding of anthelmintic resistance and will inform the development of future best practice advice for the farming community.

RINH

Jennifer Harbottle

The environmental stress response as a target for therapeutic intervention

[Talk 8 & Poster 1]

Improved health span and lifespan extension in a wide range of species are associated with the induction of the environmental stress response (ESR) regulated by the transcription factor Nrf2.

Phytochemicals which stimulate the ESR upregulate endogenous cytoprotective mechanisms and contribute towards delaying the onset of age-related disease, therefore could form part of therapeutic interventions to promote healthy ageing in humans. Thus it is of interest to identify potent inducers of the Nrf2 pathway, and to better understand how long term exposure to phytochemicals impacts upon Nrf2-target gene expression. To achieve this, a cell-based reporter system was established in human hepatocellular carcinoma HepG2 cells using a luciferase reporter gene under the control of the NQO1 promoter (a downstream target of Nrf2), and validated using sulforaphane, a potent isothiocyanate found in cruciferous vegetables. The stably transfected cell line HepG2 C1 was subsequently used to screen natural product libraries, and promising compounds were further characterised and shown to stimulate endogenous ESR gene and protein expression. The bioluminescent reporter system thus allows rapid, *in vitro* identification of novel chemicals that have the potential to improve health span through activation of the ESR. An alternative assay system using a reporter gene inserted into an ESR target gene, mediated by an adeno-associated virus (AAV) vector, is currently being generated to more closely reflect the endogenous gene environment. Finally, the epigenetic modifications of Nrf2-target genes in response to long term phytochemical exposure are being explored.

Teodora Georgescu

Characterisation of brainstem pro-opiomelanocortin neurones

[Talk 5 & Poster 2]

Pro-opiomelanocortin (POMC) neurones are an integral part of the melanocortin system, a brain network that is involved in controlling energy homeostasis and body weight. Brainstem POMC neurones are localised in the nucleus of the solitary tract (NTS). This brain structure has an essential role in the integration of gut derived factors and vagal afferents with central signals. Based on the anatomical localisation of this subpopulation of POMC neurones, we hypothesised that these neurones respond to metabolic stimuli, of both central and peripheral origin. We utilised immunohistochemistry in reporter POMC-eGFP and POMC-dsRed mouse lines to map POMC neuronal distribution and characterise their expression within the NTS. We found that POMC neurones are most abundant in the medial-caudal NTS. We observed that the energy balance regulating 5-hydroxytryptamine 2C receptor (5-$HT_{2C}R$) and leptin receptor were co-expressed in a proportion of POMC neurones. Using *in vivo* methods, we show that 5-$HT_{2C}R$ agonists and leptin directly affect the activity of NTS POMC neurones. Furthermore, using electrophysiological techniques, we reveal that these neurones possess K_{ATP} channels, which suggests they are able to respond directly to nutritional cues. These findings suggest that NTS POMC neurones are a favourable candidate for the modulation of appetite by obesity treatments.

Royal Botanic Garden Edinburgh

Sally Eaton

Landscape Scale Conservation of Epiphytes in Scottish Temperate Rainforests

[Talk 12]

Temperate rainforests are globally rare, covering less than 1% of the Earth's land surface. Scotland however has some of the best remaining examples of this habitat of anywhere within Europe. In addition, these Scottish rainforests contain a rich variety of lichen epiphytes, many of which are declining elsewhere in Europe e.g. *Lobaria pulmonaria,* thus creating a responsibility to conserve them.

The recent rescaling of nature conservation, from the site to the landscape or ecosystem scale provides many opportunities for species conservation. However it represents the most data poor resolution for small and cryptic species such as lichen epiphytes, as the surveying methods required to gather such information are too intensive to be applied over large areas. In addition, we currently have incomplete information about the spatial scales relevant to lichen population biology, with conflicting studies largely from a single species providing dispersal estimates ranging from a few meters to many kilometres.

These knowledge gaps pose a problem for decision makers, looking to provide for a variety of land-uses within a single landscape, whilst also considering the varying needs of species of conservation importance. Here, a niche modelling approach is combined with a novel method of measuring dispersal distance, in order to provide accurate distribution and dispersal data for a variety of lichen species. The study focusses on Glen Creran, a landscape of fragmentary temperate rainforest on the West coast of Scotland which is characterised by its conservation priority lichen epiphytes.

Flávia Pezzini

Phylogeny and biogeography of *Ceiba* Mill. (Malvaceae: Bombacoideae) using next-generation, targeted enrichment sequencing

[Poster 3]

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Ceiba Mill. (Malvaceae: Bombacoideae) comprises 18 mostly neotropical species and is one of the most conspicuous elements of neotropical Seasonally Dry Tropical Forests (SDTF), some of the most threatened and least studied forests in the world. *Ceiba* has a historically complex taxonomy and some problems of species delimitation remain unresolved. Previous phylogenetic analyses of DNA sequence data from the nuclear ribosomal internal and external transcribed spacers (ITS and ETS) for 14 species recovered *Ceiba* as monophyletic and showed geographical and ecological structure in three main clades: (i) a humid forest clade including *C. samauma* and *C. pentandra*, sister to the remaining species, though with weak support; (ii) a clade composed of *C. schottii* and *C. aesculifolia*, from Central American and Mexican SDTF, and (iii) a South American SDTF clade including 10 species. To investigate interspecific relationships further and to assess the biogeographic history of the genus, we are using molecular phylogenetic approaches based on next-generation sequencing techniques, specifically using targeted enrichment. This technique enables the sequencing of hundreds of independent nuclear loci, as well as loci from the chloroplast genome, which together can provide many phylogenetically informative characters. With a robust and detailed phylogeny, we can integrate molecular, morphological, and ecological data to contribute to species delimitation in *Ceiba* and use the biogeographic history of the genus to shed light on patterns of neotropical plant evolution and diversification as well as gain insights into the evolution of Neotropical SDTF.

Lorna Stoddart
Sites of Nineteenth Century Botanical Science
[Poster 5]

From working in the archive of John Hutton Balfour, distinguished botanist and Regius Keeper of the Royal Botanic Garden Edinburgh 1845-1879, a prominent theme emerged, that of the sites used for the practice of botanical science and education.

The identified sites include; published literature, teaching environments comprising the classroom, garden and 'the field' and, the public sites where botany was practiced, performed and promoted.

In and through these local sites, Balfour constructed a brand of botanical science specific to him, which although local in its making was global in its circulation. Balfour's botanical science was heavily influenced by his Christian beliefs; he endeavoured to teach a broad and deep curriculum through which it was his intention to shape his students into 'philosophical botanists'.

SRUC

Jolinda Pollock
Gut microbiota dynamics in weaner pigs in response to experimental enterotoxigenic *E. coli* challenge
[Talk 9]

The weaning transition period in pigs is linked to increased vulnerability to digestive disorders, which is partly attributed to destabilisation of the gut microbiota. Post-weaning colibacillosis is an economically important diarrhoeal disease of the small intestine, which is most commonly caused by enterotoxigenic *Escherichia coli* (ETEC) strains. This disease has been variably linked to a decreased growth rate under clinical or sub-clinical conditions, and has been associated with shifts in particular bacterial populations using culturing methods. The microbiology of the porcine gut has been a focus of study for decades, due to evidence supporting the multifaceted role of the gut microbiota in host health and development. The emergence of next-generation sequencing technologies (such as 16S rRNA gene metabarcoding) now allows higher resolution study of complex microbial communities, without being reliant on the ability to culture fastidious micro-organisms.

As part of this project, a 16S rRNA gene metabarcoding method was developed (experiment 1) to allow qualitative and quantitative measurement of gut microbiota shifts. A series of experimental ETEC challenge trials were then carried out to monitor temporal faecal microbiota dynamics in response to challenge (experiment 2) and to study the dynamics of both ileal and faecal microbiota populations in response to ETEC challenge, dietary manipulation and their combination (experiment 4).

The 16S rRNA gene metabarcoding method was validated by sequencing reagent-only controls and a mock bacterial community, which revealed that background contamination interference and sequencing error rate were low. Using this method, temporal shifts in faecal microbiota structure and stability were observed over the post-weaning period, as well as shifts in relative abundance of particular bacterial phylotypes ($P < 0.05$). This suggests that the microbiota composition changes considerably in a relatively short time frame post-weaning, highlighting the importance of this dynamic phase in shaping the porcine gut microbiota. ETEC challenge had no significant effect on faecal microbiota structure, stability or phylotype relative abundances in comparison with sham-challenged pigs ($P > 0.05$). However, when considering ETEC shedding levels, differences in community structure were observed at particular time points ($P < 0.05$). Since the site of colonisation for ETEC is the ileum, other effects on the microbiota may be localised. Therefore, analyses are ongoing to establish whether ileal microbial populations are affected by ETEC challenge and by adjustment of dietary protein levels.

Rafael Silva
Modelling sustainable intensification in the Brazilian livestock sector
[Talk 3]

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At the United Nations Framework Conference on Climate Change COP15 (2009) Brazil presented ambitious commitments or Nationally Appropriate Mitigation Actions (NAMAs), to reduce greenhouse gases emissions (GHGs) mitigation by 2020. At COP21 (2015), the country presented new commitments and a framework to achieve further mitigation targets by 2030 as so-called Intended Nationally Determined Contributions (INDCs). Both NAMAs and INDCs focus on the land use change and agricultural sectors, but the INDCs include a commitment of zero illegal deforestation in the Amazon by 2030. This research focuses on the contribution of the livestock sector to reducing GHGs through the adoption of sustainable intensification measures. We use an optimisation model to: (a) define abatement potential and cost-effectiveness of key mitigation measures applicable to the Brazilian Cerrado; (b) demonstrate the extent of cost-effective mitigation that can be delivered by the livestock sector on the INDCs, and to show a result that underpins the INDC target of zero deforestation; and (c) evaluate the consequences of reducing (or increasing) beef production on GHGs in the Cerrado. Counter-intuitively, a sensitivity analysis shows that reducing beef consumption could lead to higher GHG emissions, while increasing production could reduce total GHGs if livestock is decoupled from deforestation.

End