Australian National University

Research School of Biology Summer Scholars ePortfolio

2019/2020

ANU COLLEGE OF SCIENCE

INVESTIGATING THE PHYLOGEOGRAPHY OF AUSTRALIAN SPECIES

Eoin Nobel, ANU
Supervisor: Craig Moritz

The main objective of my project was to build a database of the genetic variation within and across a number of species. Through a meta-analytic framework, I was able to collate phylogenetic, morphological and distribution data on a wide variety of species. The results from my project will be used in the new phylolink program in the Atlas of Living Australia, providing up to date information on diversity within and between species. The information could be used by taxonomists, scientists and the general public to inform them of the variation present within a single species.

The taxonomy of species has historically been based upon morphological characteristics but with recent improvements in genetics, a revision of many species is required. A detailed knowledge of the distribution and genetic structure of a species is paramount to properly conserve and protect that species. With increased urbanization we can expect animals to become more genetically fragmented and being able to predict and understand these processes will enable conservation efforts to be maximized. Through identifying distinct lineages and even possible avenues for new species, we can strengthen management approaches and the estimates of biodiversity.

The SRS Program gave me an opportunity to gain experience and expertise in fields for which I have a strong passion. The community within RSB and the Moritz group have been extremely supportive and have made my project memorable. The skills and techniques gained in my summer project will be invaluable for any career path I take in the future!

PLANTS IN EXTREME ENVIRONMENTS

Tash Salisbury, ANU
Supervisor: Adrienne Nicotra

Throughout the summer scholar program I did fieldwork in Kosciousko National Park (alpine) and Gundabooka National Park (desert) looking at the ability of leaves from species in each location to deal with desiccation. The project follows the protocol from an international project by Leon Bravo in Chile, which aims to find plants in extreme environments that maintain a high photosynthetic rate with the ability to tolerate stress. The stress tolerance mechanisms of these plants can then be studied from a physiological to molecular level in order to understand how they maintain production under stress. With an increasingly harsh and unpredictable climate in the future, implementing these traits into crops is one way to better prepare the world's agricultural industry.

In my project, we selected 10 species from both field sites, harvested leaves and exposed them to varying levels of desiccation, and then allowed them to recover, taking fv/fm measurements and each point. On preliminary analysis, it seems that leaves from *Acasia anuera*, or Mulga (a desert species), has a high ability to tolerate desiccation stress. In the alpine, *Aciphylla glacialis* and *Nematolepis ovatifolia* seem to be the most stress tolerant. Overall, I loved the experience to collect real data and work in the field with an incredible group of researchers. The program provides such a great opportunity to be exposed to the academic world and develop new skills.

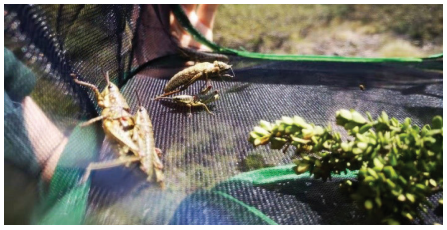
HOW DOES THE ENVIRONMENT OF PARENTS INFLUENCE OFFSPRING TRAITS?

Xinran Miao, ANU
Supervisor: Megan Head

Nowadays, warming temperature, caused by continuous high-level CO_2 emissions, lead to climate change, which is a challenge for animals' regular growth and reproduction.

I'm interested in exploring how animals to adapt to such environmental change and how increasing temperature affects organisms. Fortunately, I had the opportunity to join Megan Head's lab to take part in a project which aims to find out how the environment of parents influences offspring aging in seed beetles.

This is project involved large-scale lab experiments on seed beetles, therefore I conducted the experiments together with two other interns. At the start of the experiment, we learned about seed beetle husbandry with mung beans, how to identify their sex as well as the copulation process. After mastering the basic background knowledge and technology, we set up the parents at different environments (temperatures 25°C and 30°C) and then let the seed beetles from each temperature to copulate, allowed the females to lay eggs on beans and then waited for the offspring to hatch. Once the offspring hatched, we started to measure their traits, such as weight, metabolism, immunity, and lifespan. We also let the offspring copulate and recorded their fertility by counting how many eggs the females laid or measuring the weight of ejaculated sperm by males.



During our time in the lab, Megan also gave us opportunities to assist with other projects in her lab as well. For example, we also went to the field to collect some grasshopper samples and I learned to breed Eucalyptus beetles. This helped be to understand what research area I might be interested in studying future. Overall, this SRS program gave me opportunities to learn to use more advanced equipment, gain experience in experimental techniques and work with some excellent researchers.

The program also enabled me to meet many people from across ANU involved in the summer scholar program, make new friends and learn how to work collaboratively. All of this helped me to understand what it is like to conduct real research and helped me decide on the direction of my further studies. It was definitely a very valuable experience for me.

GENOME ANNOTATION OF A PARASITIC FUNGUS

Anna Sharp, ANU
Supervisor: Benjamin Schwessinger

The objective of my project was to compare a reference genome of the fungus *Punccinia chondrilla* with other known genomes to identify particular genes, proteins, enzymes and structural elements. These comparisons could then be used to study the evolution of the fungus since its introduction into Australia to control the introduced pest skeleton weed, *Chondrilla juncea.* This weed is a serious agricultural pest in Australia, so the fungus was introduced as a form of biocontrol, to control the spread of the weed.

To study the genome of *P. chondrilla*, I used various genomics computer programs to study the genome, and identify useful genes and other features of the genome. Through this project, I learnt an awful lot about computer programming, in particular how to use the command line to navigate another computer from my laptop. Sadly, the internship was cancelled before completion. In future, the data could be used to make phylogentic trees showing the evolution of the various fungal strains, and even determine if they have sexually reproduced with each other. By finding out what proteins are encoded in the genome, we could study how the fungus is able to infect the host plant. A key highlight for me was a workshop into computing for bioinformatics, teaching python and how to navigate the command line. The internship was a fantastic opportunity to experience university-level research, both in the field and in bioinformatics.

MONITORING THE LEAF TEMPERATURE OF ALPINE PLANTS

Kaitlyn Spooner, ANU
Supervisor: Adrienne Nicotra

Apart from assisting with other areas of the research lab, my individual project

was to monitor leaf temperature of alpine plants in the field. Leaf temperature affects fundamental processes related to plant function, such as the rates of respiration and photosynthesis. When leaf temperature differs from air temperature, it may suggest that the species has evolved the capacity to actively manipulate its temperature, whether to avoid dangerous extremes or to optimise growth rates. Variation between leaf and air temperature can also have important implications for ecosystem-scale processes such as the carbon and water fluxes.

In Kosciuszko National Park, I used thermocouples (wires connected to a data logger which can be used to measure and record temperature) to continuously monitor leaf temperature for multiple alpine species out in the field. One thermocouple for each plant was set up to measure air temperature so that the relationship between leaf and air temperature could be observed. Once analysed, this data will contribute to our understanding of the relationship between leaf and air temperature in alpine environments.

Working in the field as part of a research team was a fantastic opportunity to experience how stimulating and rewarding ecological fieldwork can be. As a research scholar this summer, I not only developed a strong understanding of thermal

Thermocouple wire inserted into a *Eucalyptus pauciflora* leaf and secured with tape in Kosciuszko National Park.

tolerance in plants, but gained insight into the life of a researcher which will be invaluable as I proceed further in my own academic career.

INVESTIGATING THE BINDING MOTIF OF KEY ANIMAL DEVELOPMENTAL TRANSCRIPTION FACTOR CDX IN SPONGE SYCON CAPRICORN

Gabrielle Smith, ANU
Supervisor: Maja Adamska

The aim of my project was to determine the binding motif of the sponge *Sycon capricorn* transcription factor Cdx using chromatin immunoprecipitation and sequencing (ChIP-seq). The ***Cdx*** gene is critically important in animal development, and it is involved in the patterning and elongation of the body axis in bilaterians. Previous work by my supervisor and lab indicates that there is likely to be a *Cdx* gene in the sponge *S. capricorn*, however due to large evolutionary distances, the identity of this gene remains disputed.

Since sponges are hypothesised to be the earliest diverging lineage from the last common ancestor of known animals, the existence of the sponge *Cdx* gene could help us gain a greater understanding of the last common ancestor's developmental processes and body form. ChIP-seq is a technique that allows chromatin bound by proteins, such as transcription factors, to be isolated from cells, which can then be sequenced and analysed to determine where in the genome the protein of interest is bound. Since ChIP-seq can be a long process, I used frozen chromatin from my previous run of this experiment during my undergraduate degree, which came from a human cell line transfected with a GFP-tagged plasmid encoding the sponge Cdx transcription factor. There were also

control cell lines with known animal *Cdx* plasmids to be used to compare results. A GFP antibody was used to isolate chromatin of interest, which was then prepared into DNA libraries, ready to be sequenced. By determining the binding motif of the sponge Cdx transcription factor, we can compare it to known Cdx motifs from other model animals and determine whether a single motif has been conserved across animal evolution.

Due to the closures of the ANU campus throughout January I don't have any results yet! We're hoping that we'll be able to identify a binding motif from peaks of mapped sequencing reads.

The summer research internship program has been a great opportunity to enjoy research in a relaxed environment without the pressure of assessments during undergraduate study. I also had the opportunity to learn more about bioinformatics and complete two short courses from BDSI in R and statistical analysis. My internship really helped me become acquainted with the lab and RSB facilities and become more independent in the lab before starting Honours.

DIRECTING THE EVOLUTION OF THE CO2-FIXING PLANT ENZYME RUBISCO

Tanya Skinner, ANU
Supervisor: Spencer Whitney

Rubisco, the most abundant protein on earth, is a key photosynthetic enzyme involved in the fixation of carbon from atmospheric CO_2. However, the carboxylation properties of Rubisco remain inefficient and this limits plant growth. In a world of increasing crop demands, the enzyme is therefore a clear target for biochemical improvement.

Previous work has successfully expressed Rubisco mutants in *E. coli* as a means of laboratory-directed evolution to improve its carboxylation properties. My project in the Whitney lab aimed to develop an improved mutant selection system for lab-directed evolution of Rubisco, escaping the natural confines of evolution. I used Golden Gate cloning to assemble plant Rubisco mutants to be expressed and evolved in *E. coli*, with the hopes of improving carboxylation efficiency beyond what is achievable in nature. Additionally, using kinetic and binding assays I showed that amino acid changes in the small subunit of the Rubisco enzyme affect its biogenesis and carboxylation capacities. This provides a rationale for using lab-directed evolution and an appropriate mutant selection system to improve the catalytic efficiency of Rubisco.

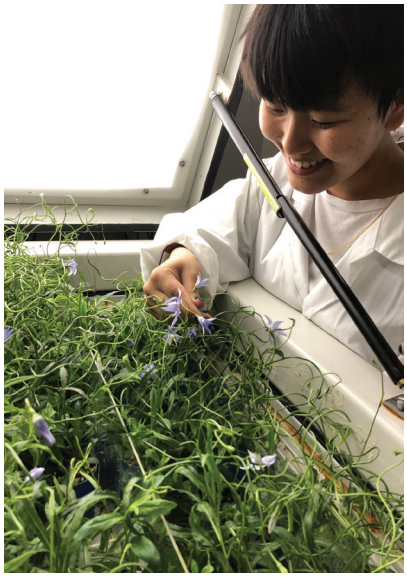
The SRI provided me with an opportunity to learn how to use a range of molecular and synthetic biology tools. Throughout my project I gained confidence in the lab and improved my skills in time management and multitasking. Most importantly however, my interest in this area of research has grown and I am looking forward to delving deeper into this project in my Honours year.

MULTITRAIT PLASTICITY: UNDERSTANDING PLANT RESPONSES TO A CHANGING CLIMATE

Tenzin Norin, ANU
Supervisor: Adrienne Nicotra

Phenotypic plasticity is the ability of an individual to alter its phenotype in response to environmental heterogeneity. Such alteration can affect the individual's performance under environmental shifts and may influence its fitness. Therefore, plasticity may play an integral role in determining the responses of individuals and populations to climate change. However, the dynamics and evolutionary roles of phenotypic plasticity are poorly understood.

My summer project aimed to address a sliver of this knowledge gap. My supervisors and I chose to study how plant traits and their respective plasticities change under contrasting thermal environments. We selected the Australian alpine herb, the waxy bluebell, *Wahlenbergia ceracea*, as our model plant. We grew hundreds of waxy bluebells under temperatures mimicking either current or future temperature conditions predicted under climate change using climate-controlled glasshouses. As the plants approached anthesis, we measured a variety of their functional, physiological and reproductive traits.

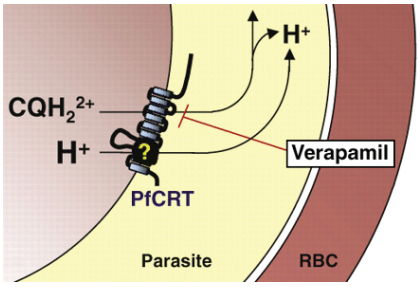


Amid my summer research, our glasshouses faced a sudden hailstorm onslaught and were heavily damaged. With holes on the roof, the plants were exposed to uncontrolled fluctuating environments for several days. We are unsure of how these conditions have affected our plants, but I will continue research on these plants through my Honours year at the Research School of Biology. This summer project allowed me to gain an appreciation of the value of team collaborations in scientific pursuits. Furthermore, I learned to navigate unpredictable mishaps in research endeavors. I'm curious to see where this research can lead us.

THERMOSTABILITY SCREENING OF CRT FROM PLASMODIUM BERGHEI

Rong Bao, ANU
Supervisor: Joseph Sydney Brock

Despite of the huge amount of research money and effort being invested in



developing new anti-malarial drugs, the remarkable potency of the parasites to evolve resistance has been an intractable obstacle to eradicate malaria. PfCRT, which is a characteristic transporter of *Plasmodium falciparum* that contributes to chloroquine resistance, is thought to be of great structural importance in understanding the resistance development. In this project, we aimed

to screen for specific interactions of lipids with the protein CRT7, a homologue of PfCRT extracted from *Plasmodium berghei*. This will be very important for future structural experiments with x-ray crystallography and cryo-electron microscopy.

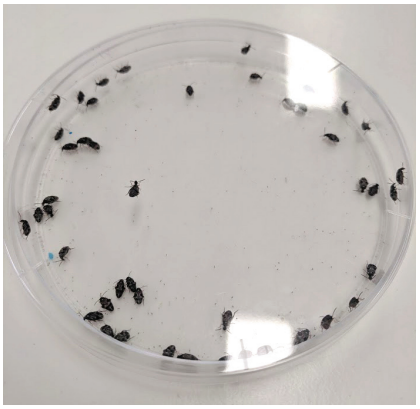
We used a simple thermal-shift assay (GFP-TS) to test the influence of various lipids on *P. berghei* CRT7. The lipids employed were pure solutions of phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and phosphatidylcholine (PC), and mixed lipids heart extract total heart bovine (HEB), liver total lipid extract bovine (LEB), brain total extract bovine (BEP) and soy extract polar (SEP). As compared to the negative control of no lipid interaction, we found that the thermostability of CRT7 is increased upon the addition of any of the lipids above. While PG increased the thermostability of CRT7 to a greatest extent. Further experiments based on this finding is required to investigate more deeply into the structural characteristics of the protein.

The project was short as it was interrupted by bushfires that closed the campus. Nevertheless I still have learned a lot, including the laboratory techniques and time management skills. Research is challenging, and can be upsetting sometimes. However, this experience has inspired me to go on doing research because of the beauty of exploring the mystery.

INVESTIGATING HOW THE ENVIRONMENT OF PARENT SEED BEETLES INFLUENCES THEIR OFFSPRING

Lydia Murphy, ANU
Supervisor: Megan Head

This summer I worked alongside two other interns and members of the Head Lab, on a large-scale experiment on seed beetles. The project aimed to investigate how the environment of parent beetles influence their offspring. Specifically, the experiment focused on how different rearing temperatures of males and females affected a number of traits in their offspring.



Initially our work involved setting up the parental generation of beetles; rearing half of the beetles in their natural environmental temperature and the other half in a hotter environment. We then performed crosses between males and females from both temperatures. When the offspring began to emerge, I was involved in measuring the male and female fecundity, metabolism, lifespan and setting up beetles for immune assays. The project is ongoing and once the measurements are completed, the data will be analysed to compare the trait measurements of offspring from different parental crosses. I am excited to hear about the results of our work later in the year.

As an undergraduate student this was my first time working in a research environment and I really enjoyed the hands-on experience. It was a big learning experience for me and the other interns, showing us some of the realities of working in research; from the hours spent weighing beetles and counting eggs to the extreme Canberra weather this summer (from smoke to hailstorms!). Despite this, everybody in the head lab was fantastic and working with them really made it a worthwhile and enjoyable experience for us.

While the seed beetle experiment was our primary focus for the summer, we got to experience a number of other opportunities both within the Head lab and in the RSB. One of my highlights of the internship was a day spent in Kosciusko National Park collecting Alpine grasshoppers for the lab. I was also able to help with looking after eucalypt beetles, enjoyed watching a number of PhD exit seminars, and met many other researchers and students working in the RSB.

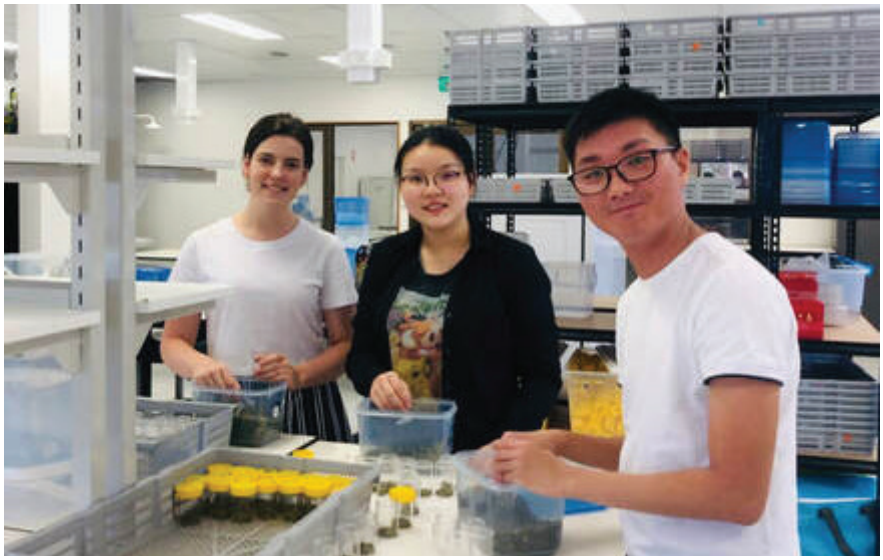
Overall, taking part in the SRI program was a really valuable experience and I would recommend the opportunity to any students interested in research.

INQUIRY INTO LONGEVITY: TRANSGENERATIONAL EFFECTS OF ANCESTRAL ENVIRONMENTS

Marvin Jin, ANU
Supervisor: Megan Head

Piqued by an interest to unravel the workings of longevity, the Head Group collaborated with the Noble Group to study the effects of parental environments on offspring performance. Using seed beetles as the study system, the team aims to tease out how different environmental temperature of parents and sex-specific effects influence a range of fitness parameters in offspring.

Climate change poses uncertainty how living conditions will be in the future. With a large proportion of organisms being ectotherms, it is crucial to understand how animals that employ ectothermy for survival respond to fluxes in temperature within the foreseeable future.



Lydia, Xinran and Jin (Larry, Mo and Curly).

In the experiment that we worked on, parent populations were subjected to different experimental temperatures and bred for several generations before offspring were tested for a range of performance parameters. These parameters served as proxies of fitness: (1) female fecundity, (2) male fitness, (3) longevity, (4) metabolism and (5) immunity. Colonies were maintained in climate-controlled insectaries of different temperatures to segregate the treatment and control groups. Besides measuring the number of eggs and ejaculate weight, the lifespans and metabolism of offspring were also measured respectively for further analysis for our hypotheses. Immunity assays would be administered to test subjects to test for melanisation, encapsulation and phenoloxidase activity.

Due to the scale of the experiments, we were unable to analyse our data before the end of the summer scholarship. However, preliminary observations do seem to imply a difference in the beetles whose parents were subjected to differential temperatures (i.e. sizes, lifespan of offspring). At the end of the internship we had completed half of the experiment and the team was embarking on the second half of data collection.

This summer has been a tumultuous one as Canberra was afflicted with adverse weather conditions. The bushfires and hailstorm definitely affected many of the staff in RSB and incapacitated many operations. However, I was lucky enough to have completed the full duration of my internship. This would not have been possible without the support from my colleagues and the school. The nature of this project presented many challenges but amidst of all the worries were the care and concerns from colleagues and the wider RSB community. Working with RSB has revealed to me the dedication and positive outlook one should adopt when conducting research. For that, I am grateful and proud to be part of the Head Group during this summer internship experience.

BIOINFORMATIC FUNGAL COMMUNITY ANALYSIS

Abigail Piscioneri,
University of Tasmania
Supervisor: Benjamin Schwessinger

Over the course of the summer research program, I had the opportunity to learn analytical techniques in bioinformatics. Using sequencing data from a mixed culture of fungi - a 'mock community' – my work aimed to help determine the best analytical pipeline for microbial material which was being collected from diseased wheat at a field trial site in Wagga Wagga, NSW. I was particularly enjoyed learning to use the bioinformatics platform QIIME2. It enabled me to investigate the mock community's species composition, relative abundance and comparative diversity, to understand whether this platform would give high-quality, correctly identified and well-analysed results without discarding a large amount of the genetic information in each sample. Because the composition of the community was already known, I could compare the results I generated to the expected ones, to understand how well my pipeline had performed. By comparing the success of this pipeline to other methods for sequencing and analysis, I was able to help determine the most effective analytical pipeline could be determined for use by a PhD candidate in their analysis of the experimental field trial samples.

I had a brilliant experience with the RSB SRS program. My supervisor and the other members of the Schwessinger lab were so friendly, and more than ready to help answer my frequent questions. A particular highlight was getting to join other members of the lab in a sampling trip to the field trial in Wagga Wagga, which really put the research into perspective. I'm very grateful for the new skills and knowledge I've developed as a result of the program, and understand microbial and genetic information in a very different way.

ADVANCED SUMMER SCHOLAR TRAINING MODULE – BIOINFORMATIC STARTER

This year saw the roll out of the first Advanced Summer Scholar training module – Bioinformatic Starter. This 3-day workshop, run by Dr Benjamin Schwessinger and Dr Jana Sperschneider, provided training in using command line, remote server connection, software installation and the use of python, pandas and github to handle large csv files and plot data.

The workshop proved to be "really helpful and valuable to have the bioinformatics tutorial. Having the one-on-one support while also being in the group environment was great" (Abi Piscioneri). The workshop was "massively helpful in helping me gain a foothold in coding and making the most of my computer. I now know how to utilize code to make my work quicker and more effective." (Jack Wess)