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## **Variation is a factor of dormancy in the Sydney Flannel Flower (*Actinotus helianthi*: *Apiaceae*)**

Nathan Emery    *Supervisors:* Dr C Offord\*, Dr M Henwood, Dr G Wardle & Professor R Overall  
School of Biological Sciences, University of Sydney, NSW 2006.

Seed dormancy may be determined by a combination of factors, including genetic origin, local environmental conditions, and the position of the seed on the plant. For the Sydney Flannel Flower (*Actinotus helianthi*) it is not clear whether one or more factors are acting alone or in combination in influencing the erratic variation in germination. A stronger understanding of how dormancy influences this variation would improve collection and *ex situ* storage protocols, thus maximising germination results and leading to more efficient use of seed for rehabilitation, restoration and horticultural practices. The main aim of this study was to determine the extent and effect of the inherent variability of dormancy both between and within umbels, and between several wild populations of *Actinotus helianthi*. The effect of storage temperature was examined to determine whether dormancy release is positively correlated with temperature during after-ripening.

Fresh seeds were collected and separated by plant umbels from five populations across the Greater Sydney region. Seeds were subsequently pooled where required before the commencement of each experiment. Germination of seeds was assessed at 15°C in water or smoke water. Germination response to after-ripening temperature (15, 30, 45 and 60°C) was also examined over ten weeks of storage followed by germination testing in water or smoke water (at ten weeks).

Poor viability was identified across all experiments, producing low numbers of germinated seeds. Greater variation in germination was exhibited in seeds from different populations than seed within the same population. Seeds treated with smoke exhibited greater germination, and an accelerated dormancy release. Dormancy release was slow during storage at 15°C and accelerated at higher temperatures (30 and 45°C). No seeds germinated during 60°C storage.

Smoke water and storage temperature influence dormancy release in *Actinotus helianthi* seeds. Seed from Flannel Flower populations should be collected and stored separately. Variability between populations is most likely a genetic response to local climate.

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## **Mapping the major QTLs that facilitate ovary activation in 'anarchistic' honey bees (*Apis mellifera*)**

Alen Faiz *Supervisors:* Professor B Oldroyd & Dr N Lo  
School of Biological Sciences, University of Sydney, NSW, 2006

In insect colonies, if a rare worker lays eggs that are reared by other workers, the laying worker has increased fitness relative to her sterile sisters. Thus the evolution of worker sterility is difficult to explain. To date, no genes directly linked to the regulation of worker sterility have been discovered. Yet such genes must exist, because in the honey bee at least, it is easy to select strains in which workers lay eggs at high frequency. For example, in the 'anarchist' strain of honey bees maintained at Sydney University, about 40% of workers have activated ovaries, compared to less than 1% in wild-type strains.

In this project we are using the anarchist strain to isolate genes that regulate sterility in the honey bee worker. Crosses show that 'anarchy' is a recessive trait. We have backcrossed an F<sub>1</sub> anarchist x wildtype cross to the anarchistic strain to produce a colony in which workers segregated for the wild-type and anarchist reproductive phenotype. From this population we selected 140 workers showing no signs of ovary activation, and 140 showing extreme ovary activation with fully-formed eggs. These workers have been screened with 162 informative microsatellite markers spread evenly throughout the genome. Markers showing strong statistical association with reproductive phenotype will be discussed.

## **The effect of diet history on the chemoreceptive attraction to food odour in an intertidal scavenging hermit crab**

Januar Harianto    *Supervisors:* Dr A Pile, Dr S Holmes & Professor S Simpson  
School of Biological Sciences, University of Sydney, NSW 2006

Many motile benthic marine species are opportunistic scavengers obtaining a portion of their nutrition from serendipitous food falls. However, for the majority of species, little is understood concerning the behavioural traits and motivations that these organisms display to exploit such food resources. My aims were to determine (1) if the choice exhibited by a common intertidal scavenging species, the hermit crab *Pagurixus handrecki*, in response to two different food sources (carbohydrate-rich vs. protein-rich) can be explained by their nutritional state, and (2) to determine the role of sensory appendages in mediating food choice.

Examination of the role of nutritional state in determining food choice was made by nutritionally conditioning hermit crabs to protein-deprived, carbohydrate-deprived or *ad libitum* (control) agar-based synthetic diets over 18 days. During the conditioning period, crabs were tested daily for behavioural responses when presented with novel odours emanating from protein-rich (squid) and carbohydrate-rich (kelp) food. Analysis of the results revealed a significant increase in attraction to squid odour in protein-deprived crabs compared to control crabs, but no significant differences in attraction to kelp odour between carbohydrate-deprived crabs and control crabs.

To determine the role of sensory appendages in effecting food choice I ablated hermit crab sensory appendages (antennae and antennules) and tested their ability to localise food odour. Control crabs were significantly better at reaching odour sources, while the ablation of either pair or both pairs of appendages resulted in significantly lower success.

The results revealed that current nutritional state could be an important factor in affecting *P. Handrecki's* selectivity to food, and also gave evidence that protein could influence diet choice more strongly compared to carbohydrates. Both the antennae and antennules are also central to successful food localisation.

## Effect of predation risk and toxins on foraging by wallabies

Daniel Issa *Supervisors:* Dr C McArthur & Dr P Banks  
School of Biological Sciences, University of Sydney, NSW 2006

Safety and nutrition are two major factors influencing the survival of living things. For animals, then, foraging optimally should involve seeking out the most nutritious food, while avoiding risky areas. Herbivores, in particular, are further caught in a struggle against plants, which strategically reduce their nutritional value by developing secondary metabolites - chemical toxins and digestibility reducers. One goal of foraging ecology is to understand how these constraints shape foraging patterns.

Using the swamp wallaby (*Wallabia bicolor*) as my focal herbivore, I had two aims. The first was to map their response to predation risk in open woodland as a landscape of fear, and to determine the landscape features associated with this fear. Secondly, I aimed to determine the effect of the eucalyptus toxin, cineole, on their foraging behaviour in high and low risk zones, and so compare the relative influence of a plant toxin against perceived predation risk.

In the first experiment, grids of feeding stations were set up at two sites, and the giving up density (GUD) was used here to quantify predation risk. Results show patchiness of fear within the two sites. In the second experiment, five concentrations of cineole were added to food at high and low risk patches (from the first trial). There was a significant effect of both cineole and patch risk (high vs. low) on GUDs, and cineole had a greater influence than predation risk.

My study demonstrates that *Wallabia bicolor* can detect and respond to small scale patchiness in perceived predation risk, possibly associated with vegetation characteristics (to be determined). They are also adverse to high dietary cineole concentration, which they identify as a greater cost than perceived predation risk in the landscape. These results contribute to foraging theory by illustrating that plant toxins and fear both have significant effects on foraging.

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## **Immuno-competent invaders: Facts and myths about amphibian eco-immunology**

David Llewellyn    *Supervisors:* Professor R Shine & Professor M Thompson  
School of Biological Sciences, University of Sydney, NSW 2006

The immune system plays an important role in fighting pathogens, but the ecological ramifications of eliciting an immune response are poorly understood for many types of animals. Current scientific literature suggests that in anuran amphibians (frogs and toads), infection will induce two types of response: an increase in body temperature and an elevation in metabolic rate. Current theory also suggests that in a species expanding its range, immune function may be compromised in invasion-front individuals due to energetic trade-offs with dispersal-enhancing traits. I experimentally elicited immune responses in invasive cane toads (*Bufo marinus*) from tropical Australia. To tease apart genetically-coded (canalised) responses, I used captive-raised (“common garden”) toads bred from adults collected from four locations across northern Australia. I injected toads with the bacterial protein lipopolysaccharide, and monitored the animal’s subsequent temperature preferences and metabolic responses. Selected body temperatures were monitored in both laboratory thermal gradients and under field conditions, to assess the extent of behavioural fever in cane toads. Contrary to prediction, injection did not induce any substantial shift in thermoregulatory behaviour of toads. Previous reports of behavioural fever in this species appear to result from experimental artefacts and behavioural changes unrelated to thermoregulation. In the laboratory, I used open respirometry to determine both resting metabolic rates and the metabolic elevation associated with an immune response. My study suggests geographic variation in both of these parameters, hinting that the invasion process has indeed modified toad immuno-competence.

## **Who learns faster? Variation in learning ability of shoaling fish, using *Gambusia holbrooki***

Kieran MacKenzie    *Supervisors:* Dr A Ward & A/Professor R Coleman  
School of Biological Sciences, University of Sydney, NSW 2006

Learning is an important mechanism that provides all animals with behavioural flexibility, allowing them to adapt and survive in a variable environment. Fish behaviour varies considerably between species, populations and even between individuals. I aimed to investigate whether individual variation also exists in the learning ability of shoaling fish, using the mosquitofish (*Gambusia holbrooki*) as a model species. Mosquitofish were trained to an associative learning task through classical conditioning where the animal is presented in each trial with a red light (conditioned stimulus, CS) which is followed by the delivery of a food reward (unconditioned stimulus, US). Over repetitive trials, the animal learns that CS predicts US which causes the animal to display food anticipatory behaviour when presented with a red light (conditioned response, CR). I found that the efficiency of the display of a learnt response from an associative learning task for a shoal of mosquitofish is dependent upon shoal size and shoal composition, where larger shoals, juveniles and males display a learnt response in fewer associative learning trials than smaller shoals, adults and females, respectively. These individual differences in learning have consequences for the diffusion of novel information through groups and populations. As information is a valuable commodity, differences in learning abilities may have important fitness consequences for individuals.

## **Cytoskeletal deletion mutants of *Arabidopsis* phospholipase D delta**

Arunima Malik    *Supervisors:* Dr J Marc, Dr N Firth & Professor D Day  
School of Biological Sciences, University of Sydney, NSW 2006

Plants are constantly exposed to a variety of environmental stress signals. Their growth and development thus relies on their ability to efficiently interact with the environment. Plants have evolved sophisticated signalling mechanisms that allow them to perceive and respond to environmental stress signals. Phospholipase D (PLD), a member of a superfamily of phospholipases, is a key signalling enzyme implicated in a range of cellular responses to hormonal and environmental stress signals including water deficit, salinity, wounding and pathogen elicitation. A PLD isotype from the model plant *Arabidopsis thaliana*, *AtPLD $\delta$* , which responds to drought and salinity, is known to associate with the plasma membrane and interact with the cytoskeleton. It is unclear, however, whether *AtPLD $\delta$*  binds directly to tubulin or actin, or whether the binding is facilitated indirectly through partner protein(s).

As a first step toward elucidating the mechanisms of action of this isotype, my project aimed at developing a set of cytoskeletal deletion mutants using a *GFP-AtPLD $\delta$*  chimeric gene and recombinant DNA technology. The strategy was to design *AtPLD $\delta$*  sequences lacking either tubulin- or actin- binding domains or both, amplify the sequences using PCR and then joining them using "Recombinant PCR". The deletion mutants were then cloned into a binary vector using Gateway-mediated recombination, followed by sequencing to determine whether the desired gene sequence was inserted into the binary vector. A total of five deletion mutants were obtained. Experiments are carried out to optimise the protocol for *Agrobacterium*-mediated transformation into an *Arabidopsis* cell suspension culture. The GFP-tag allows microscopic examination of stably transformed *Arabidopsis* cell lines, and also facilitates GFP-affinity pull-down assays followed by MS/MS to document tubulin and actin binding and to identify partner proteins.

## How do herbivores cope with the combined effects of predation risk and plant chemical defences while foraging?

Chayna Moldrich *Supervisors: Supervisors: Dr C McArthur & Dr P Banks*  
School of Biological Sciences, University of Sydney, NSW 2006

Herbivores have both physiological and behavioural mechanisms to process plant toxins. For example, they can metabolise toxins and change the size and time of feeding bouts. However, while foraging, herbivores themselves risk being eaten and this risk can influence whether herbivores stay or quit food patches. The net effect is that herbivores must balance the costs of plant toxins and predation risk, and their needs to counteract both may create a foraging dilemma.

Using a model generalist herbivore, *Trichosurus vulpecula* (the common brushtail possum), I had two aims. The first was to find factors that create a risky environment for possums. The second was to quantify the effects of both plant toxins (complimentary versus competing) at safe food patches and predation risk (high and low) between these patches on intake and movement.

In the first experiment, food was placed in an inedible matrix at patches and giving up densities (GUDs) were compared in the risky (open,  $\pm$  fox urine  $\pm$  artificial light) versus the safe (covered) patch. There was a significant fox effect; GUDs in the open patches was higher with fox urine, indicating high perceived predation risk. There was weak evidence that light exacerbated the fox effect.

In the second experiment, either complimentary toxins or competing toxins were added to food in a patch at either end of a 70 m arena. High versus low perceived predation risk was then superimposed between patches ( $\pm$  fox urine/artificial light at 10m intervals). Total intake, number of trips between patches, feeding bout lengths and other behaviours were measured. Total intake did not differ between toxin treatments. Animals may have used behavioural mechanisms to maximise their intake regardless of toxin type. Analysis of movement between patches will provide information about whether the risky environment between patches had an effect on the possum's behaviour.

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## **Chromosomal DNA segregation in *Staphylococcus aureus***

Alvina Sarosh    *Supervisors:* Dr N Firth & Dr S Jensen  
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Antimicrobial resistant strains of *S. aureus* are a major cause of hospital-acquired infections around the world. The remarkable ability of this bacterium to readily develop multi-resistance limits treatment options.

Segregation of replicated chromosomal DNA into daughter cells is an essential process in the bacterial cell cycle and represents a potential target of therapeutic intervention. Studies of chromosome segregation process undertaken in several bacteria have highlighted the involvement of two trans-acting proteins, ParB and ParA and a cis-acting centromere like DNA site *parS*. ParB is a DNA binding protein that binds to *parS* forming a nucleoprotein complex. ParA interacts with the ParB-*parS* complex and is an ATPase thought to energise the segregation process. Little is known about chromosome segregation in *S. aureus*. However, a recent bioinformatics study identified a gene for a ParB homologue and three putative *parS* sites in the chromosome of *S. aureus*, but no ParA homologue could be identified. It is hypothesised that the ParB homologue may play a role in chromosome segregation in *S. aureus*.

The aim of this study was to characterise binding of the chromosomally encoded ParB homologue to putative DNA binding sites and to determine its contribution to chromosome segregation. The *parB* gene was PCR-amplified and cloned into the *E. coli* expression vector pTTQ18 to facilitate protein over-expression as a RGS6xH-tagged derivative. Nickel-NTA resin was used to purify the protein by affinity chromatography. SDS-PAGE analysis showed that the protein obtained was 99% pure. Electrophoretic mobility shift assays with purified ParB and PCR-amplified *parS* sites revealed that the protein was able to bind to its respective binding sites. Additionally, a *parB* mutant was generated using the TargeTron Gene Knockout System. These results will facilitate ongoing studies to establish the involvement of ParB in segregation and its potential as a drug target.

## **Molecular interactions between the chromosomal replication initiator, DnaA, and origin of replication, *oriC*, in *Staphylococcus aureus***

Andrew Sawyer    *Supervisors:* Dr N Firth & Dr S Kwong  
School of Biological Sciences, University of Sydney, NSW 2006

*S. aureus* is an important human pathogen which readily acquires resistances to antimicrobial agents, resulting in strains that are increasingly difficult to treat with current medical solutions. In order to develop new antimicrobial agents to combat infection, an in depth understanding of essential cell processes is required so they can be targeted. Studies in other bacteria have shown DnaA to be an essential protein for replication of the chromosome, which initiates at a DNA site known as *oriC*, the origin of replication.

The aims of this project were to localise the *oriC* region of the *S. aureus* chromosome, and identify proteins interacting at this site. Candidate regions were identified and amplified from chromosomal DNA by PCR. However, repeated efforts to clone these fragments into *E. coli*, so they could be subsequently tested for replication proficiency in *S. aureus*, were unsuccessful. An alternative strategy based on localisation of DnaA binding sites was therefore undertaken.

The *dnaA* gene was PCR amplified and cloned under control of an inducible promoter in *E. coli* expression vector pTTQ18 RGS6. Protein expression was induced with IPTG, and RGS6His-tagged DnaA was purified by nickel affinity chromatography. Electrophoretic mobility shift assays were used to demonstrate that purified DnaA binds specifically to predicted DnaA binding sites (DnaA boxes) located adjacent to the *dnaA* gene, which likely constitute part of *oriC*. These regions were then used in DNA affinity-based pull-down experiments to isolate any proteins that interact with *oriC* and which may be involved in replication initiation in *S. aureus*.