Australian National University

Research School of Biology Summer Scholars ePortfolio

2021/2022

Working with the Australian Mountain Research Facility (AMRF) in Kosciuszko National Park and beyond!

Sabina Aitken

Nicotra Group | Supervisor: Zach Brown

I spent my summer research internship working in the Nicotra Lab. In particular I worked on the Australian Mountain Research Facility (AMRF) projects in the Australian Alps.

AMRF has constructed and maintains the Australian Mountain Observation Network (AMON) in alpine areas in the ACT, NSW, Victoria and Tasmania. These sensor arrays collect data on the climate and its effect on the surrounding ecosystems. Data includes carbon and water fluxes as well as ecosystem productivity and flammability. In addition these AMRF sites each have experimental Drought Net Shelters (shelters blocking majority of rainfall). These shelters will remain in place for 5 years and offer information on how alpine vegetation changes in response to drought. All this information is integral in understanding how our alpine ecosystems are likely to change in the future in response to climate change.

My internship started with 2 weeks in Kosciuszko National Park where I helped the team assemble 2 new AMRF sites near Charlottes Pass and Thredbo. The first day was spent organising gear that was helicoptered out to the sites. We then spent the next two weeks constructing Drought Net Shelters and observation stations.

During the internship I also got to visit AMRF sites in Victoria where we collected vegetation samples and spent time learning to identify and name many alpine species. In the lab we sorted and weighed vegetation samples as part of yearly assessment of the above ground biomass in the drought shelter plots.
Overall it was fun and rewarding to be out in Australia's unique alpine environment and contribute to this long term project. I can't wait to see what results this study brings and how they can help us better understand and preserve alpine areas.

Thermal tolerance of native plants during drought

Isabelle Bennett

Nicotra Group | Supervisors: Veronica Briceño, Pieter Arnold and Zach Brown

This project measured the thermal tolerance of plants from alpine, temperate, and desert environments which have undergone dehydration. We aimed to understand the effects of climate change on native plants during drought, as extreme weather events including

heatwaves or freezing events become more common. This information could be used to predict the impacts of climate change on native flora to understand which plants may be more vulnerable to the effects of global warming. These findings could even help to inform government policy on climate change in the future!

For this project, we collected samples of leaves from alpine, temperate, and desert plants grown in our very own green houses at ANU. These leaves were then exposed to three different levels of dehydration to simulate drought conditions. We then measured the chlorophyll florescence in the leaves as they were heated or cooled to extreme temperatures. Chlorophyll florescence was measured using a thermoelectric Peltier plate heating/cooling system to tell us the critical temperature of the leaves. This is the temperature where the leaf has accumulated a critical amount of damage to the photosystem II, a crucial step of photosynthesis which is necessary for the function of the leaf itself. Unfortunately, due to a covid outbreak in the lab, we didn't get to finish the experiment but hopefully it will continue in the future to establish the effect of drought on the thermal tolerance of plants from different environments.

The main highlight of this program was getting the opportunity to meet and work alongside great researchers. Zach and Piet, as well as everyone involved on the lab were super helpful, and Vero especially was incredible, encouraging the summer scholars to be involved in using new equipment and organising for me to get to go to the snowy mountains to do some field work, which was another highlight for me! I was fortunate enough to spend 10 days up in the Snowy Mountains as part of a team helping UTS honours student Lisa Danzey, learning some of the techniques we would be using later in the experiment in the lab. It gave me an opportunity to meet more amazing women in the field of ecology, who were undertaking masters and PHDs and had lots of advice to share with me as an undergrad considering further study. Overall, the summer scholar program was an incredible experience, which I would highly recommend!

Are psyllids motivated by smell?

Lucy Bruck

Head Group | Supervisors: Megan Head and Madison Fink

Our objective was to answer the question: "Do *Cardiaspina albitextura* utilise olfactory chemicals to locate favourable *Eucalyptus blakelyi* leaves?"

Cardiaspina albitextura –commonly known as psyllids -feed on *Eucalyptus blakelyi* leaves. In doing so, they remove leaf compounds necessary for photosynthesis. *C. albitextura* currently overcrowd *E. blakelyi* communities which causes wide spread loss of photosynthetic capacity that can lead to tree death. Our project aimed to increase knowledge about this insect-plant relationship by determining how *C. albitextura* locate suitable host trees.

We conducted olfactory choice tests using a Y-shaped tube. This provided the psyllids with two stimuli which they could move towards and choose between. We additionally conducted oviposition choice tests, where psyllids were mated and given a choice of leaves for oviposition. By monitoring the psyllids through these tests we aimed to see whether the psyllids are able to distinguish between susceptible and resistant *E. blakelyi* trees from olfactory signals.

C. albitextura showed no significant choice between susceptible and resistant leaves across all of our experiments. Most often in the Y-tube olfactory tests, the psyllids made no choice toward either leaf and stayed in the middle, seeming uninterested in the olfactory stimuli. Additionally, the mating and oviposition choice tests showed no significant findings, as the psyllids showed no interest in either of the leaves given. These results possibly indicate that the psyllids do not use olfactory chemicals to locate trees, which has been reported in other species of psyllids. However, it could also be due to inconsistencies within our experimental set up.

My personal highlight from the Summer Research Internship was having the opportunity to do field work. I was able to understand the full scale of this problem and visualise firsthand how the research I conducted will help to answer questions on how to reduce the tree death occurring from *C. albitextura.*

Diversity patterns of micromammals in islands around the world

Metta Chalapati

Moritz Group | Supervisor: Octavio Jimenez Robles

The aim of my project was to test island biodiversity patterns of rodents and bats against predictor variables, in order to explain broader patterns in alpha and beta diversity.

As islands shift and develop over time, biodiversity patterns have reflected these changes. Research into contributing variables can be used to maintain diversity levels and inform decisions, particularly relating to climate change effects on islands.

Previous theories such as the Theory of Island Biogeography (TIB) (MacArthur & Wilson, 1967), have placed emphasis on physical characteristics such as island area and distance to the mainland. I included both in my analysis using GIS tools in R, in addition to other factors such as elevation, sea depth and the mainland species pool, expecting to find differences between rodents and bats due to their differing traits of dispersal and reproduction. To test the effect of these variables, I used two alpha diversity measures, species richness and phylogenetic richness, as well as both compositional and phylogenetic beta diversity to compare dissimilarity between islands and the species pool in their closest point in the continent. I then used R to perform multimodel inference, based on generalised linear models to test our hypotheses to explain the different biodiversity measures.

I found that in general, the predictors behaved as expected. Area and distance to the mainland were the most consistently significant variables. Interestingly, the range of elevation in an island, hypothesised to indicate carrying capacity, was the least important. Bats were also not affected by distance to the mainland, likely due to their more efficient dispersal mode. Notably, the results also showed that spatial turnover increased with area, distance and sea depth, possibly indicating the work of speciation processes (island evolutionary radiations), not predicted by the original TIB.

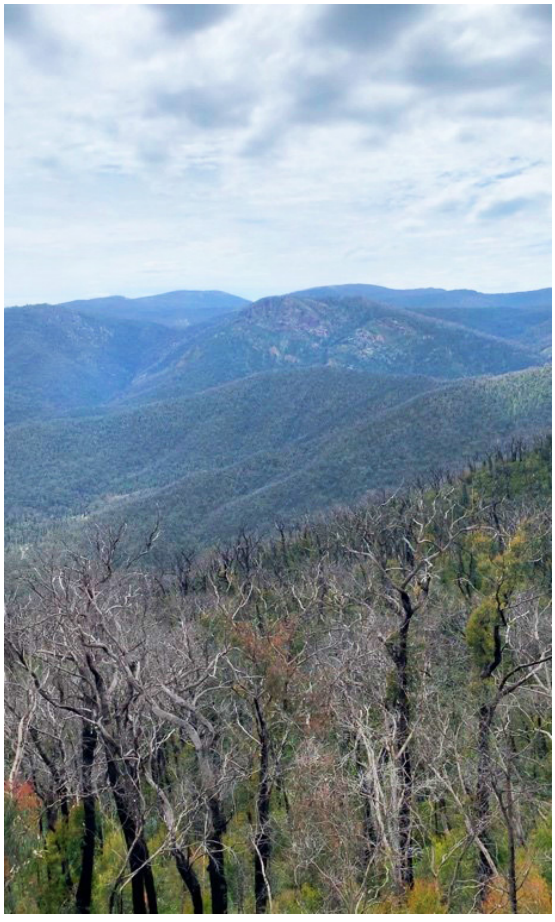
Throughout the program, I really appreciated the welcome and support I received from members of RSB, particularly from my supervisor. This allowed me to refine and improve my project immensely, especially as I widened my scope to global datasets. I particularly enjoyed developing my coding skills in R and gaining experience troubleshooting and adapting data to overcome unexpected hurdles.

Koalas: The enigmatic inhabitants of Kosciusko National Park

Ashley Davies

Foley Group | Supervisor: Karen Ford

Koalas are one of Australia's most iconic and well-loved species, but over the years, we've watched their habitat disappear and their food resources diminish. The aim of this summer research project was to find out whether koalas are still living in Kosciusko National Park by undertaking acoustic and spotlighting surveys.



Kosciuszko National Park

This project is significant for many reasons, first and most importantly, Kosciusko National Park has also never been formally surveyed for koalas, despite having viable koala habitat. The federal government, therefore, is attempting to establish a National Koala Monitoring framework, which requires information about where koalas occur. The presence of koalas can also provide information about the types of eucalypt species within a forest, as koalas prefer some species over others (symphomyrtus). Therefore, a forest will contain these species if a sizeable koala population is present, and this can shape policy and conservation efforts.

Firstly, we deployed acoustic recorders throughout the park, at locations where koalas had been reported before. These acoustic recorders were set up to record for 12 hours every night to listen for koala mating calls. The data from the recorders was collected and sent to a lab in NSW Department of Primary Industries to be processed via an automated koala call recogniser. If a sound signal similar to a koala mating bellow was detected, it was tagged, and then we were tasked with confirming whether the call was really a koala.

For observer spotlighting, two researchers each 150m apart, walk along a 300m transect in the dark with a spotlight and see if any koalas can be spotted through eye shine. This has the added benefit of providing numbers for other threatened arboreal marsupials such as yellow-bellied gliders and greater gliders. Tree surveys were also conducted at the location of the acoustic recorders, to get a better idea of the species in the area and whether they could support koala habitation.

We found some koala scat at one location, and the processed recordings are still being checked to confirm koala bellows. At this point there were no koalas sighted by our team in Kosciusko, but hopefully in time we might just spot one.

Overall, this project was truly a pleasure to work on. Not only for its significance to koala conservation but also its importance for the future environmental health of Australia. It was also amazing to work with a team of many hardworking and dedicated researchers, who showed me the importance of getting out in the field. I learnt many valuable and lifelong skills both in terms of research and my worldview and I won't soon forget my summer spent koala hunting.

Antipredator behaviours of immunocompromised guppies

Tina Gopalan

Head Group | Supervisor: Megan Head

In the beginning of the summer internship program, I worked alongside another intern, and members of the Head lab on psyllids. However, due to covid-19 regulations and changes in the weather (being the rain), we were unable to continue researching psyllids as much as hoped and so I switched to working on guppies instead. The project I took up was to investigate the antipredator behaviours of immunocompromised guppies when compared to a control group.

Throughout the summer, I focused on collecting data related to the hiding behaviour of guppies when immunocompromised with a bacterial strain. This data collection was done from pre-recorded videos. Behaviour was recorded for each guppy before and after being immunocompromised. As well as the hiding behaviour of guppies, I also measured guppy colouration before and after bacterial strain injection to see if immunocompromised affected male investment in their sexual display signals. For this, and also measuring guppy length I learnt to use ImageJ.

During the summer scholar program, I mainly focused on data collection which taught me how research can be meticulous and repetitive but definitely a very important process in discovery. The unique and varying lengths, hiding behaviour and colouration of each guppy also made me realise that every measurement is as important as the collective data when analysed. Future analysis of the data is required to determine if the guppies had significant behavioural changes after bacterial inoculation.

Taking part in this program has also taught me the proper etiquette in a research group setting. For the first time, I have experienced what it is like to be part of a team with like-minded researchers in a university setting. In short, I had a brilliant time during the RSB SRI program with my supervisor and other members of the lab being so friendly and kind. I am truly grateful for the new skills, friends, and knowledge I have gained.

Conducting habitat surveys to determine tree availability at two locations in the Southern Tablelands

Riley Guyatt

Foley Group | Supervisor: Karen Ford

Aim: To compare two different habitat survey methods and to generate data on tree availability that can support previous work on koala behaviour in the area.

This summer I worked alongside PhD candidate Murraya Lane on a field-based project. We used two survey methods (spot and transect) to determine composition and key characteristics of eucalypt trees across two locations in the Southern Tablelands of New South Wales.

This data will be used for two key purposes. Firstly, comparing the results of the two survey methods will

show whether they yield similar or different results, which could inform habitat survey methods in the future. Additionally, the data will support previous studies on koala behaviour and habitat quality in the area, allowing a comparison between which trees are used by koalas and which are actually available in the environment.

To collect this data, Murraya and I applied the two different survey methods at 30 sites over two locations. At every site we recorded data on species, location, diameter and canopy type of 30 trees for each spot survey, and 32 trees along a 420m transect intersecting the spot. We also collected leaf samples along several of the transects to support other ongoing research.

The project is not yet complete, with a few sites left to survey and data analysis to be completed. However, from our experiences in the field we expect there to be differences in data collected between spot and transect surveys, particularly given the way that transects are able to intersect more small concentrations of particular species (e.g. around a creek or on a different side of a slope) leading to a potential to record more species diversity.

I really enjoyed this project. Not only did I appreciate it as a great opportunity to get some experience doing fieldwork and get familiar with scientific research methods, I also found wandering around the bush looking at trees to be a surprisingly fun way to spend my summer break. I learned some valuable skills, and by the end of the project I was finally able to estimate the diameter of a tree to within 1cm and spot a spider web before walking straight into it.

Hormonal treatments and motion tracking of skinks

Zachary Hewertson

Noble Group | Supervisor: Daniel Noble

Climate change threatens both the developmental stages and metabolic function of ectothermic organisms. These organisms require external factors to regulate their metabolism, and are susceptible to changes in the environment. Quantifying the metabolic responses of ectotherms to differing conditions can reflect changes within an ecosystem, making this research paramount for projections of population health into the future. Our skink species, *Lamopropholic delicata* (Rainbow skink) was the perfect model specimen due to their ectothermic nature and rapid egg production, providing many early-developmental forms which could undergo experimental manipulation. The main objective of my project was to hormonally manipulate the developmental stages of *L. delicata,* and determine if there were changes to both the metabolic function and physiology of the lizards that hatch.

My first duty in this project included extracting 'behavioral data' from videos of the skinks' behavior without stimuli. I extracted the data using the motion tracking software EthoVision XT. This data will act as a control to view the lizard's behavior in undisturbed conditions, and provide a comparison to the 'antipredator response' data previously extracted. This will allow individual skink behavior to be analyzed, and it can be compared to any offspring the skinks produce in the future. My second duty was egg collection and hormone treatments. This was my favorite part of the internship as it was exciting to see so many lizard eggs, and for such small creatures, skinks have lots of personality which was fun to see! Once the eggs were collected, hormone treatments began. The hormone used was cortisol, a stress response hormone, in ethanol solution. Hormone treatments of differing strengths were randomly assigned and applied topically to each egg, before being placed in one of two incubators at different temperatures (23 and 28°C). In turn, this data can ascertain how metabolic changes due to experimental manipulations can accumulate to both the fitness and life-history of a population, especially during a period of increasing environmental alteration.

Due to the nature of working with live animals, results are not instantaneous and we will not know whether the hormone treatments produced significant results or not until the eggs hatch and the growth of the young can be analyzed. However, we hypothesize that the primary impact of increased cortisol in the egg will result in increased hatching speed, so hopefully we will have a lounge of baby lizards very soon! The SRI program gave me the opportunity to gain skills in large-scale research projects, as well as animal husbandry and motion tracking software. This program has allowed me to meet and work with passionate people and researchers like Dan, experience the amazing RSB facilities and grow my laboratory skills as I delve further into my studies.

De novo assembly of *Serendipita talbotii* genome and identification of mating type loci

Felix James

Schwessinger Group | Supervisors: Benjamin Schwessinger and Celeste Linde

My project aimed to extract, sequence and assemble the genome of a recently discovered Australian fungus that forms forms a mycorrhizal association with orchids. After assembling the genome, I planned to identify the mating type locus of the dikaryotic sample.

Mycorrhizal fungi are crucial to the maintenance of robust forest ecosystems, and as such, are becoming increasingly valuable in conservation biology. In order to understand the population structure and diversity of Australian mycorrhizal fungi, we must investigate their genomes and mating mechanisms.

DNA was extracted using fungal-specific methods developed by Ashley Jones and Benjamin Schwessinger, and sequenced using the Oxford Nanopore MinION technology. Basecalling, trimming, assembly, polishing and other processing steps were performed using a wide range of software packages. The genome was annotated with BRAKER2 and read mapping was visualised with Integrative Genomics Viewer (IGV). I performed a BLAST search to find the fungal mating type loci.

My genome assembly was made with high quality, long reads and was ~51 megabase pairs. I ran into issues with phasing: while the fungus is dikaryotic, meaning it has two nuclei, my assembler only partially phased my data, so many contigs were collapsed" together where they should have been separated. Next time, I would use an assembler better suited to dikaryotic genomes. I identified a few candidates for mating type loci, and was able to demonstrate heterozygosity – essentially showing that the fungus had two mating types.

This program has been invaluable to my understanding of bioinformatics and research. I now feel prepared to tackle research at higher levels with confidence. The highlight for me was learning about the intricate steps of DNA extraction and sequencing in the lab, and assembling the first genome of a new species.

Parasite transporters responsible for the uptake of purines in *Toxoplasma gondii*

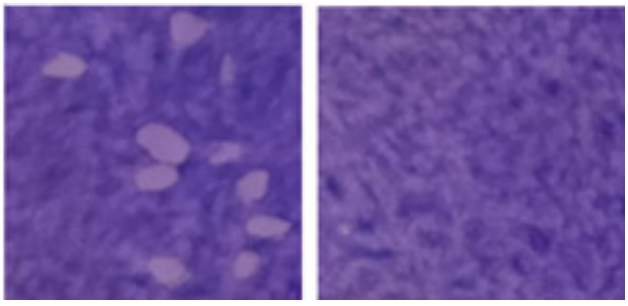
Rachel Leonard

van Dooren Group | Supervisor: Giel van Dooren

Toxoplasma gondii is an obligate intracellular parasite, and model organism of the apicomplexan phylum of intracellular parasites. *T. gondii* is the causative agent of toxoplasmosis and the life-threatening encephalitis, infecting a wide range of hosts including animals and up to 1/3rd of the adult human population worldwide. Unfortunately, the few pharmaceutical drug treatments for T. gondii have side effects and are unable to kill the persistent stage of the parasite life cycle.

Purines are essential components of nucleotides that comprise molecules such as DNA and RNA, and which serve as energy carrying molecules such as ATP. Given that *T. gondii* is unable to synthesise such important molecules the transport of these substrates is particularly interesting. However, despite an enormous incidence of infection and the importance of purine

uptake, how these parasites scavenge purines from their host remains poorly understood.



I sought to identify the parasite transporters responsible for the uptake of purines into the parasite scavenged from the host organism. In humans, equilibrative nucleoside transports (ENTs) transport certain nucleosides across the cell membrane. My project focussed mostly on three ENT homologues encoded in the *T. gondii* genome that I termed *Tg*ENT1, *Tg*ENT2 and *Tg*ENT3. To understand the importance of *Tg*ENT3 for parasite proliferation, I generated a parasite strain where I could "turn off" *Tg*ENT3 expression upon the addition of a small molecule; anhydrotetracycline (ATc). Using fluorescence and plaque assays, I was able to determine that the loss of *Tg*ENT3 expression resulted in a severe defect of parasite growth. This indicates that *Tg*ENT3 is required for the survival of these parasites. Many questions still remain including the localisation of *Tg*ENT3 and determines the substrates that this protein transports.

Investigating reactive oxygen species in early nodulation

Alex Lu

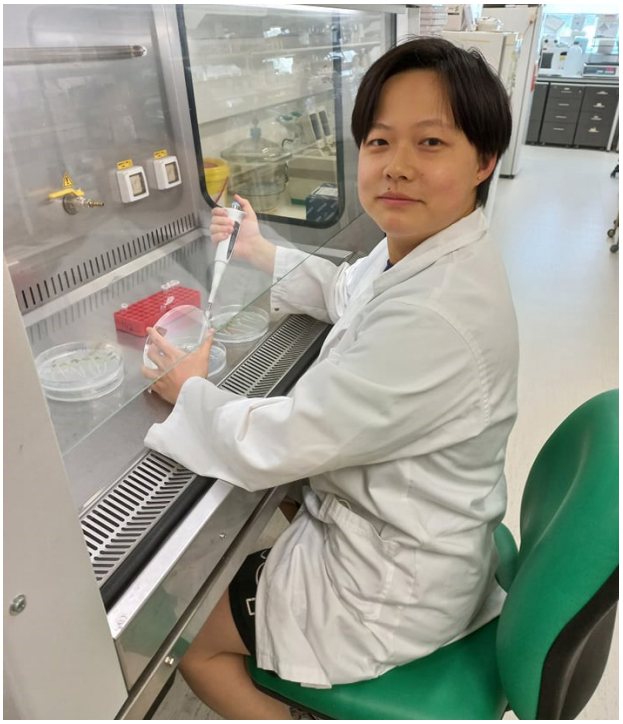
Mathesius Group | Supervisors: Angus Rae, Ulrike Mathesius

The main aim of my summer research internship was to determine the factors affecting reactive oxygen species (ROS) production during the early stages of nodulation in the model legume *Medicago truncatula.* ROS is associated with the symbiotic relationship between legumes and nitrogen fixing rhizobia. To understand this nitrogen fixing symbiosis in legumes as well as potentially implement it in other crops, it is important to learn the function of the ROS response in early nodulation.

We inoculated *Medicago truncatula* seedlings with 4 different *Rhizobia* treatments: Positive control (wildtype *Sinorhizobium meliloti* 1022), negative control (BMM media), ExoY (immunity-activating exopolysaccharide-deficient strain), and NodC-(Nod factor-deficient strain). To assay ROS levels, roots were stained with the ROS-reporter dye CM-H2DCFDA and viewed under a fluorescence microscope. To study the role of flavonoids in ROS production during nodulation, we performed a 'hairy root' CHSi transformation to create roots which could not produce flavonoids and then performed a rescue assay to check if ROS can be restored with specific flavonoid candidates.

Compared to wildtype, ExoY showed a positive response but not stronger than the wildtype, while NodC-had a negative response. This indicates that Nod factors might be essential for initiating ROS but not the activation of immunity. The flavonoid candidate, Afromosin, restored ROS response in half samples. However, ROS response in control was much higher than expected, so we could not make a conclusion favouring its role in producing ROS.

The summer scholar internship made this summer special and valuable to me. It was the first time that I learnt trouble shooting in research, and I really enjoyed the process of solving problems and getting our protocol to work! Everyone in my lab is so nice and friendly, and they are always patient to answer my dumb questions. During the whole project, I learnt time management skills, experiment skills, and even got career advice from people in my lab!



Genetic mechanisms underlying changes in flower colour of *Glossodia major*

Grace Marsh

Peakall Group | Supervisor Darren Wong

I was fortunate to explore the genetic mechanisms governing the change in flower colour of the local orchid species, *Glossodia major*, between the common purple and rarer white form of the plant. I assembled the transcriptome of the two floral colour forms of

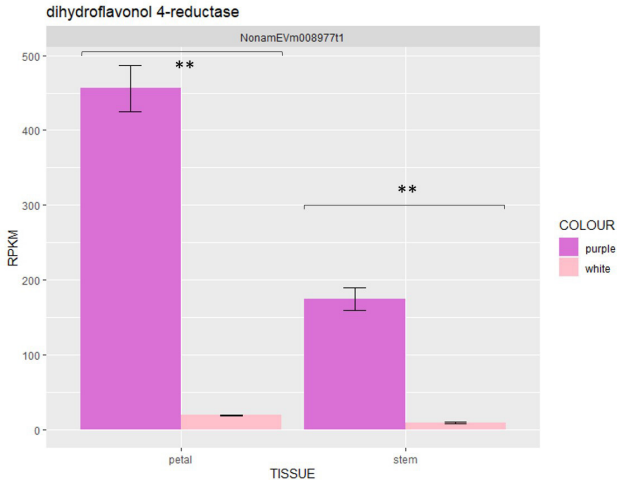
White and purple form of the orchid *Glossodia major*

this species using sequenced reads, and performed several quality control measures and functional gene annotation. Ultimately, I searched for differentially expressed genes relevant to the anthocyanin biosynthesis pathway, to find key genes involved in this observable colour difference of the orchid.

Flower colour is an important aspect of pollinator attraction, with changes in flower colour resulting in ecological impacts driven by the plant and pollinator interaction. Understanding the mechanisms of colour change in flowers requires insight into specific pigment biosynthesis pathways, as explored in this system through genes involved in producing anthocyanin, a blue to red pigment often responsible for the purple colour of flowers.

Sequenced petal, stem and leaf tissue samples, of the purple and white *G. major* orchid, were provided, allowing me to investigate this colour change at the molecular level. Investigation of these samples led me on a journey through a varied array of bioinformatic programs, from processing reads with FastP, creating de novo assemblies with Trinity and SPAdes, and the assessment of these assemblies by BUSCO gene completeness scores. Further, the Trinity assembly was clustered by the Corset and Evigene programs, with the latter used for read alignment by Bowtie2, and read quantification by FeatureCounts. This expression was then compared between the purple and white samples for both tissue types concerning the anthocyanin biosynthesis pathway, using DE analysis programs, EdgeR and DESeq2, in addition to the annotated assembly by Mercator.

From a large number of structural anthocyanin biosynthesis pathway genes scored, the differential expression of the dihydroflavonol 4-reductase (DFR) gene was the only candidate correlating with tissue specific distribution of purple colour. This distribution was observed as significantly reduced expression of DFR in the white samples across both tissue types, suggesting the involvement of this gene in the colour change of *G. major.* This was an exciting find, as the DFR gene has known effects on petal colour in other flowering plant species, and is regarded as a mutational hotspot. I was amazed to learn that there are even examples of the transgenic introduction of DFR in other flowering plants, allowing the curation of flower colour. Exploring the mechanisms underlying the colour change of *G. major* was a rewarding journey, from understanding new biological systems to harnessing the programs involved in this investigation. Not only were these bioinformatic programs entirely new to me, but I had never coded from the command line before, and I greatly appreciate the guidance I received from my supervisor, allowing me to use the high-performance computing servers to unravel the genetic data of the *G. major* orchid. Most of all I enjoyed the immersion into real research that this experience provided, and the supporting environment of other researchers at RSB.



Designing synthetic CO_2-fixing protein nanocages

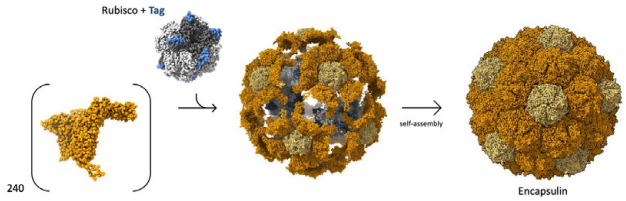
Nathan Paul

Whitney Group | Supervisors: Spencer Whitney and Tim Rhodes

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key photosynthetic enzyme involved in catalysing carbon fixation from atmospheric CO_2. Plant growth is often limited by the slow carboxylation properties of Rubisco and its competitive inhibition by O_2. In nature, many organisms have evolved carbon concentrating mechanisms (CCM) that concentrate CO_2 around Rubisco to maximise the rate of carboxylation and mostly prevent O_2 inhibition. In cyanobacteria, the CCM includes housing Rubisco in a protein organelle called a carboxysome, whose multi-protein componentry makes it difficult to fully reproduce in heterologous hosts such as leaf chloroplasts.

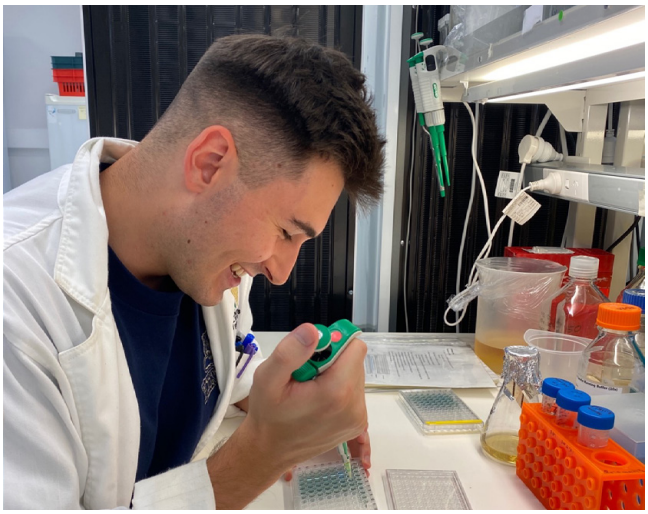
My Summer project aimed to use *Escherichia coli* protein expression to test whether Rubisco could be packaged into an encapsulin cage, an emerging class of structurally simple, self-assembling protein nanocages produced in many archaea and bacteria comprising homo-oligomeric protein shells. To test this, I appended a short encapsulation signal peptide (ESig) to the small

(S) subunit of Rubisco. While I found the appended ESig epitope slightly impaired the capacity of the S-subunits to assemble with the large (L)-subunits into functional hexadecameric L8S8 complexes, it did successfully direct the packaging of functional Rubisco into the encapsulin protein nanocages. These preliminary findings pave the path towards the development of structurally simpler synthetic CO_2-fixing protein nanocages of potential future use in ongoing ventures to enhance plant and algae photosynthetic productivity.



The *Quasibacillus thermotolerans* encapsulin self-assembling around tagged Rubisco

The program allowed me to develop a range of molecular biology tools and techniques, such as Golden Gate cloning, protein expressions, PAGE analysis and immunoblotting, all of which will greatly assist my transition into a Biochemistry Honours.



Modelling contemporary evolution

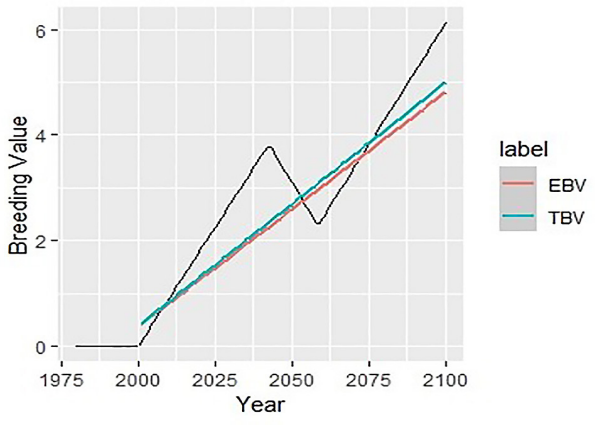
Daniel Pavlich

Jennions Group | Supervisor: Timothee Bonnet

The project I undertook for my summer scholarship was concerned with the statistical modelling of contemporary evolution. Climate change is causing more extreme fluctuations in environmental conditions as well as altering the average conditions observed. Thus, it would be reasonable to assume that evolution is likely fluctuating in conjunction with the environmental conditions. Despite this hypothesis, most contemporary research uses linear models to predict the evolution of a population. The project I was involved in seeks to determine how effectively linear models of a fluctuating environment can predict evolution, and hence works towards understanding the possible changes we may see in plant and animal populations in the future.

Our methodology was relatively simple. I used the statistical animal model with a MCMC (Markov Chain Monte Carlo) algorithm to estimate the breeding values of a simulated population. After repeating this simulation 100 times on the NCI (National Computer Infrastructure), I changed the environmental input and ran 100 more repeats. These environmental situations that I constructed differed from each other via the duration of the deviations from their overall trend. We found that while our models contained no bias, they became less precise as the duration of the fluctuation increased. Thus, using linear models to predict the future evolution of animal populations in response to fluctuations in climate is flawed, and other methods should be explored.

This project taught me many completely new skills, such as coding and data analysis in R, workflow documentation in RMarkdown, how to interact with the command line and use the NCI, and how to collaborate with others in Github. I was also given a taste of independence in the research environment that I had not previously experienced, being truly treated as an equal by my supervisor Timothee. I had a truly incredible time for the whole duration of the program and can't recommend it highly enough to those looking for research experience.



A novel assay for plant defence detection

Elise Rawlinson

Schwessinger Group | Supervisors: Benjamin Schwessinger and Salome Wilson

Wheat forms a staple of most people's diets, contributing to the foods containing carbohydrates that are on the bottom of the food pyramid, as well as being a key industry in Australia worth around $6 billion. However, plants susceptible to fungal infection cause large amounts of economic losses and waste despite the use of pesticides. Genetic modification of commercial wheat to confer innate resistance would reduce these costs and wastage. Mechanisms of pathogen virulence are also very important to understand for pathogen monitoring, something that is currently being applied by the Schwessinger lab to identifying variants of COVID-19.

My project involved developing an assay capable of detecting when a defence response is induced by a pathogen in wheat protoplasts. The assay harnesses advances in synthetic biology to construct a switch mechanism that fluoresces when a candidate gene activates a defence response in wheat. This allows for rapid screening of avirulence and resistance genes in fungi and wheat respectively. A key point of the assay is the use of synthetic genes in the plasmid to create an amplification loop that increases the sensitivity of reporter expression and allow for easily observable results. I used Golden Gate cloning to construct a library of 14 candidate plasmids to be used in this protoplast assay. These are then to be tested and compared to optimise the assay's detection and sensitivity. This will facilitate the rapid and accurate screening of any pathogen avirulence genes or host resistance genes that are thought to be involved in fungal infection of wheat.

I've really valued this experience and the opportunity to contribute to an important and relevant field of research. I'm grateful for the skills that I have learned, the support I have received from the Schwessinger lab and the RSB, and the new appreciation I have for the complexity of our world.

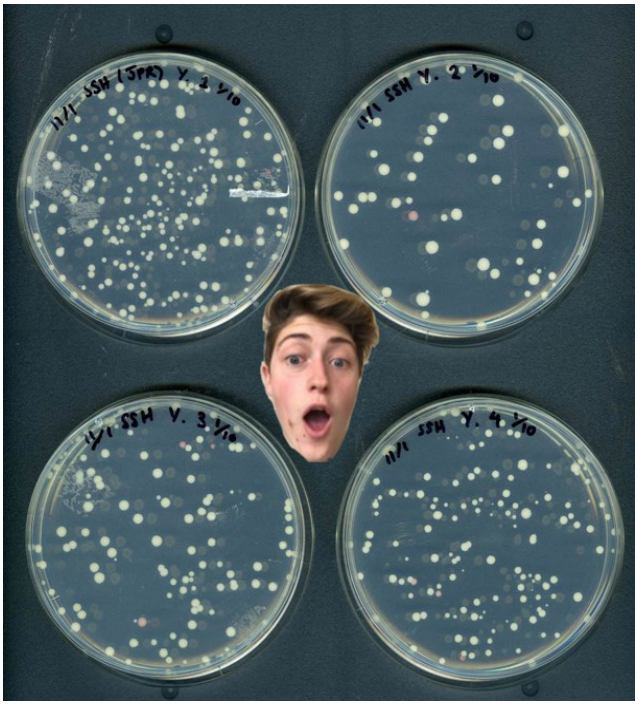
Keeping up with the pathogens: Assaying interactions between cereal crop resistance genes and pathogen effectors

Sissi Scott-Hickie

Rathjen Group | Supervisor: Xiaoxiao Zhang

Over the summer, my project aimed to create a system in yeast to test for interactions between resistance proteins and effectors. I made and modified constructs of the proteins of interest to be used in a yeast mating based split ubiquitin system (MbSUS) which is a split protein assay where interactions between proteins of interest can be selected for by yeast growth.

With cereal crops comprising 50% of the world's dietary intake, ensuring high yield crops is essential for continuing food security. One way to safeguard this resource is to better understand and build upon plant's natural resistance to pathogens to prevent the spread of disease in crops.



My research consisted of two parts, making the bait and then the prey constructs to be used in the MbSUS system. To make the bait constructs I has to perform an SDM on the original vector to insert restriction enzyme sites to allow the insertion of signalling membrane anchor protein to anchor the construct to the cell membrane. I then made prey constructs by transforming vectors and inserts of effectors or R proteins into yeast. Successful bait and prey constructs were selected for through digestion with specific enzymes and gel analysis as well as sanger sequencing results.

Over the course of my project, I was able to successfully insert the desired restriction enzyme sites into the plasmid at a specific location. I also created prey constructs containing either effectors or R proteins to be used in the MbSUS system. I also attempted to insert the Exg2 anchor protein into the bait construct plasmid but was unsuccessful. Hopefully, with the progress I have made towards creating the constructs for the system, my supervisor can create the final system to be used for further research.

The Covid-19 pandemic has reduced the number of hours I've been able to spend in a lab over the past two years of my undergraduate degree. However, the SRS program allowed me to gain valuable experience not only in a lab environment but also in the context of a real work and research environment. While it goes without saying I've met some great people, I know the skills and experiences I've acquired over the summer will be indispensable in the years to come.

Investigating the function of an essential enzyme (dhodh) in *Toxoplasma gondii*

Paremila Shanmuga Nathan

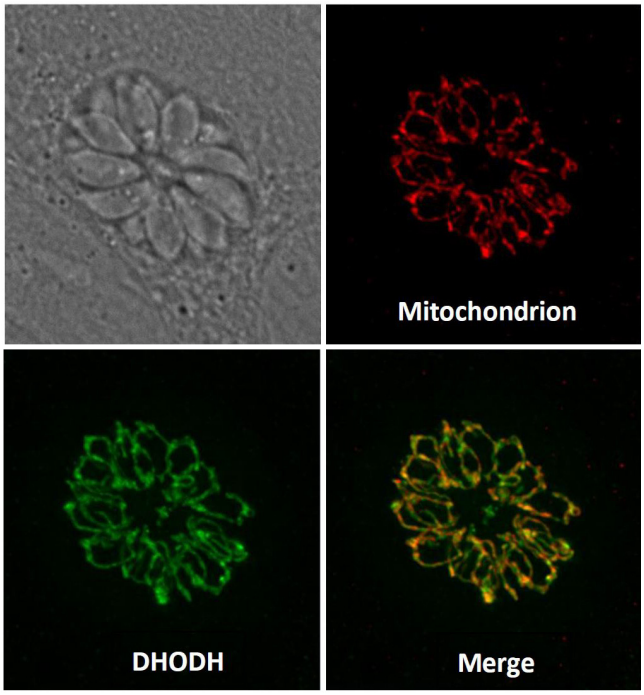
van Dooren Group | Supervisor: Giel van Dooren

Toxoplasma gondii is an intracellular parasite that is capable of invading and replicating in any warm-blooded nucleated cell of a host. Toxoplasmosis can be fatal in immunocompromised individuals and congenital infection in pregnant woman could give rise to miscarriage and fetal abnormalities. Current approaches in treating toxoplasmosis includes a combination of anti-parasitic drugs in which effect of drug toxicity in patients and drug resistance is a constant issue. Thus, essential proteins needed to be studied to potentially discover new drug targets for a reliable treatment.

Many clinical drugs target pyrimidine metabolism because it is essential for parasite growth during infection. For instance, the electron transport chain (ETC) inhibitor atovaquone kills malaria-causing parasite *Plasmodium falciparum*. This is because the ETC is required for the activity of the protein dihydroorotate dehydrogenase (DHODH) which is involved in the pyrimidine biosynthesis pathway. If exogenous pyrimidine (e.g. uracil) is supplied, *P. falciparum* is no longer susceptible to atovaquone. Further, *P. falciparum* lacking DHODH can only survive in the presence of excess pyrimidines, suggesting DHODH is an essential enzyme and potentially a promising drug target. However, the function of DHODH is unclear in *T. gondii* because DHODH-knockout approach has been unsuccessful to date.

My main objective during my summer project was to characterize the function of DHODH. Since *P. falciparum* is less sensitive to atovaquone when there is excess uracil supplied, I questioned if it's the same in *T. gondii*. I performed a growth assay with the presence or absence of uracil to test the susceptibility of *T. gondii* to atovaquone. I found that the parasite was equally susceptible in both conditions, suggesting that, unlike malaria parasites, the ETC is vital in *T. gondii* for other functions than pyrimidine biosynthesis pathway.

Next, I set out to determine whether DHODH is expressed in *T. gondii.* I introduced a FLAG epitope tag into the native locus of *Tg*DHODH using CRISPR/Cas9 genome editing. I then performed an immunofluorescence assay and found that *Tg*DHODH localized to the mitochondrion (merge picture above). To test if *Tg*DHODH is part of a mitochondrial multi-protein complex, I undertook blue native page to separate the *Tg*DHODH protein and did western blotting to detect them. Interestingly, I found that *Tg*DHODH is, in fact, part of 2 complexes unlike in other organisms where this protein exists in monomeric unit. My future aims to achieve are characterize the complexes and identify their functions.



Determining the thermal tolerance range of alpine, desert and temperate plants under dehydration stress

Sarah Woodcock

Nicotra Group | Supervisors: Veronica Briceño Rodriguez and Pieter Arnold

The objective of this project was to determine the thermal tolerance range of plant species from alpine, desert and temperate biomes after different levels of leaf dehydration. The thermal tolerance range is the temperature range within which the leaf can function (e.g. -20 – 40 degrees).

This was done by dehydrating whole leaves in falcon tubes with different salt solutions to create 100%, 90% and 85% humidity conditions.

Leaves were then placed under an imaging PAM, a machine that measures chlorophyll fluorescence, with a Peltier plate set to reach either -20 or 60 degrees. The imaging PAM detected a large jump in fluorescence, which occurred when the leaves responded to either freezing or heating. From this we can see the temperature at which different leaves respond to temperature to determine the thermal tolerance range of various species from three different biomes.

These experiments will help us understand how changes in weather and overall climate will affect various species and whether plants from different biomes are more or less tolerant to temperature changes. The project is still in progress so there are no findings as of yet.