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Characterising potential symbiosome membrane proteins essential to the legume-rhizobium symbiosis

ELLA BREAR *Supervisors: Dr P Smith & Professor R Overall*
School of Biological Sciences, University of Sydney, NSW 2006

Legumes are important to society as a food source and are grown as alternatives to synthetic fertiliser application. A contributing factor to their importance is their ability to develop symbiotic relationships with nitrogen fixing bacteria called rhizobia. Rhizobia provide the legume with fixed nitrogen in return for energy and nutrients required for bacterial metabolism. The bacteria are housed in nodules where they are surrounded by a plant derived membrane called the symbiosome membrane. This membrane contains protein transporters that facilitate nutrient exchange between symbiotic partners. Our current knowledge of how nutrients are exchanged is limited. In order to fully appreciate this economically important symbiosis increased understanding is vital.

In this study I aimed to characterise potential symbiosome membrane proteins in soybean (*Glycine max*). I chose candidate proteins that were identified in a proteomic study of the symbiosome membrane, were predicted membrane proteins and had high nodule expression. The first section of my project involved using quantitative real time PCR to measure expression of candidates during nodule development. I showed expression increased before nitrogen fixation began and continued to increase in nitrogen fixing nodules. Suggesting the proteins are required during nitrogen fixation. To elucidate potential roles for the candidate proteins I attempted to reduce protein abundance by silencing gene expression using artificial microRNA. I also attempted to confirm localisation to the symbiosome membrane by internally fusing green fluorescence protein and observing location of fluorescence. Both microRNA and GFP constructs have been introduced into soybean and transgenic nodules are developing.

The expression levels during development suggest candidates are required for the symbiosis. Hopefully artificial microRNA silenced plants will demonstrate potential roles, facilitating the design of future functional studies.

Plasmid stability determinant of pSK1 from *Staphylococcus aureus*

HELENA CHAN *Supervisors: A/Professor N Firth, Dr. A Brzoska, Dr. S Jensen*
School of Biological Sciences, University of Sydney, NSW, 2006

Staphylococcus aureus is a bacterium that is a common cause of hospital-acquired infections and is also becoming a significant cause of serious community-acquired infections. Many clinical strains of *S. aureus* carry extrachromosomal DNA elements called plasmids, which often encode genes conferring antimicrobial resistance. Plasmid segregation mechanisms such as active plasmid partitioning systems ensure that low copy-number plasmids are accurately segregated and inherited by progeny cells, even in the absence of selection. Such systems underlie the stability of resistance-conferring plasmids and the persistence of antimicrobial resistance.

The staphylococcal multiresistance plasmid, pSK1, contains a gene, *par*, which increases plasmid segregational stability, possibly via an active plasmid partitioning system. pSK1 *par* is unusual because it encodes only a single protein, Par, rather than two proteins encoded by recognised partitioning systems. Despite this, studies have shown that Par exhibits both the DNA-binding and filament-forming activities that characterise active plasmid partitioning systems.

This project focused on the disordered and highly-charged C-terminal domain of pSK1 Par. To determine the possible functions of this domain, molecular cloning techniques were used to generate a pSK1 mini-plasmid that encodes a Par C-terminal mutant. Plasmid segregational stability assays in *S. aureus* showed that this plasmid was lost from a growing population at a similar rate as plasmids that do not carry any *par* gene, suggesting that the C-terminal domain is crucial for pSK1 plasmid segregational stability. An overexpression plasmid encoding the pSK1 Par C-terminal mutant was also generated to enable purification of the mutant protein for further functional analyses. However, repeated attempts to overexpress this protein in *E. coli* have so far been unsuccessful. Determining the impact of mutations in the C-terminal domain of pSK1 Par will further our understanding of the mechanisms of plasmid segregational stability, and provide insights into controlling and preventing the spread of antimicrobial resistance in bacteria.

An Endophytic Enigma: Exploring interactions between dark septate endophytes and plants

YVONNE CHANG *Supervisors: A/Professor P McGee & Dr Jenny Saleeba*
School of Biological Sciences, University of Sydney, NSW 2006

Melanised Ascomycetous fungi isolated from roots, termed dark septate endophytes (DSE), frequently colonise healthy plants. The ubiquitous presence of DSE in a broad range of plant species and environmental conditions, and their ability to provide plant benefits under some conditions, has led many to conclude that DSE provide important ecological benefits to their host. However, the distribution, ecology, host effects and the mechanisms of action of even the most studied DSE, *Phialocephala fortinii*, remain enigmatic.

DSE may act like mycorrhizal fungi; whereby the fungus increases mineral uptake by its host via direct transfer of minerals especially phosphate across the fungal-plant interfaces. Alternatively, DSE may influence plant function such that increased plant growth is a consequence of indirect factors, not direct mineral uptake.

The aims of my project were to:

- (1) Determine whether the presence of DSE increases plant biomass as a consequence of increased uptake of minerals.
- (2) Examine whether DSE change the environment in which seeds germinate and grow.

Trifolium subterraneum (forms arbuscular mycorrhiza) and *Brassica rapa* (non-mycorrhizal) were inoculated with one of three different DSE isolates, selected because they had previously been shown to increase growth of subclover. Plant biomass, shoot phosphorus, shoot chlorophyll and soil pH were measured at 3, 5 and 7 weeks.

The effects of DSE on plant growth and phosphorus uptake varied depending on the identity of the plant and the fungus, mineral availability and developmental stage of the plant. The differential growth responses under different mineral conditions, suggests that the fungus might increase plant mineral acquisition. However, the DSE only colonised plant roots to a very low extent and there was no reduction in root biomass compared to total biomass. Furthermore, isolates 48B and 222A significantly decreased or had no effect on soil pH. Both DSE isolates 48B and 222A had complex interactions with their hosts which are unable to be explained by the mycorrhizal model.

The effect of fire and post-fire rainfall on *Triodia basedowii* and small rodents in a desert system

AMY CHEN Supervisors: Professor C Dickman & A/Professor G Wardle
School of Biological Sciences, University of Sydney, NSW 2006

Fire can have long-lasting effects on desert biota as recovery depends on subsequent rain and on the pulses of primary productivity that it stimulates. I studied post-fire recovery of a dominant species of grass, spinifex *Triodia basedowii*, in the Simpson Desert of south-western Queensland, and also how native rodents respond to post-fire vegetation in this system. My aims were to identify how rodents use *T. basedowii* in areas of different fire history and also whether food or shelter resources influence rodent activity. Three species of rodents were used, *Notomys alexis*, *Pseudomys hermannsburgensis* and the larger *Rattus villosissimus*.

Over two sampling periods (May 2011 and July 2011), 39 animals were tracked using the spool-and-line method in both burnt and unburnt areas. Both small species foraged in areas with large amounts of ground-level seed, but *P. hermannsburgensis* moved preferentially under dense cover whereas *N. alexis* preferred to move in the open. Neither species, nor *R. villosissimus*, showed any response to areas with different fire history. To distinguish whether the availability of seeds or cover influenced the observed patterns, I placed dishes (4 dishes a station, 20 stations, 2 sites) in open and closed cover areas, with and without seed, and measured rodent activity using an index of their spoor. Both food and cover determined rodent activity.

The results suggest that 1) post-fire rainfall had stimulated sufficient growth of *T. basedowii* to support similar levels of rodent activity in the burnt and unburnt areas, and 2) the three species have different habitat preferences but seek sites where their foraging returns are maximised. The latter result indicates further that the rodents were food-limited. My findings show that post-fire rainfall readily ‘resets’ spinifex grassland and its rodent communities, but caution that more work is needed to identify the effects of long-term drought.

Patterns of lipoprotein lipase expression in the uterus of the Australian lizard the southern grass skink (Pseudemoia entrecasteauxii, Family: Scincidae)

OLIVER GRIFFITH Supervisors: Professor M Thompson & A/Professor K Belov
School of Biological Sciences, University of Sydney, NSW 2006

Nutrient provision across the placenta (placentotrophy) has evolved in as few as four lineages of amniote vertebrates (birds, reptiles and mammals), with three of these lineages belonging to the skink lizards (Family: Scincidae). In placentotrophy, a net uptake of a range of nutrients occurs, including lipids, which are the most important source of energy for developing reptilian embryos. I studied the Australian lizard, *Pseudemoia entrecasteauxii*, which provides approximately 60% of the lipid for embryonic growth and metabolism to embryos across the placenta. Lipoprotein lipase is an important enzyme in lipid transport in mammals, so I tested the hypothesis that lipoprotein lipase is expressed in the uterus of pregnant *P. entrecasteauxii*. I used reverse transcription quantitative real time PCR to measure the expression of the lipoprotein lipase gene. I examined lipoprotein lipase expression in the uterine tissue of non-pregnant as well as early, middle and late pregnant *P. entrecasteauxii*. I compared uterine lipoprotein lipase expression in the two placental regions of the uterus. Expression of the lipoprotein lipase gene is significantly higher in the uterus of late pregnant compared to non-pregnant and early pregnant *P. entrecasteauxii*, indicating a greater capacity for lipid transport towards the end of pregnancy. This result correlates with the time that developing embryos are undergoing the greatest growth and have the highest metabolic rate. Lipoprotein lipase gene expression was significantly higher in the uterine tissue adjacent to the yolk sac than the uterine tissue adjacent to the embryo, providing support for the role of the yolk sac placenta in lipid transport.

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Estimating feral honey bee (*Apis mellifera*) colony density in South Eastern Australia using linked microsatellite loci

ELOISE HINSON *Supervisors: Professor B Oldroyd & A/Professor M Beekman*
School of Biological Sciences, University of Sydney, NSW 2006

Crop pollination by honey bees (*Apis mellifera*) and other wild pollinators is an essential ecosystem service that ensures sustainable agriculture. Recent declines in the abundance and diversity of pollinating insects, particularly bees, indicate that pollination services may be at risk, which would have negative consequences for both agricultural and natural ecosystems. Feral honey bees perform the majority of crop pollination in Australia, but there are concerns that feral bees may have significant negative impacts on native fauna and flora. Robust estimates of feral and wild honey bee colony densities are essential for understanding the role of honey bees in an ecosystem and for management decisions.

Due to their cryptic nesting behaviour, estimating the density of wild honey bee colonies on foot is time-consuming and error-prone. Fortunately, a new genetic method is available that allows rapid assessment of the density of feral colonies. Using a set of five tightly linked microsatellite loci I genotyped male honey bees (drones) to estimate the number of drone-producing colonies within several Australian landscapes. Samples were collected from paired natural and agricultural sites within each landscape. Natural sites were located within national parks and included the riparian woodland of Wyperfeld National Park in north-west Victoria, the mugga ironbark woodland of Grenfell National Park in the central west of New South Wales and the dry eucalypt and rainforest of Barrington Tops National Park. Disturbed sites were located at least 10 km outside the national parks and were cleared for agricultural or horticultural purposes.

Preliminary results show that colony densities in these areas range from between 0.06–0.09 colonies/ha in natural areas and 0.03–0.08 colonies/ha in disturbed areas. These densities are far below the recommended number of hives for successful pollination, which is between 2–6 colonies/ha, depending on the cropping system.

Understanding the processes determining the spatial distribution of two temperate damselfishes

AMANDA HUEN *Supervisors: Dr P Smith & Professor P Waterhouse*
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Plants possess sophisticated genetic mechanisms for responding to environmental stresses. MicroRNAs are a class of small RNAs which regulate gene expression through post-transcriptional silencing. Certain microRNAs are present in the phloem during nutrient starvation, and may be translocated as long-distance signalling molecules.

My project examined the mobility of several nutrient starvation-induced microRNAs, and their potential structure during long-distance movement. Using quantitative reverse-transcriptase PCR, I measured the abundance of the phosphate starvation-induced microRNAs miR399d and miR827 in shoot-root graft combinations between wild-type and microRNA biosynthesis mutant *Arabidopsis*. I confirmed previous findings that miR399d moves between shoots and roots and demonstrated the shoot to root mobility of its complementary (star) strand. miR827 was mobile from shoots to roots but its star strand was present at negligible levels under the same conditions. The contrasting abundance of the star strands for these two microRNAs suggests that mobile microRNAs can undergo long-distance travel independent of the star strand.

I additionally determined that miR398a was mobile from shoots to roots, but its specific overexpression in copper-starved wild-type shoots grafted to microRNA biosynthesis mutant roots suggested that it is usually induced in the shoots during copper starvation, but subsequently repressed by a mobile root-derived factor. This factor appears to be induced via a microRNA-related pathway. I propose that miR398a is part of a novel autoregulatory loop requiring long-distance communication between shoots and roots, and acts to maintain miR398a expression at basal levels during copper starvation. This pathway may serve as a genetic fine-tuning strategy for the target genes of miR398.

My findings reveal the potential mechanisms of microRNA transport during the plant response to nutrient starvation, as well highlighting as the multi-layered nature of microRNA regulation in plants. Understanding these complex processes has applications for developing crop varieties with more efficient nutrient uptake strategies during nutrient-poor conditions.

Phylogenetics and Biogeography of the Australian terrestrial isopod *Spherillo grossus*

TIM LEE *Supervisors: Dr N Lo, Dr S Ho & Dr G Wilson**
School of Biological Sciences, University of Sydney, NSW 2006

At the beginning of the Oligocene, the rapid aridification of the Australian continent led to the contraction of the mesic biome (rainforests and sclerophyll forests) to the east coast and the south-west. During the Miocene and Quaternary, fluctuating climatic conditions resulted in the fragmentation and, in some cases, reunification, of mesic habitats. The phylogeographic effects of these habitat fluctuations have been studied in mammals, birds, and plants, but rarely in terrestrial invertebrates. Populations of small, slow-moving, endemic terrestrial invertebrates should show the effects of changes in the mesic zone over the past 30 million years.

My study species is *Spherillo grossus*, the most common endemic terrestrial isopod on the east coast of Australia. This isopod is wingless and is expected to have low dispersal ability. I collected *S. grossus* specimens from five localities in coastal NSW, and used specimens from museums (including the paratype) and other collectors from eight further sites in NSW and QLD, for a total of 91 specimens. I extracted, amplified, and sequenced DNA (16s, COI, 18s genes). Using phylogenetic methods, I investigated the intra-specific relationships in *S. grossus* and estimated the time of population divergences. This genetic study was complemented by morphological data, which I collected from seven representative specimens.

My results show that *Spherillo grossus* is a highly genetically variable species or perhaps a species complex. Up to 30% genetic divergence was found among specimens in the mitochondrial 16S rRNA gene. Molecular clock analyses indicated that the last common ancestor of the examined specimens existed some 30 million years ago. Relationships among specimens from different sites typically did not follow any clear geographic pattern. This probably reflects the changing range of *Spherillo grossus* over the past 30 million years, in response to the fragmentation and reunification of the mesic biome. I have also found strong evidence for human effects on the distribution of *S. grossus* populations, which are overlaid on the ancient lineage distribution. My scanning electron microscopic results show that there are no significant morphological differences between lineages.

A more complete understanding of the biogeography of endemic terrestrial invertebrates on the east coast of Australia will have implications for conservation and the response of the biota to future climate change, as well as giving us insights into the development of Australian invertebrate fauna in the Miocene and onwards.

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Role of actin cytoskeleton in abscisic acid signalling

JOHN LODGE *Supervisors: A/Professor J Marc*

School of Biological Sciences, University of Sydney, NSW 2006

The actin cytoskeleton forms a dynamic filamentous protein network that constitutes a major component of the plant cytoskeleton. The network functions in coordinating cell polarity where it directs the plane of cell division and cell wall synthesis, in positioning organelles within the cell, and in forming tracks for actomyosin-based cytoplasmic streaming. Abscisic acid (ABA), a plant growth hormone which elicits responses to environmental stresses including cold temperature and salinity, has been shown to induce long-term reorganisation of the actin cytoskeleton, although the early events in the process are unknown.

This project aimed to investigate how the actin microfilament network reorganises in response to ABA in various cell types in live cells in transgenic *Arabidopsis thaliana* (Columbia ecotype) seedlings stably expressing actin reporter gene *GFP-fABD2* (green fluorescent protein fused to the second actin binding domain of fimbrin 1). ABA was flooded directly over the seedlings and configurational changes of the actin cytoskeleton were monitored over time using either conventional fluorescence microscopy or confocal microscopy. In addition, immunolabelling of actin microfilaments in aldehyde-fixed tissue was performed to complement the results obtained with GFP-actin reporter gene technology. Wildtype *A. thaliana* (Columbia ecotype) seedlings were either grown on agar nutrient media supplemented with ABA or the seedling roots immersed directly in ABA solution. Whole roots were fixed with aldehyde solution, permeabilised with Triton X-100 and cellulose-digesting enzymes, and processed for indirect immunolabelling using mouse anti-actin antibody and FITC-conjugated sheep anti-mouse second antibody. Labelled actin microfilaments in the roots were examined using confocal microscopy.

Distinct actin microfilament arrays labelled with the GFP-fABD2 reporter in the transgenic *A. thaliana* seedlings were observed in cotyledon/leaf epidermal pavement cells, hypocotyl, root meristematic cells, epidermal cells in the root elongation zone, and in root hairs. The pavement cells typically showed thick endoplasmic cables and fine cortical filaments, which often formed parallel arrays arranged either transversely or longitudinally to the long axis of the cell. The fine filaments rapidly depolymerised within 25 min of exposure to ABA and subsequently formed thick cables, indicating extensive reassembly of microfilaments and bundling. Similar sequence was seen in the root elongation zone. Intriguingly, young root hairs showed unique, spirally arranged actin microfilaments, which rapidly depolymerised in response to ABA. In long, mature root hairs the actin microfilaments formed long spirals at shallow angles to the side walls and appeared to be more resilient to the ABA treatment. Overall, the results indicate that the earliest response to ABA in epidermal cells is rapid depolymerisation of the fine cortical microfilaments, followed by reassembly and bundling. Immunolabelling was only partially successful and would require optimisation of the protocol, although it confirmed the formation of the thicker cables seen with the GFP-fABD2 reporter.

The EAC Dude: Physical oceanography of the East Australian Current and its effects on larval fishes

PALOMA MATIS *Supervisors: Dr W Figueira & A/Professor R Coleman*
School of Biological Sciences, University of Sydney, NSW 2006

The East Australian Current (EAC) is a dominant force driving oceanographic processes off the east coast of Australia. Knowledge gaps are evident on the ecological effects of the EAC on the coastal shelf region. This study explores the role of oceanographic processes in the early life history of various fish species. Larval fish assemblages were sampled aboard the Southern Surveyor research vessel between the EAC separation zone, cold core and warm core eddies present at that time. Identification of larval fish to family or species level showed the assemblage offshore NSW is taxonomically diverse. Assemblage composition differed among the oceanic features and between locations within each feature, thus supporting the original hypothesis.

Otolith analysis was used to determine growth of fish sampled. Counting the number of increments on the otolith and dividing it by the length of the fish determined average growth per day. There was a relationship between growth and the oceanic environment, thus supporting the original hypothesis. Results would indicate the high food availability due to cold water upwelling in the cold core eddy produces optimum conditions for growth. However there are high levels of predation associated with these conditions. In contrast, low levels of nutrients and lower predation risk associated with the warm core eddy, provide poor conditions for growth but are advantageous for survival. These organisms play a pivotal role in ocean food webs and a main food source for human populations. An understanding of larval fish assemblages in the EAC is crucial considering climate change predictions a warming and strengthening of the EAC, which could significantly change the distribution and abundance of many fish species.

The distribution of limpets in a barnacle world

BEN MUSCAT *Supervisors: A/Professor R Coleman & Dr Richard Murphy**
School of Biological Sciences, University of Sydney, NSW 2006

Ecosystems are comprised of numerous organisms and processes functioning at a variety of spatial and temporal scales. Ecologists are charged with unravelling this variability to deepen our understanding of the underlying patterns that contribute to maintaining ecosystem function. Rocky intertidal ecosystems have a diverse range of organisms and numerous abiotic processes, all of which exhibit variability. Limpets are common in most intertidal ecosystems and function primarily as grazers, controlling algal growth along the shoreline. Barnacles are also common to rocky shores and among other roles contribute to the structural variability of the shoreline. Much is known about the distributions of both these organisms and the factors influencing their distribution. However, few studies have explored the way each organism's distribution is affected by the other. The primary aim of this study was to further characterise the distribution of limpets in areas of the rocky intertidal dominated by barnacles. Various models explaining the observed patterns were then tested. These included factors such as movement, surface complexity and food. All experiments were conducted at the Cape Banks Marine Scientific Research Reserve. The study found that limpets do inhabit areas with barnacles, though in significantly lower numbers than areas without barnacles. It also found that the percentage cover of barnacles can to a certain extent predict the number of limpets, and that the movement of limpets between areas with and without barnacles plays a role. A considerable amount of variability was also observed, suggesting that whilst barnacles may affect limpet distribution, there are likely numerous other factors involved.

** Dr Richard Murphy - Aust Centre for Field Robotics School of Aerospace, Mechanical & Mechatronic Engineering USyd*

The role of seed banks in plant population models

VUONG NGUYEN *Supervisors:* A/Professor G Wardle
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Seed banks of dormant viable seeds are critical for buffering plant populations against fluctuating environments. Seeds can be incorporated into a matrix population model to assess the persistence or time to extinction for threatened or invasive species. Despite this, the problematic nature of collecting field data for seed banks has resulted in its exclusion from many of the 400 published plant population models. In this study, I aimed to determine the following: 1) To what extent has the seed bank been excluded? 2) How does the seed bank influence population dynamics? 3) Are there any patterns that can be used to predict beyond the species level?

I added 75 new models to an existing database of >200 terrestrial plant species. A comparative review revealed that 49% of the studies since 2008 excluded the seed bank stage from their model. Numerical simulations on species from the database showed that populations of *Atriplex acanthocarpa* would persist longer with a seed bank in poor years, in contrast to other species where the influence of the seed bank was minimal. Changes in population growth rate for *Atriplex acanthocarpa* were also highly dependent on the germination rate used in the model. To test for patterns among different taxa, I produced a phylogeny of the species in the database and performed phylogenetic least-squares analyses to test for relationships between vital rate elasticities and sensitivities. I compared statistical models with and without phylogeny and found the model without phylogeny was preferred in all but one case.

These results highlight a substantial knowledge gap in our understanding of the effect of seed banks on population dynamics. While management of threatened and invasive species will be improved by incorporating the seed bank, my simulations demonstrate the need for field data on seed survival and germination to produce accurate models.

The implications of nutrient variation in arthropod food webs for carnivores

MICHAEL NORRIS *Supervisors: Professor S Simpson & Dr S Wilder*
School of Biological Sciences, University of Sydney, NSW Australia 2006

Carnivores can have direct and indirect effects on plant assemblages, prey populations and ecosystem processes. Whilst prey scarcity can lead to opportunistic foraging, recent evidence also suggests that carnivores can modify their foraging to regulate their intake of nutrients. I examined the role of trophic level in explaining phylogenetic variation in insect nutrient content and the potential consequences of this nutrient variation for carnivorous arthropods, especially spiders. Samples were collected from seven field sites in NSW and NT, representing 588 species from 25 orders and ranging in dry weight from 0.5-642 mg. My results indicated that carnivorous arthropods are confronted with a heterogeneous nutritional environment, with substantial variation in lipid (0 – 0.51 mg/100mg dry weight) and protein (0.02 – 0.89 mg/100mg dry weight). Some carnivorous prey, especially common spider prey, can have lower lipid content.

I then conducted focal studies of Australian wolf spiders (*Lycosa godeffroyi*) using phylogenetic pairs of herbivorous and carnivorous prey. Spiders ingested significantly more lipid and less protein when feeding on herbivorous compared to carnivorous prey. Furthermore, I found that the low amount of lipid in carnivorous prey can limit the lipid available for predators to assimilate into their body.

Trophic variation in the nutrient content of prey and the nutrients that carnivores can extract from prey has significant implications for food webs. Depending upon the nutritional requirements of carnivores, there could be strong selective pressures for carnivores to engage in omnivory or intraguild predation, which may affect food web stability, food chain length, trophic cascades or nutrient cycling.

Do some like it hot? The cost of thermal acclimation in *Gambusia holbrooki*

Nicky Rollings Supervisors: A/Professor F Seebacher, Professor M Olsson & Dr E Miller
School of Biological Sciences, University of Sydney, NSW Australia 2006

Organisms require the ability to adjust to changes in their environment. However, every adjustment may come at a cost. For example, acclimation may require an increase in metabolism to adjust at a cellular level and maintain performance. The main aim of this study is to assess the costs incurred as a result of temperature acclimation in the Eastern mosquitofish, *Gambusia holbrooki*, at the molecular level and, more specifically, with respect to telomeric shortening. Newly born mosquitofish were exposed to either 20 or 30 °C for 10 days. After this time, half of the fish from each treatment were randomly selected and gradually exposed to the opposite temperature to assess the cost of compensatory growth. The standard length of the fish (mouth to tail base length) was measured every five days to assess growth. The fish were maintained at these temperatures until their muscle tissue was harvested between 25 and 75 days after birth. These samples were analysed using real time quantitative PCR, and the telomeric sequences were amplified using specific oligonucleotide primers. Relative telomere length was calculated as a ratio (T/S) of telomere repeat copy number (T) to control single gene copy (S). Significant variations in growth rate were found across the different treatments, with the group exposed to 30 °C growing at a faster rate than those exposed to 20 °C. These results will be discussed in more detail. This study will demonstrate how variations at the organismal level can correlate with variations at the molecular level.

The importance of odours in horse fear communication

ALISA SANNIKOVA *Supervisors: Dr M Crowther & A/Professor Paul McGreevy*
School of Biological Sciences, University of Sydney, NSW 2006

Horses (*Equus ferus caballus*) kill or seriously injure more Australians than any other animal, posing significant risks to the people that interact with them. The major cause of injury is due to the horse's unpredictability due to sudden frights. The causes of fear or anxiety in horses has previously been studied in relation to behavioural traits and non-conspicuous factors in the environment. However, little work has been done to determine whether and how horses can transmit fear between each other, to match studies done on other domestic ungulates.

To determine whether horses consider odours important to investigate, I released horses into a round yard containing fresh horse faeces, fake clay faeces or nothing, and recorded their behaviours for 10 minutes. All horses investigated the enclosure olfactorily, and spent more time investigating the enclosure itself and the ground than the faeces, when either real or fake faeces were present. This confirmed that horses actively examine the smells in their environment whether or not visual cues are present, and may be susceptible to odours that cause make them frightened.

I collected urine and faeces from young foals immediately before and after weaning. The foals showed behavioural and physical signs of distress, and cortisol levels were elevated in the urine collected after weaning (before $11.8 \pm 4.0 \times 10^{-6}$, after $27.3 \pm 9.8 \times 10^{-6}$). Young horses were accustomed to entering a round yard while wearing a heart rate monitor, and will be observed as they encounter the samples collected from the weanlings. It is expected that heart rate will be elevated on encountering the post-weaning samples, indicating that horses do pick up on the fear of other horses from odours and care should be taken in high-traffic horse facilities to reduce the effect of odours as a fear-causing agent.

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Mammalian predator-prey interactions in arid Australia

SAMANTHA TESORIERO *Supervisors: Professor C Dickman & Dr M Crowther*
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Introduced predators are regarded as a major cause of declines and extinctions of native species in Australia. To determine the role of predators in driving declines of Australia's desert mammals, my project aimed to explore interactions between native desert rodents and the introduced red fox (*Vulpes vulpes*) and feral cat (*Felis catus*) in the Simpson Desert of south western Queensland. I first confirmed that rodents form a major part of the predators' diet, and then employed novel tracking methods to identify which habitat components ('microhabitats') the predators use to hunt their prey. I then investigated rodent behaviour in microhabitats that provide varying risks of encounter with the predators.

To determine whether predators selectively traverse different microhabitats, I followed predator tracks and compared them to random transects. Predators utilised open microhabitat in greater proportion to its availability, and avoided closed microhabitats. Placement of prey models in varying microhabitats also confirmed that predators attack prey selectively in open microhabitat.

I then investigated the effects of predation risk and microhabitat on the foraging behaviour of native rodents. I assessed the intensity of foraging by establishing feeding stations in open and closed microhabitats, and recording the amount of food eaten by rodents from the stations after nightly bouts of foraging. Predation risk was manipulated using artificial illumination and predator odour. Foraging activity, for the most part, was lowest in open microhabitats where predation risk had been elevated, and greater where perceived risk was low.

My study confirms previous general observations that hunting by foxes and cats often takes place in open areas where prey animals have little refuge, and that small mammals are more sensitive to the risk of predation in open than in covered habitats. The results advance our knowledge of predator-prey relationships in arid environments and supply reliable guidance for conservation management.

Daring to be different: The evolution of NK receptors in the endangered marsupial, Tasmanian devil

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One important group of immune genes that display tailoring to particular species' niches are the genes encoding cell-surface receptors on innate natural killer cells. These receptors may be classified into two different families, which are encoded in two genomic clusters: the natural killer complex (NKC), and the leukocyte receptor complex (LRC). Although the form and function of these are relatively maintained throughout evolution, members of these receptor clusters can vary significantly between species. This is thought to be driven by the need to combat different pathogens. While NK receptors have been well studied in placental mammals, comparatively less is known about them in marsupials, with only the opossum studied so far.

Given their evolutionary position between placental mammals, and reptiles and aves, and the primate-rodent-like evolutionary gap with the opossum, Tasmanian devils (*Sarcophilus harrisii*) present an interesting new case study. Devils display a predisposition to cancerous growths, and, as marsupials, are born with an underdeveloped immune system that develops outside of the womb. With the recent sequencing of the Tasmanian devil genome, I sought to characterise the natural killer receptor gene complexes in the devil, examine their evolution, and determine whether any marsupial trends were observable in the evolution of these receptors.

Through a combination of conserved protein domain searches, gene prediction and phylogenetic analyses, five NKC orthologs and an expansion of immunoglobulin (Ig) domains were identified in the Tasmanian devil genome. The expansion and clustering of Ig domains suggests that the Tasmanian devil predominantly utilises immunoglobulin-like receptors for NK cell function, rather than C-type lectin receptors, and reaffirms the existence of marsupial-specific Ig-like receptors. These results provide insight into the evolution of these receptors in mammals, and provide a valuable foundation for future functional and diversity studies into these genes in the Tasmanian devil, for which these results are already being used.

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Adaptive foraging strategies in slime moulds

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Slime moulds are unicellular, multinucleate organisms from the phylum Protista. Despite their apparent simplicity, these extraordinary organisms are capable of surprisingly complex behaviours such as solving mazes. This project investigated adaptive foraging behaviour in slime moulds. Animals using adaptive foraging strategies use information on the quality, density and composition of past resources to determine future behaviour. While adaptive foraging behaviour is well documented in a wide range of animals, it is not clear whether brainless, decentralised organisms exhibit similar behaviour. The aim of my project was two-fold. Firstly, I investigated the effect of differing food qualities on foraging behaviour in *Lepidoderma trigrinum* and *Didymium iridis*. I assessed the search intensity of slime moulds after consumption of food sources of varying quality. If slime moulds use adaptive foraging strategies, search intensity should increase with increasing food quality. My results suggest the use of adaptive foraging strategies by *Didymium iridis* but not *Lepidoderma trigrinum*. The second aim of my project was to investigate the effect of past food environments on the diet preferences of *Physarum polycephalum*. Slime moulds were reared on 3 diets; an optimum diet (that maximised growth), a protein biased diet, and a carbohydrate biased diet. I tested whether or not past experience influenced diet preference by offering slime moulds from each diet treatment a choice between an optimum, protein-biased or carbohydrate-biased food source. My results suggest past food environments do not influence the foraging strategies of slime moulds.