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Anthracnose disease of common bean: *Colletotrichum lindemuthianum* and the switch from biotrophy to necrotrophy

ALEX BENECKE *Supervisors:* Professor D Guest* & Professor R Overall
School of Biological Sciences, University of Sydney, NSW 2006.

Anthracnose diseases, caused by the fungus *Colletotrichum lindemuthianum*, can lead to total crop losses of *Phaseolus vulgaris* (common bean). *C. lindemuthianum* is a hemibiotroph that exhibits two stages of parasitism: (1) an initial asymptomatic biotrophic stage, followed by (2) a destructive necrotrophic stage. The mechanisms regulating the switch in the bean/*C. lindemuthianum* interaction are not understood, but temperature has been shown to be involved, with spreading necrotrophic growth occurring at 17°C but not 25°C. In other *Colletotrichum* spp., ammonia secretion and host cell alkalinisation is involved in the switch from biotrophy to necrotrophy. This project aimed to exploit this temperature-induced switch to necrotrophy in order to examine whether host cell alkalinisation and ammonia secretion is involved in the *C. lindemuthianum*/bean interaction.

Detached bean hypocotyls were inoculated with *C. lindemuthianum* spores and incubated at 17°C and 25°C. Interestingly, the bean cultivar and Australian fungal isolate used in this study showed spreading necrotrophic growth at both temperatures, and the timing of the switch to necrotrophy appeared to be developmentally regulated and independent of temperature.

Ammonia secretion and host alkalinisation were analysed *in vitro* and *in planta* at a single temperature (17°C) using a phenol-hypochlorite assay for ammonia and a combination of pH probes and indicator stains to measure pH. *C. lindemuthianum* increased the pH of filtered bean decoction broth from pH 5 to pH 7 over 6 d, and it increased the pH of bean agar from pH 4 to over pH 5.5 several millimetres ahead of the hyphal front, thus supporting the original hypothesis. However, the concentration of ammonia in the bean medium fluctuated, and was not consistent with the steady increases in pH. No change in ammonia was detected in infected bean hypocotyl tissue at the switch to necrotrophy. Little or no alkalinisation was detected in infected cells or around fungal biotrophic hyphae using the pH sensitive fluorescent dye Carboxy SNARF-1 and confocal microscopy.

C. lindemuthianum is a pathogen of growing tissues of bean and appears not to secrete ammonia to alkalinise host cells in order to facilitate the switch to necrotrophic growth, as do post-harvest *Colletotrichum* spp.

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Potential mediators of phase polyphenism: the Heat Shock Proteins

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Locusts are distinguished from other acridid species, such as grasshoppers, by their ability to change their morphological and behavioural characteristics in response to population density. During periods of increased population growth, contact between shy solitary locusts becomes unavoidable, leading to aggregation and consequently the formation of swarms. The mechanisms underlying locust swarming in the Australian plague locust, *Chortoicetes terminifera* (Acrididae: Oedipodinae) are currently being investigated, however the discovery of phase specific gene expression in the closely related Migratory locust *Locusta migratoria* (Acrididae: Oedipodinae) has given rise to the hypothesis that a phase polyphenism may be governed by molecular signals in *L.migratoria*, *C.terminifera* and other locusts species.

In *L. migratoria* heat shock protein (HSP) mRNA has been shown to be expressed in a phase specific manner, with gregarious locusts having a higher level of protein expression than their solitary counterparts. This study aimed to determine whether *C.terminifera* phase change is similarly mediated by HSPs. I therefore measured the effects of population density on the expression of one HSP of interest; HSP70, by the use of electrophoresis and Western blots. My results illustrate that HSP70 expression in *C.terminifera* is highly localised only found in the hind legs and thoratic tissues of both solitary and gregarious locusts with no significant differences in protein expression between phases. These results indicate that HSP70 expression is not altered by phase change in *C. terminifera* and that other molecular signals (or other HSPs) may govern phase polyphenism in this species. This study lays the foundation for future work into the molecular mediators of phase polyphenism where techniques such as RT-PCR and immunohistochemistry can be used to further unravel what role various HSPs have in mediating phase polyphenism in one of Australia's most detrimental pests.

Spatial positioning and Leadership in fish shoals

ALICIA BURNS *Supervisors:* Dr A Ward & A/Professor G Sword
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In social animal groups, an individual's spatial position is a major determinant of both predation risk and foraging rewards. Additionally, the occupation of positions in the front of moving groups correlates with initiation of group movements. Whether some individuals are predisposed to consistently occupy certain positions and therefore to consistently lead groups is, as yet, unresolved. Using the mosquitofish (*Gambusia holbrooki*) as a study species, I examined the consistency of an individual's spatial position within a moving group over successive trials. I found that certain individuals consistently occupied lead positions in moving groups and also that it was these individuals that initiated group decisions. Whilst it has previously been shown that leadership is state and context dependent, this is the first study to show that leadership is also an intrinsic property of the individual. Furthermore my results show that the occupation of lead positions in moving groups is not correlated with dominance, size or sex, suggesting that certain individuals are natural leaders. This has important implications for our understanding of collective behaviour in animal groups.

Foraging in a nutritionally complex world: tests using agent-based models and locusts

XIMONIE CLARK *Supervisors:* Dr F Clissold & Professor S Simpson
School of Biological Sciences, University of Sydney, NSW 2006

All animals are faced with the challenge of matching the demand for obtaining multiple nutrients from the environment or suffering fitness costs. Plants, as a whole, contain all the necessary nutrients for herbivores, but individually do not possess the required ratios. Thus, herbivore nutrient balance can be maintained by adjusting the selection and amounts consumed of various plants. For herbivores, two general foraging strategies have been identified. These are defined by the degree to which a herbivore will consume foods that deviate in composition from the required optimal balance of nutrients (intake target). A nutrient generalist will consume a range of foods that differ largely in nutrient content from the animal's intake target, whereas nutrient specialists eat foods that are very similar to that required. Which strategy a herbivore adopts will depend on previous dietary history, based on the nutritional range of foods encountered when foraging. Foraging strategies are shaped by evolution; however, behavioural plasticity allows animals to optimise fitness in variable environments. The aim of this study was to investigate whether the nutritional environment encountered could alter the foraging strategy of a nutrient specialist, *Locusta migratoria*. First, an agent-based model was used to model the fitness outcomes for each foraging strategy associated with eating imbalanced foods in a continuum of nutritional environments. The agent-based model indicated that increasing consumption of a highly imbalanced food, the nutrient generalist strategy, produces the best fitness where highly imbalanced foods are encountered with high frequencies. Second, a laboratory experiment using *L. migratoria*, was conducted to ascertain the conditions under which *L. migratoria* shifted from being a specialist to a generalist. After being subjected to temporal variations in the supply of food sources, *L. migratoria* adopted a more generalist strategy. However, further work is needed to translate temporal conditioning into a spatial environment.

A taxonomic investigation of the native plant species complex; *Ajuga australis* using phenetic and molecular phylogenetic methods

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School of Biological Sciences, University of Sydney, NSW 2006

Species complexes present considerable and sometimes puzzling morphological variation throughout their geographic range. Multivariate phenetic analyses, in addition to DNA sequence data, can assist in determining the taxonomic structure in such complexes, and can lead to a better understanding of the processes causing the variation.

Ajuga australis R.Br. is a plant species complex found across New South Wales, in a wide variety of habitats. Throughout its distribution, *A. australis* exhibits a wide range of variation in terms of habit, leaf shape and size of reproductive and vegetative parts. The lack of consensus concerning past recognition of separate taxa within *A. australis*, makes this an ideal model on which to conduct a robust taxonomic investigation.

A multivariate phenetic analysis was conducted on 57 individuals of *A. australis* collected across New South Wales. Through the use of cluster analysis (UPGMA) and ordination methods (Semi-strong hybrid non metric multidimensional scaling), several taxonomic groups could be distinguished, some of which correspond to previously recognised taxa.

Two DNA markers were sequenced from a subsample of individuals, with two exotic *Ajuga* and one outgroup species. Sequences of the non-coding cpDNA region; *psbA-trnH*, were invariant across the sample. The nuclear ribosomal internal transcribed spacer region (ITS1 and ITS2) however, showed some variation resulting in the differentiation of one phenetically distinct morphotype. Both markers revealed that *A. australis* as currently recognised is monophyletic within Australia.

Justification for the reinstatement and recognition of several taxa within *A. australis sensu lato* will be presented.

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Red light perception in *Acaryochloris marina*

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Photosynthetic organisms that produce oxygen utilise the redox potential of chlorophyll *a* to oxidise water. *Acaryochloris marina* is the only known exception to this rule. This unique cyanobacterium lives in environments depleted in the typical photosynthetically active wavelengths of red light and has adapted to these far-red light enriched conditions by evolving a red-shifted chlorophyll, chlorophyll *d*. It is likely that *A. marina* has a light perception mechanism that is highly adapted to its niche light conditions. Phytochrome is the photoreceptor in plants and bacteria responsible for detecting and responding to changes in red light. A search of the *Acaryochloris* genome reveals a gene homologous to phytochrome, designated here as *amrbphp* (*Acaryochloris marina* bacteriophytochrome photoreceptor). I aimed to determine the evolutionary position of *AmrBphP* in the phytochrome superfamily and study the function of *AmrBphP*. I generated amino acid sequence alignments of conserved modular domains of representative phytochromes and used these to generate phylogenetic trees. Interestingly, *AmrBphP* is more closely related to phytochromes from non-photosynthetic bacteria than cyanobacterial phytochromes. Additionally, *AmrBphP* contains a bilin lyase domain characteristic of biliverdin (BV)-binding bacterial phytochromes. Compared to phycocyanobilin (PCB)-binding cyanobacterial phytochromes, BV-binding phytochromes have a red-shifted absorbance spectrum. A BV-binding phytochrome would be suitable for photosystem regulation in the far-red light enriched environments *A. marina* inhabits. I studied the function of *AmrBphP* by recombinant protein expression in *Escherichia coli*, followed by pigment-protein reconstitution. Unfortunately, expressed *AmrBphP* was not very soluble, but a fortuitously expressed truncated form was more soluble. Assessing pigment-binding was problematic and the methods will need to be refined in the future.

Understanding the processes determining the spatial distribution of two temperate damselfishes

RONEN GALAIDUK *Supervisors:* Dr W Figueira & Dr B Curley
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Many factors both biotic and abiotic affect the distribution and abundance of marine species. At larger spatial scales temperature is among the most important of these given its direct effects on survival, reproductive success, dispersal and behaviour. At local scales processes such as competition have been shown to be quite important for population regulation of marine species. However the impact of temperature on all metabolic processes means that these processes are likely to interact to ultimately determine the distribution and abundance of marine organisms. This project studied two temperate damselfish species *Parma microlepis* and *Parma unifasciata* whose ecology is very similar and whose geographical ranges only partly overlap. Temperature regimes were recorded in the field at different depth intervals in order to monitor species/size related patterns in the distribution of studied species at these depths. A temperature manipulative experiment was conducted in controlled laboratory conditions in order to derive optimal growth conditions for each of the studied species. Field and laboratory competition experiments were performed to study relative aggression between the species and any temperature dependency of this. Processing the data showed that *P.microlepis* lives much longer (max. recorded age of 37 years old) than *P.unifasciata* (max age 12 years old). The analysis revealed as well that *P.microlepis* is the more aggressive species and it completely dominates at warmer temperatures. My study demonstrates that different temperature regimes affect various physiological and ecological aspects of interspecific interactions. These findings indicate the importance of understanding community interactions (competition in this case) in concert with abiotic factors when trying to predict possible outcomes of distribution shifts due to global climate change.

Effects of storm-water runoff on algal epifauna

GIULIA GHEDINI *Supervisors:* A/Professor R Coleman & Dr J Klein
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Urbanised coastal areas are subject to numerous anthropogenic disturbances that may influence natural assemblages. Storm-water discharges following rainfall events are common disturbances in subtidal habitats, affecting physical and/or chemical variables of the water in proximity of the outfalls. Although laboratory bioassays have established negative effects of storm-water effluents on a range of different species, little is known on the effects on marine assemblages in the field. I, therefore, investigated effects of storm-water discharges on the composition and structure of epifaunal assemblages associated with habitat-forming macro-algae. A preliminary investigation showed that assemblages in locations with drains differed from assemblages in locations without drains. To test the model that differences were caused by the presence of storm-water drains, I transplanted assemblages from three control (no drain) to three impacted (with drain) locations, including the appropriate procedural controls. Artificial units of habitat (*i.e.* pot-scourers) were used to reduce the physical variability of habitat among locations, which may potentially confound the results. Storm-water discharges had variable effects on different taxa and on assemblages among locations. At two locations transplanted assemblages differed in composition and structure from those in areas without drains, but they also differed from those in impacted locations. In one of these locations, however, the procedure used for transplantation had significant effects on assemblages. Thus, observed changes could not be clearly attributed to the presence of the drain. No differences were observed in the third location. Storm-water runoffs appear to influence epifaunal assemblages, but these effects are highly variable in space. This variability may be caused by differences in the catchment areas of storm-water drains and/or by differences in the amount of rainfall, which can vary at small spatial scales among locations. Further experimental manipulations at several spatial and temporal scales are, therefore, necessary to clarify the effects of storm-water drains on epifaunal assemblages.

What the bees can teach us about sex

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

It is rare for a species to do without sex, only ~ 0.1% of animals show any form of asexual reproduction. The complete absence of sexual reproduction is even more rare. Yet in some species, exclusively asexual (parthenogenetic) lineages exist. Their endurance is considered an evolutionary mystery, particularly given that some forms of parthenogenesis incur an additional cost: loss of heterozygosity, which leads to effective inbreeding in asexual lineages.

I examined levels of heterozygosity in a parasitic asexual lineage of *Apis mellifera capensis* (hereafter *Capensis*). In this honeybee subspecies the parthenogenetic production of females (thelytoky) is predicted to result in $\leq \frac{1}{3}$ loss of heterozygosity per generation. Yet previous studies have shown that heterozygosity is maintained at a level much higher than predicted. Two hypotheses have been put forward to explain this observation: suppression of meiotic recombination and selection against homozygous recombinants.

My work showed that levels of homozygosity were significantly higher in eggs than in larvae and pupae. This suggests that homozygous recombinant progeny are at a selective disadvantage and are less likely to survive early development. As a result homozygous recombinant offspring are underrepresented in larval and pupal progeny. I also found some evidence for a reduction in recombination, however at a rate lower than previously reported. I constructed a deterministic model to investigate whether selection against homozygous recombinant offspring is a plausible mechanism for the long-term maintenance of heterozygosity. In the absence of selection heterozygosity declines to zero; while reduced recombination will slow this decline, it does not prevent it. Heterozygosity is only maintained when homozygous genotypes are less fit than heterozygous genotypes (overdominance). Selection against homozygotes thus results in the maintenance of heterozygosity, however it incurs a per-generational cost. Because the *Capensis* lineage is parasitic, it can endure these costs while non-parasitic lineages will quickly be sent extinct.

Soil respiration at cold temperatures: what is the influence of plants?

MICHAEL KOWALCZYK-BARKER *Supervisors:* Dr C Warren & Dr M Jenkins*
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Cold ecosystems are important reservoirs of organic carbon but are also susceptible to increases in temperature, due to the positive feedback system. When temperatures increase, especially in winter, plants increase their rates of photosynthesis which means more products of photosynthesis are created, released via plant roots in the form of substrates and utilised by soil microbes. The microbes in turn respire more, causing the amount of CO₂ leaving an ecosystem to outweigh that coming in, and so cold ecosystems can re-enforce the greenhouse effect.

Studies in Australian cold ecosystems are lacking, but in parts of the Snowy Mountains it has been found that there is a change in soil respiration rate in winter at around 2°C, something which could be useful in determining the positive feedback potential of these alpine areas. Such a change in respiration rate at a ‘threshold’ temperature has been found in other parts of the world but the cause for the one in the Snowy Mountains is unknown, and so in this study I collected soil from these sites for a series of respiration experiments.

Soil microbes were found to respond to temperature, but they were especially sensitive to the cold. When substrates were added, microbes increased their respiration rates, even at cold temperatures. With the presence of Snow Gums (*Eucalyptus pauciflora*) soil respiration rates increased, but it wasn’t until the plants began photosynthesising that there was a noticeable increase in soil respiration, even at cold temperatures. So plants can increase microbial respiration rates via substrates in cold temperatures, and this could very well lead to the ‘threshold’ observed in the field (other explanations were deemed unlikely). This could have important ramifications for the future of climate change effects in Australia, and is a good reason as to why more studies in Australia are needed.

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Molecular aspects of caste determination in the termite *Coptotermes lacteus*

BINSHUANG LI *Supervisors:* Dr N Lo, Dr B Ujvari & Professor B Oldroyd
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Caste of eusocial insects has long been believed to be determined by environmental factors such as nutrients, pheromones and temperature (environmental caste determination; ECD). Recent studies show an alternative - genetically-influenced caste determination (GCD) - in which the genotype of an individual has a significant influence on what caste it will become.

Using microsatellite genotyping, researchers have found numerous cases of GCD in ants. One example is the little fire ant *Wasmannia auropunctata*, where new queens arise from parthenogenesis, while the workers come from sexual reproduction. The first evidence of GCD in termites has come from crossing experiments in the Japanese termite species *Reticulitermes speratus*. The GCD model predicts that *R. speratus* termite kings and queens should arise from inbreeding. Using microsatellite genotyping, I examined an Australian termite, *Coptotermes lacteus*, to test for ECD vs GCD. The results showed neither parthenogenesis nor inbreeding exist in *C. lacteus*. Thus, GCD may not apply to this species.

Recent studies suggest that DNA methylation may regulate caste differentiation in another eusocial insect, the honeybee, by silencing crucial genes involved in caste formation. DNA methylation has not yet been reported in termites. I used a modified amplified fragment length polymorphism (AFLP) technique with isoschizomers *Hpa* II and *Msp* I which display differential sensitivity to cytosine methylation to test for the presence of DNA methylation in *C. lacteus*. The result of 21 head samples from 3 colonies showed consistent methylation patterns, and represents the first demonstration of DNA methylation in termites. Although colony-specific methylation patterns were found, no caste differences in methylation were detected. This may be because the DNA in tissues of termite heads is not differentially methylated among castes, or because different tissues within the head contain different methylation patterns, leading to difficulties in detection.

Many eyes: is the distribution of vigilance shared, or are some simply better at the job?

DELUXMI LOGENDRAN *Supervisors:* Dr A Ward & A/Professor M Beekman
School of Biological Sciences, University of Sydney, NSW 2006

Larger groups of animals can often detect an approaching predator from further away than smaller groups and individuals. Commonly called the many-eyes concept, previous studies have been based primarily on terrestrial animals, and it is not known whether the many-eyes concept applies to groups of fish. I aimed to test whether (a) larger groups of fish will detect a predator at a greater distance than smaller groups and individuals, and (b) if vigilance is distributed evenly between individuals in a group.

Groups of one, two, four, eight and sixteen mosquitofish (*Gambusia holbrooki*) were placed in a clear plastic cylinder within a larger experimental tank. Fish movements were recorded using a high-speed camera whilst a motorised model predator approached the cylinder at 5cm/second. One randomly chosen fish from each replicate was tracked in ImageJ, and its maximum turning angle (in radians) was calculated in Matlab. A response (the point at which fish turned and oriented towards the approaching predator, before turning away sharply) was recorded at the maximum turning angle in each trial, and the distance from the predator at which this occurred was calculated. To test whether vigilance was shared equally amongst group members, six groups of four fish (each uniquely tagged) were repeatedly tested twenty times (each replicate three days apart to minimise learning by the fish) using the same methodology.

Preliminary analyses showed there was no difference in the distances at which groups of different sizes responded to the predator. Additionally, there was no hierarchy of vigilance amongst group members; vigilance was distributed evenly in all replicate groups.

The results suggest that vigilance (and its underlying mechanisms) may interact differently in underwater systems compared with terrestrial systems. My study provides valuable first insight into how the many-eyes concept applies to such underwater systems, and future studies should be directed towards determining how this concept works under different conditions i.e. marine vs. freshwater environments, where communication modes can vastly differ.

The effect of fire regimes on small mammals in the Greater Sydney Region

EMILY MOWAT *Supervisors:* Dr M Crowther & Dr J Webb
School of Biological Sciences, University of Sydney, NSW Australia 2006

Fire is a key ecological process influencing the population dynamics of small mammals in the Australian environment. However, the way in which small mammal populations respond to differences in fire frequency – an important component of fire regimes – is poorly understood. In this study, I investigated the effect of fire frequency on small mammals at two locations (Gosford and Yengo) within the Greater Sydney region. In each of these two regions, I used replicate sites which differed in the number of times burnt during the last four decades (one, two, three or four fires); all were last burnt in a wildfire during 2002-2003.

I carried out live trapping on three occasions over five months. I found that fire frequency did not affect the abundance of bush rats (*Rattus fuscipes*), yellow-footed antechinuses (*Antechinus flavipes*), common dunnarts (*Sminthopsis murina*), or the overall abundance of small mammals. Whilst in the Gosford region the number of fires did not appear to affect abundance of brown antechinuses (*Antechinus stuartii*), in the Yengo region significantly more *A. stuartii* were captured in the single burn sites, compared with all other fire frequencies. A possible explanation for this pattern is competition between *A. stuartii* and *A. flavipes*, the latter being found only in the Yengo region. Proportionally, I captured greater numbers of *A. stuartii* in less frequently burnt sites and greater numbers of *A. flavipes* in more frequently burnt sites. It is possible that fire mediates competitive interactions between these two species, which are both at the edge of their ranges in Yengo National Park.

To elucidate further the patterns of small mammal abundance, I measured a range of structural vegetation characteristics at all sites. I then used model-averaging of generalised linear models to compare mammal abundances with structural vegetation characteristics.

This study provided insights into the complexities of small mammal responses to fire, and suggests that competition may be important in regulating these species responses. Species responses to differences in fire regimes can vary across regions, which has important implications for the implementation of fire management plans for conservation.

The relationship between genetics, morphology and geography in the Victorian Christmas Bush (*Prostanthera lasianthos*: Lamiaceae)

KIRSTIN PROFT *Supervisors:* A/Professor M Henwood & A/Professor Barry Conn*
School of Biological Sciences, University of Sydney, NSW 2006

Species that are distributed across a wide geographic range are often morphologically and genetically complex. Over time, this complexity can lead to the evolution of distinct groups that might ultimately become new species. The Victorian Christmas Bush (*Prostanthera lasianthos*) is an excellent species in which to study the interaction between morphology, genetics and geography. It is widespread in south-eastern Australia, from southern Queensland to Tasmania, and is very morphologically variable.

In this study, I addressed the following questions: 1) Are there distinct morphological groups within *P. lasianthos*? 2) Is there genetic structure within *P. lasianthos*? 3) Does the genetic structure within *P. lasianthos* correlate with any morphological groups and/or reflect a geographic pattern? I performed multivariate analyses of seven vegetative and 14 floral characters, which showed seven morphological forms within *P. lasianthos*. I sequenced one nuclear and two chloroplast markers, and performed phylogenetic reconstructions using maximum parsimony and Bayesian inference methods. These analyses revealed considerable structure within *P. lasianthos*, which was congruent with the geographic origin of samples. Notably, individuals from the northern tablelands of New South Wales formed a well-supported clade, which, along with samples from Queensland, was sister to the remaining individuals. When I mapped the morphotypes on to the phylogeny, some corresponded to genetic clades, whereas others did not.

These results show that there is complex genetic and morphological structure within *P. lasianthos*. Genetic groups are generally linked to specific geographic regions, and morphological forms reflect these genetic and geographic groups in some instances. Formal taxonomic recognition should be given to the genetically and morphologically distinct groups identified by this study.

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Visualizing Actin in the Plant Cell Cortex

JACQUELYN SIMPSON *Supervisors:* Professor R Overall & Dr D Barton
School of Biological Sciences, University of Sydney, NSW 2006

Actin microfilaments form a vital cytoskeletal network in the plant cell cortex. They are involved in processes such as pathogen defence, environmental acclimation and wound response. So far it has not been possible to entirely image the actin filament network using electron microscopy or investigate how they interact with the cortical microtubule array. A method has been developed for visualisation of cortical microtubules which uses confocal microscopy and field emission scanning electron microscopy (FE-SEM). This project aimed to optimise this technology for the fixation and imaging of actin microfilaments in *Tradescantia virginiana* leaf epidermal cells. This would lead to the investigation of the interaction between actin microfilaments and cortical microtubules.

In order to do this the original technique was modified with pre-fixative solutions, omitting steps or changing the duration of certain steps. The cells were fixed using a Gluteraldehyde and Paraformaldehyde fixative solution, cracked open and extracted. Two antibodies were then applied, the primary antibody Anti-Actin and the secondary antibody Fluoronanogold. The fluorescently labeled actin microfilaments were then observed with the confocal microscope. The cells in which actin filaments were seen were then prepared for FE-SEM.

Using this method I have been able to visualise actin microfilaments bundled together after pre-fixing with MBS and in a separate treatment I was able to visualise microtubules on which gold particles were found after allowing the cells to recover overnight. From these results I can see, with a little certainty, the structure of actin microfilaments and a possible co-alignment between actin microfilaments and microtubules. The results are promising to the study of actin, however, further work is required before conclusions can be drawn on actin-microtubule interactions.

Mitigating the impact of cane toads on an Australian predator - The Goanna

CHRIS SPRAGGON *Supervisors:* Dr J Webb, Dr M Letnic & Professor R Shine
School of Biological Sciences, University of Sydney, NSW 2006

Invasive species are a key threat to global biodiversity. Impacts of invaders on native species can occur through a variety of pathways. The cane toad, *Bufo marinus*, is a major threat to many native predators, which often die following ingestion of this highly toxic pest. Crocodiles, snakes, quolls and goannas have all shown decline following cane toad invasion; goannas (genus *Varanus*) have suffered declines exceeding 90% in some areas. Australia's diverse array of goanna species – almost all of which have a current or predicted future range overlap with the cane toad – comprise more than half of known species worldwide. Conditioned taste aversion was recently shown to benefit the survival of Northern Quolls (*Dasyurus hallucatus*) imperilled by cane toads. My study aimed to determine 1) if goannas can be taught to avoid cane toads and 2) if toad aversion persists in the wild and benefits individual survival. I collected goannas from irrigation channels in Kununurra, WA, and assessed their feeding responses to toads as well as other prey in the lab. I then fed them toad aversion baits and assessed their responses to live toads before release. Goannas treat cane toad chemical cues as prey in the same way as native frogs or fish, which comprise their regular diet. Following conditioning, *V. mertensi* showed a strong aversion to cane toads, while *V. mitchelli* showed moderate aversion. Efforts to control and eradicate cane toads have met with limited success. Methods to mitigate the impact of this toxic invader on predators may provide a more promising avenue for protecting native fauna. Broad-scale application of conditioned taste aversion to *V. mertensi* populations has the potential to safeguard this species from cane toad induced mortality.

The effect of marine park zoning on fish assemblages and potential interactions with fur seals, wave exposure and water temperature, within the Batemans Marine Park NSW

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The implementation of marine parks has the ability to reduce the negative impact of fishing on fish assemblages by increasing the diversity, abundance and size of fish within protected zones. Environmental variables, such as wave exposure, water temperature, habitat and marine predators, may also influence fish assemblages, and fluctuate with location within a marine park. The presence of top carnivores, such as seals, may alter or inhibit recovery trajectories of protected areas due to their continual foraging on fish species. Fur seals haul-out on Montague Island, which is an offshore island within the Batemans Marine Park (BMP), NSW. I investigated the effectiveness of the Sanctuary Zones (SZ) within the recently zoned BMP by comparing fish assemblages among sites within the SZ, where no fishing is permitted, and sites where fishing is allowed, around the mainland and the island using Baited Remote Underwater Videos (BRUVs). Habitat type differed strongly between mainland and island sites, and may contribute to the differences in fish assemblages observed between the two locations. Fish assemblages differed between SZ and fished areas; and this effect varied between mainland sites and island sites. Around Montague Island, the difference between zones was driven by a greater abundance of species such as the one-spot puller (*Chromis hypilepis*), silver sweep (*Scorpiis lineolata*) and striped mado (*Atypchthys strigatus*) within SZ. The difference in fish assemblages between zones at mainland sites was largely due to a greater abundance of yellow-tail scad (*Trachurus novaeandiae*) and silver trevally (*Pseudocaranx georgianus*) within SZ. Seal activity was significantly lower within SZ around Montague Island, but higher in SZ around the mainland. There was a negative correlation between seal activity and species richness around Montague Island. Wave exposure did not have a significant effect on fish assemblages; however sea surface temperature and species richness were positively correlated at mainland sites. My results suggest that the abundance and diversity of fish within SZ is greater than fished areas; however there is the potential for seal foraging to effect fish communities locally.

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