

ANU Research School of Biology EMCR Conference

September 3rd 2019

The Sciences Teaching Building (136), level 3
Linnaeus Way, ANU, 0200



plant energy biology
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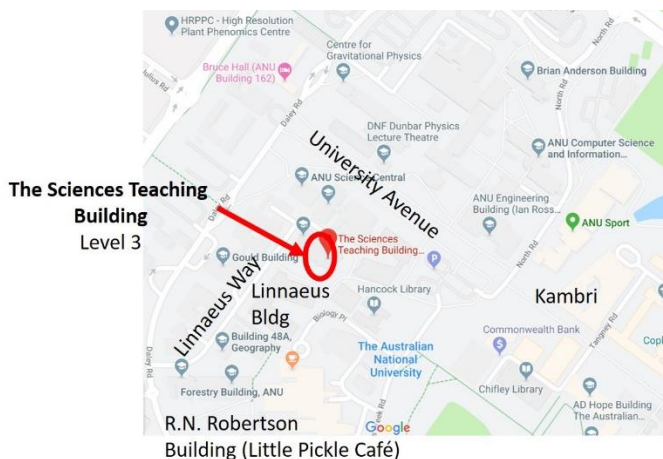


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RSB EMCR Conference September 3rd 2019

- 8:30 – 9:00 Registration
- 9:00 – 9:10 Welcome
- 9:10 – 10:20 Research Talks - Session 1
- 10:20 – 10:50 Morning Tea
- 10:50 – 11:30 Research Talks – Session 2
- 11:30 – 12:30 Funding Masterclass
- 12:20 – 13:00 Lunch
- 13:00 – 13:50 EMCR Ideas Rally
- 13:50 – 15:00 Research Talks – Session 3
- 15:00 – 15:20 Afternoon Tea
- 15:20 – 16:20 Career Pathways Panel
- 16:20 – 16:30 Closing
- 16:30 – 18:30 Happy Hour



Research Talks – Session 1

9:10 – 10:20 am (Chair: **Rebecca Bathgate**)

Living on the edge: How do plants in extreme thermal environments cope when pushed to their limit?

Verónica Briceño Rodríguez (E&E)

Of all the climatic factors determining species distributions on Earth, temperature is arguably the most important. It is extreme temperatures – rather than averages – drive species evolution, so it is concerning that extreme temperature events are increasing in frequency and intensity. All organisms have a characteristic thermal tolerance range; that is, an upper and lower temperature threshold beyond which physiological damage occurs. In general, species in cold regions can withstand colder temperatures than species in hot regions and vice versa, but there is variation within a given region.

This thermal tolerance breadth, however, is not static; organisms acclimate by shifting damage thresholds up or down in response to prevailing conditions. Desert and mountain species live at the thermal limits of biological processes. Unlike plants in more moderate, temperate regions, plants in these extreme environments are exposed to both very low and very high temperatures. Studies on alpine plants around the world have mainly focused on cold tolerance and in desert plants research on heat tolerance predominates, thus we lack an understanding on the whole thermal tolerance range for alpine and desert species. In this talk I will explore how we are planning to tackle this gap using seasonal field and laboratory measurements at different plant developmental stages and the relevance of this data to forecast effects of climate change on our unique alpine and desert flora.

Revisiting carbon isotope discrimination in C₃ plants: Respiration rules when photosynthesis is low

Florian Busch (PS)

Stable isotopes are commonly used to study the diffusion of CO₂ inside photosynthetic tissues of plants. The standard method to interpret the observed preference for the lighter carbon isotope in C₃ photosynthesis involves the model by Farquhar, O'Leary and Berry, which relates carbon isotope discrimination to physical and biochemical processes inside the leaf. However, the model frequently returns unreasonable results for mesophyll conductance to CO₂ diffusion (gm), especially when rates of photosynthesis are low. A derivation using modified assumptions related to the isotope effect of mitochondrial respiration yields a new isotope discrimination model that returns much more reasonable gm values than those obtained with the old model.

Heteromeric solute carriers: modular, domain-based formation and evolution

Stephen Fairweather (BSB)

Solute carriers form one of two major superfamilies of membrane transporters in humans. Following several decades of molecular characterisation, multiple solute carriers that form obligatory heteromers are emerging as a distinctive group of membrane transporters. Heteromeric solute carriers are composed of a, generally, larger translocating subunit and a smaller ancillary

subunit and are represented in humans by the SLC3-SLC7 amino acid exchangers, SLC16 monocarboxylate/H⁺ symporters and basigin/embigin, SLC4A1 (AE1)/glycophorin A exchanger, and SLC6 heteromeric symporters among others. We have recently reviewed experimentally established heteromeric solute carriers and, in the process, undertook a systematic analysis of their structural architecture and phylogenetics. Here, I make the case that heteromer formation is based on the underlying modular structure of the proteins and this modularity allows for functional diversity, especially within the ancillary subunits of heteromeric carriers. Put another way, discrete domains within the protein subunits are responsible for discrete function, including heteromer formation, and these separate domains are loci of different evolutionary selection pressures. This modularity is also observed in the underlying exon/intron organisation of the protein-encoded genes. These principles are exemplified by the case of glycophorin A from red blood cells, which appears to have two distinct protein regions: one involved in antigenic variation as an adaptation to malaria parasite invasion, and a second responsible for stabilising mutations in its heteromeric partner AE1. A proposed model for this modular-based evolution will conclude the presentation.

Effector protein trafficking of fungal rust pathogen *Melampsora lini* **Xiaoxiao Zhang (PS)**

Rusts are obligate biotrophic fungi that cause devastating diseases and yield losses on crops. During infection, the rust fungi develop a specialized cell structure, called haustorium, within the plant cells to obtain nutrients and secrete a set of proteins, called effectors, to suppress host immunity to aid colonization. The haustorium penetrates host cell wall but is separated from the host cytoplasm. Flax (*Linum usitatissimum*) confers resistance to flax rust (*Melampsora lini*) through the induction of hypersensitive cell death response at the haustorium formation sites, governed by direct recognition between host resistance (R) proteins and pathogen effector proteins. These effector proteins are inferred to be delivered into host cells, based on their recognition by host intracellular R proteins. How the rust effectors are secreted from the haustorium and delivered into the cytoplasm of host cells remains elusive. We have developed an *Agrobacterium*-mediated genetic transformation procedure for flax rust. Selection of the transgenic rust strains is based on silencing of the AvrL567 effector that is recognised by the flax L6 R protein. Using this system, we have further generated flax rust strains expressing the AvrM effector fused to a fluorescent protein tag. We showed that the AvrM protein is delivered into host cells during rust infection in flax. This method allows us to further investigate effector protein trafficking through haustorium in living plant cells.

Fast freezing and frying leaves: measuring plant thermal tolerances using chlorophyll fluorescence **Pieter Arnold (E&E)**

Thermal tolerance has far-reaching implications for the persistence and distribution of plants, both natural and cultivated, as the climate continues to warm and generate more stochastic extreme temperature events. Leaves that are exposed to temperature extremes accumulate damage to their photosynthetic apparatus, which impairs their ability to function and may be lethal to the plant. I will showcase some of our ongoing experimental work using chlorophyll fluorometry: a technique that measures temperature-dependent changes in basal chlorophyll fluorescence while freezing or heating leaves beyond their tolerance limits. The critical temperature at which fluorescence rapidly increases signals when the photosystems are incurring significant damage, and describes potential temperature limits for that plant's persistence. But there are many challenges to taking useful measurements of thermal tolerance. For example, in nature, cold

transitions occur much more slowly than heat transitions and the rate of temperature change may affect the protective mechanisms that are induced in response to thermal stress. In the lab, how much does the rate of temperature change matter for obtaining biologically realistic estimates of thermal tolerance that are also comparable across species? I will present recent findings that demonstrate how important it is to consider details such as this to improve our understanding of plant thermal tolerance, and why this field of research is crucial for our rapidly changing world.

Dissecting genetic variation in gene expression at the single-cell resolution.

Adam Reddiex (PS)

Expression quantitative trait loci (eQTL) mapping studies are concerned with finding variants in the genome that underlie patterns of gene expression. However, the tissue sampled in such studies contains a heterogeneous mix of different cell-types and it is unclear to what degree the effects of genetic variants are consistent across cell-types. To address this, we used single-cell RNA sequencing to measure RNA expression of root tips in 150 accessions of *Arabidopsis thaliana*. In this presentation we present the preliminary results of eQTL style analyses conducted in a cell-type specific manner.

Mining impacts on coral reefs - time to clear up our mess!

Jennie Mallela (E&E)

Does mining impact coral reefs? Surprisingly, there are very few studies out there so we decided to find out more. Phosphate mining activities on Christmas Island began in the late 1800's providing a unique, long-term case study in which to assess the impacts of mining on coral reef development. We used a range of multidisciplinary techniques to identify potential pollution "hotspots" on the reefs, this included watershed modelling and pollution analysis of reef sediment. Phosphate rich mining pollution flowed from local watersheds onto nearshore coral reefs with extreme levels of up to 54,000 mg/kg of total phosphate recorded in reef sediment at sites next to mining infrastructure. Pollution negatively impacted coral reef building communities, in particular, branching corals and small encrusting, reef building organisms, critical to the future survival of coral reef ecosystems. These findings highlight the importance of stopping runoff and pollution from catchment based mining activities and protecting reefs for the future.

Morning Tea 10:20 – 10:50 am

Research Talks – Session 2

10:50 – 11:30 am (Chair: **Stephen Fairweather**)

How does the rate of carbon dioxide uptake into leaf cells vary across the canopy, and as leaves age?

Tory Clarke (PS)

Mesophyll conductance is an important photosynthetic parameter that influences the amount of carbon dioxide (CO₂) available for fixation by Rubisco, the central enzyme of photosynthesis.

Variation in mesophyll conductance throughout the plant leaf canopy is not well understood, but has implications for how we model photosynthesis in crops. We investigated how mesophyll conductance, and other photosynthetic and leaf anatomy parameters, varied throughout the canopy of *Nicotiana tabacum* var. Samsun plants. Assimilation rate and stomatal conductance all decrease as you move down the canopy from young fully-expanded leaves through to older, shaded leaves at the base of the canopy. Leaf thickness and cell-wall thickness were also influenced by leaf position, with older leaves at the base of the canopy thicker and with wider cell walls than those higher in the canopy. Despite these increases in physical barriers for CO₂ transfer into chloroplasts, mesophyll conductance surprisingly remained constant. We are now investigating the photosynthetic biochemistry to further understand the relationships between these parameters across leaf canopies.

Eggshell collection genomics: approaches and applications to the study of Australian birds

Alicia Grealy (E&E)

Museum specimens offer one of the only opportunities to examine how diversity has changed across time and museum eggshells in particular are an untapped resource of genomic information for thousands of avian species, including rare and extinct taxa. Over the past few years we have been developing techniques to non-destructively retrieve both mitochondrial and nuclear DNA from museum eggshell specimens. Here, I will detail how we are going about this, and explain how these new methods can be applied to help solve previously intractable biological questions, from the identification of unknown specimens to the reconstruction of avian evolutionary history.

Dynamic adjustment of wheat respiration and net assimilation rate to warming and their contribution to yield resilience

Onoriode Coast (PS)

Heat waves and increased warming significantly affect crop yield. Understanding the capacity of crops to dynamically adjust physiological processes, such as dark respiration (R_{dark}) and net assimilation rate (A_{net}) to warming - and identifying genotypic variation in these processes - could contribute to global food security. To quantify variation in acclimation to warming, we measured leaf gas exchange, and used a novel high-throughput method to measure leaf and spike R_{dark} of a range of wheat genotypes at anthesis in the field. The genotypes consisted of commercial cultivars and breeding lines varying in putative heat tolerance. We exposed plants to natural warming by sowing at three time points at one-month intervals during the 2017 and 2018 growing seasons. As such, later-sown plants developed under warmer conditions. We hypothesized that, when measured at a common temperature (25°C), wheat grown under warmer conditions would: (i) have lower leaf and spike R_{dark} , (ii) show increased leaf A_{net} , (iii) display smaller degrees of variation in acclimation ($R_{\text{dark}}/A_{\text{net}}$ ratio), and (iv) that these traits would be associated with higher yields. To understand the drivers of the temperature responses of R_{dark} and A_{net} we explored relationships of both traits with leaf nitrogen and leaf mass per unit area. In this presentation, we will discuss how the diverse responses to warming in wheat may improve the resilience of wheat yield under future warmer conditions.

The cyanobacterial solution for high performance plants

Britta Forster (PS)

The world population is growing every second. Our ability to feed everyone adequately does not increase at a matching pace. Increasing the productivity of our staple crops can be part of the

solution. One avenue to more productive plants is to enhance their uptake carbon dioxide for use in photosynthesis and production of energy-rich sugars or carbohydrates while losing less water in the process. Cyanobacteria have evolved a powerful system to actively take up inorganic carbon as carbon dioxide as well as its hydrated form bicarbonate and effectively increase the CO₂ concentration at the enzyme Rubisco that is responsible for CO₂ fixation as sugars. In short, a CO₂ concentrating mechanism or CCM that enables cyanobacteria to grow very efficiently in most diverse environments. We aim at introducing the cyanobacterial CCM into the leaves and chloroplasts of crop plants, using a transgenic approach. Critical to success is an in depth understanding of the regulation of the CCM genes and proteins to achieve coordinated function in higher plants. I will present some snapshots of our current understanding of regulation of the inorganic carbon uptake proteins of the CCM.

Funding Masterclass – Sponsored by PEB 11:30 – 12:30 am (Chair: **Xiaoxiao Zhang**)



ANU Research Management, ARC Grants

Michele Lamb

Joyce Das

Industry Funding - **Professor Stefan Bröer**

ARC Grants - **Professor Michael Jennions**

Understanding Piezo1's Relationship with Lipids

Amanda Buyan (BSB)

Mechanosensation is a transduction mechanism which is integral in various higher physiological processes in eukaryotes, such as touch and hearing. To be able to hear and sense touch correctly, cells need to be able to sense and react to mechanical stimuli caused by these physiological processes. These forces are detected via membrane-embedded channels. One way these channels detect these forces is through deformation of the lipid bilayer, deemed the “force-from-lipids” principle. Bacterial homologues, such as MscL and MscS, exemplify this principle and have been studied for the past few decades, and have contributed greatly to our understanding of mechanically gated channels. However, understanding the underlying molecular force sensing mechanisms, and how similar bacterial and eukaryotic mechanosensitive channels are in terms of their gating mechanisms, remains elusive.

The recent discovery and structure elucidation of the first eukaryotic mechanically gated channels, named the PIEZO family, allows for the mechanisms of mechanical gating to be studied in higher organisms. Since their discovery, PIEZO channels have been implicated in many cellular

processes, but the gating mechanism and the role that lipids play in PIEZO's mechanics remain elusive. To this end, we are using a combination of electrophysiology and molecular simulations to understand protein-lipid interactions between Piezo1 and relevant lipids in model mammalian bilayers, and ultimately the role that lipids have on Piezo1's activation. We are able to show that piezo has specific interactions with a number of membrane components that likely play a role in mediating the bilayer-protein interaction.

Lunch 12:20 – 13:00

EMCR Ideas Rally – Identifying RSB EMCR issues

13:00 – 13:50 pm



Research Talks – Session 3

13:50 – 15:00 pm (Chair: **Onoriode Coast**)

How fast are wild vertebrate populations evolving today?

Timothée Bonnet (E&E)

Given rapid anthropogenic changes it would be useful to know how fast populations are currently evolving by natural selection and adapt to their environments. But how to measure adaptive evolution? How to summarize it across all traits and genes? The good news is that there is a theoretical solution: By Fisher's fundamental theorem of natural selection, additive genetic variance in relative fitness is the rate of on-going adaptive evolution. The bad news is that this parameter is available for very few systems in the wild, and has mostly been estimated using unrealistic assumptions. Estimating additive genetic variance in fitness requires large datasets with high-quality relatedness information, but also complex analyses that are just reaching maturity.

We assembled data from 20 long-term monitored wild populations of birds and mammals, with high-quality pedigrees, and analysed them with quantitative genetic "animal models" using recent conceptual and methodological improvements to better deal with non-Gaussian traits. We first illustrate how generalized linear animal models provide directly interpretable parameters and improved statistical inference. We then show the diversity of rates of adaptive evolution estimated in our analyses, and discuss the relevance of these rates for the fates of populations.

Functional characterisation of the necrotrophic effector ToxA from wheat pathogen *Parastagonospora nodorum*

Bayantes Dagvadorj (PS)

The wheat necrotrophic fungal pathogen, *Parastagonospora nodorum*, secretes effector proteins to manipulate host immunity and promote successful infection. One of the major determinants of the disease is ToxA effector protein, which causes cell death in the presence of wheat susceptibility gene Tsn1. This gene-for-gene interaction appears to be indirect. Previous studies have identified host proteins that interact with ToxA including ToxA Binding Protein 1 (ToxABP1), plastocyanin and the Pathogenesis-Related 1 protein (PR1-5). Studies to date though in the Solomon laboratory have been unable to reproduce these interactions. As such, the molecular function of ToxA during infection remains poorly understood.

To elucidate the molecular mechanisms of ToxA effector, we are searching to identify potential host target proteins for ToxA. To do this, we use two high-throughput and large-scale approaches to identify protein-protein interactions for ToxA. First, host binding partners were detected by Yeast Two-Hybrid using a prey library made from infected leaves and also ToxA-infiltrated wheat cultivars carrying the ToxA specific susceptibility gene, Tsn1. An alternative to this approach, we will use co-immunoprecipitation (Co-IP) and liquid chromatography-tandem mass spectrometry to find interacting protein complexes from infected ToxA susceptible cultivars by using tagged ToxA. After validating shortlisted interactions by in-planta Co-IPs and western blots, we will undertake assays that combine cell biology, genetics and biochemistry to determine their roles during necrosis. The knowledge gained from this study will be valuable not only for researchers working on plant necrotrophic pathogens but also for scientists engaged with understanding and improving plant disease resistance.

Ants in VR orient themselves in relation to whole panoramas, not individual landmarks

Trevor Murray (E&E)

With the completion of the Antarium (insect catered VR system), we are now about to interrogate the processes that underlie visual navigation in ants. We project panoramic snapshots to tethered ants and record their navigational decisions. In experiment 1 we test ant's ability to respond to whole scene rotations when virtually displaced to familiar, unfamiliar and unstructured locations. We find that ants perform better when in familiar than unfamiliar environments, and are unable to orient in unstructured scenes. In Experiment 2 we independently rotate the ant's foraging tree and the rest of the visual scene. These ants are able to follow whole scene rotations but appear to ignore movement of the foraging tree, and instead continue to follow the rest of the scene.

Investigating the interaction between Tox3 and PR1 proteins in the *Parastagonospora nodorum*-wheat pathosystem

Megan Outram (PS)

Parastagonospora nodorum, the causal agent of Septoria nodorum blotch is an important necrotrophic pathogen of wheat. *P. nodorum* secretes small, cysteine-rich proteinaceous effectors (ToxA, Tox1, and Tox3) that interact with corresponding dominant host sensitivity gene products, rendering the plant susceptible to disease and promoting cell death in an inverse gene for gene manner. Whilst it is understood that these effectors are required for disease, there is limited understanding of their function. Tox3 elicits a strong necrotic reaction in wheat lines containing the sensitivity gene Snn3, and has been shown to interact with wheat pathogenesis-related 1 (TaPR1)

proteins. Given the challenges associated with this host system, we developed protocols to heterologously express Tox3 and TaPR1 proteins, and protein-mediated in planta phenotyping assays to dissect these interactions. Here, we report that infiltration of purified TaPR1 proteins represses infection by *P. nodorum* and induces upregulation of several defence related genes, which is dependent on the release of the CAPE1 peptide. Co-infiltration of recombinant Tox3 and TaPR1 proteins abrogates this protective mechanism. To understand the molecular basis of this interaction, we determined the high-resolution crystal structures of Tox3 and TaPR1 proteins and identified a proline at position 173 in Tox3 that is required for interaction with TaPR1 proteins. Infiltration of purified Tox3P173S into Snn3-containing wheat lines resulted in necrosis development, uncoupling the Tox3-Snn3 and Tox3-TaPR1 interactions. These results suggest that Tox3 has a dual function in disease by inducing Snn3-mediated necrosis, and repressing host defence through its interaction with TaPR1 proteins.

How do landscapes generate diversity? What can imaginary species tell us?

Dan Rosauer (E&E)

Macro-ecological process models are showing great potential to increase understanding of how the interaction of historical climate, geography and evolution have shaped the spatial distribution of biodiversity. When simulating expected biodiversity, a relatively simple set of parameters can shed light on the complex dynamics which generate and sustain biodiversity.

Here I present a novel macro-ecological simulation to focus on the phylogeographic scale, where new species arise through repeated processes of divergence and reintegration, isolation and secondary contact. Varying just three parameters: dispersal speed, rate of local niche evolution and the rate at which genomic divergence leads to reproductive isolation, this model elicits emergent properties observed in nature.

Improving crop yield with cyanobacterial CO₂-concentrating mechanism

Wei Yih (Wil) Hee (PS)

One promising approach to increasing photosynthetic efficiency and crop yield is to incorporate the cyanobacterial CO₂-concentrating mechanism (CCM) into crop plant chloroplasts. Our studies focus on the structural formation of carboxysomes, one component of the bipartite CCM, within tobacco chloroplasts. Formation of structural and functional carboxysomes requires coordinated expression of around a dozen proteins, highlighting that their transgenic construction is a complex engineering task. In this study, we successfully synthesised simplified carboxysomes, derived from the source organism *Cyanobium*, within tobacco chloroplasts. We replaced the endogenous Rubisco large subunit gene with a gene cassette expressing cyanobacterial Form-1A Rubisco large and small subunits, and two key α -carboxysome structural proteins, CsoS1A and CsoS2. Our results demonstrate the first evidence of plant growth dependent on Form-1A Rubisco, as well as the successful encapsulation of this Rubisco in chloroplastic carboxysomes.

The influence of fossil uncertainty on the evolution of kangaroos

Ian Brennan (E&E)

Studying organismal evolution on deep timescales provides us opportunities to identify the processes driving patterns in diversity and forms. Macroevolutionary studies of trait evolution however, often fail to account for sources of artifactual variation in the data—be it phylogenetic, temporal, or other. In some instances, this may not affect our evolutionary understanding, and

accounting for sources of uncertainty may only subdue confidence in our inferences. In more dramatic cases, narrow views of trait uncertainty may result in conclusions that are misleading. Because macroevolutionary analyses are built atop a number of preconceived hypotheses regarding the relationships between taxa, origination and divergence times, and intraspecific variation, it is important to incorporate and present this uncertainty. Here I use a dataset for Australian kangaroos to demonstrate the importance of incorporating uncertainty when testing patterns of diversification. After accounting for fossil age uncertainty, I provide evidence that a proposed Pliocene origin of *Macropus* kangaroos is at odds with the results of some molecular and morphological dating methods. While a Pliocene radiation of macropodines exists within the realm of plausible realities, there are other scenarios more consistent with the data, and I was unable to reproduce ages as young as those proposed. Depending on the estimated crown age of kangaroos, the evolution of hypsodonty is as likely caused by the continental expansion of C4 grasses as it is by increasing windborne dust levels or paleotemperature. These results suggest that previous interpretations of the radiation of modern kangaroos are not as bulletproof as we believe, and that multiple factors have likely influenced their remarkable diversification across the Australian continent. This example demonstrates the importance of incorporating uncertainty in comparative evolutionary studies, and I encourage us as a community to better understand our data, the methods we employ, and the assumptions that both contain.

Afternoon Tea 15:00 – 15:20

Career Pathways Panel – Sponsored by CEAT
15:20 – 16:20 pm (Chair: **Melanie Carmody**)



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Mary Kelly (CEAT) Don't hide, life happens!

Hannah Osborn (Australian Department of Agriculture)

Julie Christie (CSIRO) When the side quest becomes the whole game

Simon Williams (ANU)

Closing Ceremony 16:20 – 16:30 pm followed by
Happy Hour 16:30- 18:30 pm

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