1

Toward population genetics of dark matter in the microbial universe

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Human bodies are luxury condominiums for bacteria, populated with billions if not trillions of microbial cells. These microbes have crucial roles in metabolism and protection from pathogens that we are just beginning to appreciate. Next generation DNA sequencing and in particular metagenomics has been instrumental in revealing the previously hidden diversity of microbes in our bodies and characterising their roles in human health and the environment. Until now, most studies of bacterial population genomics have focused on cultured isolates. From these studies we have learned that acquisition of genes via lateral transfer and swapping of alleles via homologous recombination can dramatically alter how microbes interact with the environment and with hosts. This type of fine scale genetic variation in bacterial populations is important, but still poorly understood outside laboratory culture.

Recent work in my own group and others around the world has led to new molecular tools for resolving the fine-scale genetic structure in wild bacterial populations. Among these are single-cell genome sequencing,single-molecule long read sequencing, and metagenomic Hi-C. These new data types hold great promise to reveal the private lives of microbes,but also pose a major analytical challenge because the data violate common assumptions of existing models and population genetic inference frameworks.

2

The 10 megabases of Arabidopsis centromeric sequence behave as a single locus over evolutionary time

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Eukaryotic centromeres are comprised of several megabases of short tandem repeats that adopt a higher order structure required for proper chromosomal segregation. Generally, the centromeric repeats within one species are more similar to each other than to related repeats in neighbouring species, consistent with a process of concerted evolution.

Making use of the full sequence from the 1001 *Arabidopsis* genome project we show that this within species homogenisation has been absolute. As a result, every centromeric repeat within the population shares a common ancestor more recently than the species origin. Essentially, while centromere position and function has been maintained between species, their underlying sequence has undergone complete replacement.

We follow the subsequent birth and spread of new repeat variants within and between centromeres using linkage disequilibrium in the natural population, and segregation in F2 crosses. This rapid sequence evolution is not easily explained by current models of repeat turnover, and has interesting population genetic consequences.

3

Genome sequencing, assembly and annotation of *S. aurantiacum*

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Scedosporium aurantiacum is an emerging pathogen of humans and animals. It is commonly present in urban environments. Animal studies, using the *Galleria mellonella* model, have revealed virulence differences among strains. To establish a molecular basis for future studies to understand the origin of those differences, we have sequenced and assembled for the first time the genome of *S. auarantiacum* strain WM 09.24 using different genome assemblers. To project the genome annotation and develop gene models we performed whole-genome alignment with the next closest species for which an annotated genome was publically available, *Trichoderma virens*. However, this species is still so distant that only approximately 30 % of the two genomes could be aligned through whole-genome alignment. Therefore, neither direct-gene projection nor BLAST searches could be applied in order to annotate the *S. aurantiacum* genome. To overcome this problem we performed RNA-sequencing of strain WM 09.24 under different growth conditions (to maximise the number of transcribed genes), and used the JAMg pipeline to annotate the genome. The annotated genome of strain WM 09.24 was then used to annotate three additional *S. aurantiacum* strains with varying virulence to enable a whole-genome comparison to identify genes or genetic signatures that are associated with virulence.

4

Novel therapeutics for complex diseases from genome-wide association data

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Background

Human genome sequencing has enabled the association of phenotypes with genetic loci, but our ability to effectively translate this data to the clinic has not kept pace. In silico tools such as candidate gene prediction systems allow rapid identification of disease genes by identifying the most probable candidate genes linked to genetic markers of the disease or phenotype under investigation. Integration of drug-target data with candidate gene prediction systems can identify novel phenotypes which may benefit from current therapeutics. Such a drug repositioning tool can save valuable time and money spent on preclinical studies and phase I clinical trials.

Methods

We previously used Gentrepid (www.gentrepid.org) as a platform to predict 1,497 candidate genes for the seven complex diseases considered in the Wellcome Trust Case-Control Consortium genome-wide association study; namely Type 2 Diabetes, Bipolar Disorder, Crohn's Disease, Hypertension, Type 1 Diabetes, Coronary Artery Disease and Rheumatoid Arthritis. Here, we adopted a simple approach to integrate drug data from three publicly available drug databases: the Therapeutic Target Database, the Pharmacogenomics Knowledgebase and DrugBank; with candidate gene predictions from Gentrepid at the systems level.

Results

Using the publicly available drug databases as sources of drug-target association data, we identified a total of 452 candidate genes as therapeutic targets for the seven phenotypes of interest and 2,130 drugs feasible for repositioning against the predicted novel targets. All three drug databases made significant contributions to target identification, with the highest contribution from DrugBank (400), followed by TTD (156) and PharmGKB (61). We also found 428 novel therapeutic targets accounting for almost 94% of the identified targets. Potential drugs associated with novel targets may be used for further evaluation directly in phase II clinical trials

Conclusions

By integrating genetic, bioinformatic and drug data, we have demonstrated that currently available drugs may be repositioned as novel therapeutics for the seven diseases studied here, quickly taking advantage of prior work in pharmaceutics to translate ground-breaking results in genetics to clinical treatments.

5

Scottish Fold cats: an animal model for human digital arthropathy

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The distinctive and defining physical trait of the Scottish Fold cat breed is the characteristic ear phenotype. Scottish fold cats have ears which fold forward, this presumably reflecting lack of resilience of the pinna and auricular cartilages. There is convincing clinical, radiologic, histologic and genetic evidence that Scottish Fold cats have an underlying congenital defect which affects the structure and function of cartilage, resulting in progressive bone, joint and cartilage abnormalities that subsequently lead to progressive dysfunction. Cats develop a variable osteochondrodystrophy causing abnormal bone development likely through defective endochondral ossification, progressive osteoarthritis and lameness. From pedigree analyses and breeding experiments the phenotype has been shown to be inherited as autosomal monogenic dominant trait with variable expression. Thus, cats with two copies of the abnormal gene invariably have severe disease from an early age, whereas cats with one copy of the defective gene have disease which may vary from mild to moderate severity (in terms of extent of involvement and clinical progression). We applied a whole-genome SNP association mapping approach using a total of 78 cats (53 Scottish fold cats and 25 Scottish shorthairs). DNA samples were genotyped with the feline Illumina 63kSNP genotyping microarray. A genome-wide significant association on chromosome D3 has been identified and confirmed with fine structure mapping. The region contains a positional candidate gene involved in a wide range of inherited skeletal dysplasias in humans.

6

RNAnexus: A framework for RNA data mining and visualization

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RNAseq has become the technology of choice for understanding the transcriptome profile in all organisms. It also allows for far more accurate measurements of RNA levels and reveals the complexity of RNA content in a given biological system. More importantly, it offers easier access to the nucleotide sequences of RNA that can be used for studying the nucleotide evolutionary patterns in organisms in lieu of the genome sequences. There are ~42,000 raw RNAseq datasets deposited in the NCBI Short Read Archive database from vertebrates; almost half of them being non-model organisms. However, this data is not readily accessible because sophisticated analysis is required for the assembly, annotations and visualization of the RNAseq data. We have utilized large compute facility available at the National Computational Infrastructure (NCI) to assemble and annotate vast amounts of RNAseq data available for non-model organisms. We have also constructed gene trees to annotate the RNA and understand the species-specific expansion of gene families in vertebrates. Simultaneously, we have developed a web-accessible database for visualization of the RNAseq data. I will present my work on the developments of this database and discuss opportunities and challenges for a systematic effort to collate such dataset.

7

De novo assembly of the scabies mite mitochondrial genome from metagenomic sequencing reveals haplotype structuring and relationship between scabies varieties

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The scabies mite, *Sarcoptes scabiei,* is a parasite of the skin that infects humans and other animal species, causing scabies, a disease that is characterised by rashes and extreme itching. It can also cause a severe form known as crusted scabies in individuals with impaired immunity. Scabies infections are a major health problem in remote indigenous communities in Australia, where co-infection of scabies wounds by Group A streptococci and *Staphylococcus aureus* is thought to be responsible for the high rate of rheumatic heart disease and chronic kidney disease.

As part of the scabies mite genome project, we have collected and sequenced mite DNA from several sources, including: pools of thousands of whole mites from a laboratory pig model, and 2 clinical isolate pools from human patients living in different regions of Northern Australia. This sequencing samples the metagenome of the mite, its gut flora and the wound micro-environment. We have applied a bait and assemble algorithm to this pooled sequence data to assemble the mitochondrial (Mt) genome. We have annotated the Mt genome and used SNP information to infer haplotypes. This provides insight for the first time into the genetic diversity of scabies infections.

8

The dynamic genome: allelic variations in the genomes of single cells from an invertebrate metazoan

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The diversification of immune receptor genes provides a powerful mechanism for the detection of a large array of non-self determinants. This strategy is best exemplified the immunoglobulin superfamily genes of the vertebrate adaptive immune system. Each circulating lymphocyte genome contains only one functional allele at the Ig/TCR locus, which is 'constructed' from a large array of gene segments. Although diversifying immune response gene families have been reported in some invertebrates, little is known about the nature of the molecular mechanisms that underpin such gene diversification. Previous work has demonstrated that sea urchins possess and express a wide repertoire of 185/333 genes in response to immunological challenge. Each individual may possess more than 100 alleles of this gene family in its genome. We report here on the manifestation of somatic gene diversity of the sea urchin 185/333 genes. This study investigated the diversity of 185/333 alleles in the genomes of individual blood cells. Fluorescence Activated Cell Sorting was used to collect individual blood cells into the wells of a multi-well plate. 185/333 sequences were then analysed using allele-specific primers. Our results showed that individual blood cells contained unique haplotypes of 185/333 alleles. This diversification was apparent in specific subpopulations of blood cells. Taken together, our data suggest that sea urchins somatically diversify their 185/333 gene repertoire. It also lends support to the notion that there may be different, but as yet undiscovered, flavours of somatic diversification amongst the metazoans.

9

Climate change and evolutionary adaptation: where are we?

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In this talk I provide a brief of what has been learnt from evolutionary genetic studies of climate change adaptation in natural populations of animals. I briefly outline some examples of recent rapid adaptation and provide summary of the two divergent views that have developed in the literature about the likelihood of evolutionary adaptation occurring in natural populations. Factors that contribute to these different views are briefly covered, including the nature of selection, genotype-environment interactions, population processes and the genetic architecture of traits. I then discuss the components of biodiversity that are most likely and least likely to evolve under climate change, and new insights that are starting to emerge from comparative genomics and population genomics. Implications of these findings for conservation are also mentioned.

References

Franks, S.J. Hoffmann, A.A. (2012) Genetics of climate change adaptation. Annual Review of Genetics 46:185-208

Shoo, L. et al (2013). Making decisions to conserve species under climate change. Climatic Change 119:239-246

Coleman, R.A. Weeks, A.R. Hoffmann, A.A. (2013). Balancing genetic uniqueness and genetic variation in determining conservation and translocation strategies: a comprehensive case study of threatened dwarf galaxias, *Galaxiella pusilla* (Mack) (Pisces: Galaxiidae). Molecular Ecology 22:1820-183

10

The genomic basis of local adaptation to climate in conifers

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Local adaptation is common in widespread conifer species and current reforestation policy reflects this through local seed sourcing and breeding programs. However, as the climate changes local tree populations may become mismatched to their local environments. Our goal is to identify the genes responsible for climatic adaptation in western Canada's two most economically important conifers, lodgepole pine (*Pinus contorta*) and interior spruce (*Picea glauca*, *P. engelmannnii*, and their hybrids). As the genomes of these species are very large (>20Gb), we have used sequence capture methods to target our sequencing efforts to regions of interest. To identify these regions, we developed a *de novo* transcriptome for each species and conducted an RNAseq expression study. Using these data and early drafts of the white spruce genome (SMarTForests Project) and the loblolly pine genome (PineRefSeq Project) we developed sequence capture probes for exons in >28K genes in lodgepole pine, and for >35K genes in interior spruce. Over 600 seedlings per species have been re-sequenced for these exons as well as for control, non-coding regions. We have sequenced these same individuals using a genotype-by-sequencing approach. Associations between provenance climate, phenotypes and SNP genotypes are being used to identify the climatic drivers of local adaptation in both species. Our results will be important for designing reforestation policies that consider future climates, and for understanding the genetic capacity of natural populations to rapidly adapt to new climatic conditions.

11

Effective population size and migration along a steep environmental gradient: Insights from niche models and population genomics

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Understanding the how species respond to climate change is essential for the maintenance of biodiversity. Historically climate has shaped the distribution of species; with oscillations between glacial maximum resulting in population expansions and contractions. The rate of anthropogenic climate change has exceeded initial predictions. Australia will be hotter and drier with more intense and frequent droughts and heat waves. To address how species will respond to these challenges we employ ecological niche models and population genomics using NSW Waratah (Telopea speciosissima) as a case study.

Our previous work has demonstrated that coastal and upland populations have genetically and ecologically differentiated, despite evidence of genetic exchange at intermediate elevations. Here we present correlative models predicting the distribution and abundance of coastal and upland populations under LGM and future climate scenarios. NGS gemonic and transcriptomic are used to independently estimate effective population sizes and migration rates in coastal and upland populations. This study highlights the importance of historical and ecological processes in determining the capacity for species to respond to climate change.

12

Exploring full mitochondrial genomes in search of signatures of positive and purifying selection

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Directional selection imposed by environmental variation can reduce intraspecific gene flow and promote population divergence. This is especially true if selection acts on traits with large fitness effect, for example, metabolic pathways. Mitochondrial and nuclear genetic structures over much of the species range of an Australian bird, the Eastern yellow robin (*Eopsaltria australis*), are perpendicular in space. This is inferred to be the result of strong natural selection mainly in females affecting one or more of: mitochondrial genes, W-chromosome genes, other nuclear genes or interactions among these, notably mitochondrial-nuclear interactions. Given that mtDNA variation in this species is strongly correlated with hottest summer temperatures, we hypothesize that selection has acted on the Oxidative Phosphorylation (OXPHOS) pathway to energy production that is co-encoded by mitochondrial and nuclear genes. As starting point in testing this, we have analysed patterns of purifying and positive selection in complete mitochondrial genomes of two sister species of yellow robins, *E. australis and E. griseogularis*, and outgroups, focussing on the 13 mitochondrial-genes coding for OXPHOS proteins. Analysis of patterns of amino acid replacements, including changes in their physicochemical properties and projected protein structure, revealed that some regions of the *E. australis* mitogenome may have been shaped by positive selection, and this has potentially impacted protein functioning (i.e. may be locally adapted). These results contribute important information to testing for co-adaptive evolution of mitochondrial and nuclear genomes, for which genomic variation in nuclear-encoded OXPHOS genes will be collected across environmental transects.

13

Evolution of a butterfly mimicry locus through modular regulation of an input-output gene

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Publish consent withheld

14

Incest vs. abstinence: reproductive tradeoffs between mate limitation and progeny fitness in a self-incompatible invasive plant.

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A plant's mating system influences its success in invading new environments. Self-compatible species benefit from reproductive assurance during the early phases of the invasion process when founding populations are small, but may suffer the fitness costs associated with selfing. In contrast, self-incompatible (SI) plants often suffer mate limitation in small populations but maintain progeny fitness. SI plants often have complex dominance interactions among S-alleles that increase mate availability by allowing mating among half- and full-sib relatives. Such biparental inbreeding generally has smaller fitness effects than selfing. In this study we assessed if promotion of biparental inbreeding through selection for intermediate dominance S-alleles is a ecologically and evolutionarily viable strategy for SI invaders that simultaneously maximises mate availability and progeny fitness. Specifically, we explored the tradeoff between mate availability and progeny fitness in five Australian populations of the SI weed wild radish (Raphanus raphanistrum). To do this we used a two-generation controlled cross experiment to generate self, full-sib, half-sib and unrelated individuals and measured their life-time fitness under field conditions. Diallel crosses were conducted to estimate S allele numbers, frequencies of dominance interactions and mate limitation in each population. We found large negative effects of selfing on fitness in all populations across a broad range of vegetative growth and plant reproductive characters e.g biomass, flowering, seed set. Biparental inbreeding consistently resulted in smaller but significant negative fitness effects. Diallel crosses showed dominance among S alleles contributed to increased mate availability through facilitation of mating among relatives, though the degree of this effect varied among populations. Interestingly the importance of dominance in freeing up mate availability in a population was positively correlated with the observed severity of inbreeding depression expressed in selfed individuals. Taken together these data suggest that dominance among S alleles may play an important role in freeing up mate availability in small populations of wild radish. As biparental inbreeding was demonstrated to be less severe in its effects on fitness than selfing, selection for intermediate dominance alleles may provide a novel ecological strategy to maintain population viability during the colonisation process, while preserving self-incompatibility.

15

Sexual mimicry in sympatric orchid species promotes outcrossing, multiple paternity and reproductive isolation.

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Most flowering plants engage animals to carry out the essential service of pollination. The majority of these plants have evolved flowers that advertise rewards for this service via visual and chemical cues such as petals and scent. There are however a number of species whose false advertisements draw pollinators to rewardless flowers. Among them are the sexually deceptive orchids, which employ a precise chemical mimicry of female wasp sex pheromones to attract male wasps for pollination. This study utilizes neutral genetic markers to examine the consequences of sexual deception for mating patterns in two sympatric Australian orchids. We show through behavioural and population genetic analysis that the chemical mimicry crucial to sexual deception is also responsible for pre-pollination reproductive isolation and potentially even speciation. We also report paternity and mating system analyses that demonstrate that sexual deception results in near exclusive outcrossing despite clonality as well as multiple paternity–a rarity for orchids. We show that this pollination strategy is an adaptive solution to the problem flowers face of simultaneously attracting pollinators and persuading them to leave quickly.

16

Timing of antimicrobial use influences the evolution of antimicrobial resistance during disease epidemics

Mark Tanaka, Ben Althouse, Carl Bergstrom

While the emergence and spread of antibiotic resistance has been well studied for endemic infections, comparably little is understood about this problem in the context of epidemic infections such as influenza. The availability of antimicrobial treatments for epidemic diseases raises the urgent question of how to deploy treatments in a population to achieve maximum benefit despite resistance evolution. Recent simulation studies have shown that the number of cases prevented by antimicrobials can be maximized by optimally delaying the use of treatments during an epidemic. These studies focus on the indirect benefit of antimicrobial use: preventing disease among untreated individuals. Here we identify and examine the direct benefit of antimicrobial use, namely, the number of successfully treated cases. We develop mathematical models to study how the schedule of antiviral use influences the success or failure of subsequent use due to the spread of resistant strains. The direct benefit of drugs is maximized when their use is postponed, even if an unlimited supply of the drug is available. This occurs because the early use of antimicrobials disproportionately drives emergence and spread of antibiotic resistance, leading to subsequent treatment failure. However, in the case of antimicrobials with low effect on transmission, the benefits of delaying antimicrobial deployment are only modest and can only be reaped if the trajectory of the epidemic can be accurately estimated early. Given the uncertainties faced in most epidemic situations it will usually be sensible to initiate widespread antimicrobial use as early as possible.

17

One parasite and two unexpected hosts: Using transcriptomics to understand host-parasite adaptations of two strains of *Tritrichomonas foetus*

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Few, if any, protozoan parasites are reported to parasitize two very different host species and exhibit extreme organ tropism like the flagellate *Tritrichomonas foetus*. In cattle, this parasite causes extensive damage to the uterine lining which results in abortion while infection in domestic cats causes chronic diarrhoea. Whilst single-gene comparisons and cross-infectivity studies suggest that these two strains fall under the same *T. foetus* species-level umbrella, little is known of their strain-specific characteristics. Analysis of the transcribed RNA offers an ideal means of investigating regulatory patterns and preferentially expressed genes. In the absence of a *T. foetus* genome, we employed a *de novo* approach to characterize the whole-cell transcriptome of a bovine and feline strain with the aim of providing a more dynamic approach of understanding cell-wide processes and virulence factors in *T. foetus.* This provides the first comprehensive cell-wide transcriptomics analysis of the bovine and feline *T. foetus* strains and aims to identify to what extent the expressed *T. foetus* genomereflects the host from which it was isolated.

18

Contrasting dispersal and inbreeding in a fig-pollinating wasp and its parasitoid

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Figs (*Ficus* spp.) and their pollinators (Hymenoptera: Agaonidae) are a classic example of obligate symbiosis, having evolved completely interdependent life histories. Their symbiosis is exploited by host-specific parasitoid wasps, which kill pollinator offspring and can thus reduce fig pollen transfer. Interactions between the figs, pollinators and parasites play an important role in the coevolution of this system. Pollinator dispersal mediates gene flow of the host fig, and therefore influences fig species boundaries. Furthermore, attack by parasitoids may actually help stabilise the mutualism in the long term by reducing seed predation by pollinator offspring. Previous studies indicate that some fig-pollinating wasps are capable of long-distance dispersal, but little is known about the dispersal and mating systems of their parasitoids. Differential dispersal capacity between pollinator and parasitoid may affect fig – pollinator dynamics in the short term, particularly in isolated fig populations, as well as long-term coevolution. Here, we present the first comparative microsatellite analysis of a fig-pollinator and its parasitoid.

We collected *Pleistodontes imperialis* sp. 1, a pollinator of Port Jackson figs (*Ficus rubiginosa*), and its parasitoid (*Sycoscapter* sp. A) at a series of sites located from about ten to several hundred km apart in eastern Australia, and genotyped all wasps at six microsatellite loci. Preliminary results indicate that *P. imperialis* sp. 1 comprises two distinct genetic populations, separated geographically by a ~600 km gap between Mackay and Atherton in Queensland. Pairwise F_{ST} values between sites were lower for *P. imperialis* wasps (0.003 – 0.031) in the southern population compared to co-sampled *Sycoscapter* wasps (0.000 – 0.054). This supports the prevailing but untested idea that pollinators disperse further than their parasitoid enemies. Despite this, Bayesian clustering analysis determined that *Sycoscapter* forms a single genetic population over hundreds of kilometres. Inbreeding was higher in pollinators, consistent with our prediction that fewer pollinator than parasitoid females contribute eggs to each fig, limiting the diversity of mates in the next generation.

Fig-pollinator populations are large and geographically expansive. Fig and pollinator gene flow thus occur over large distances and may prevent the evolution of local genetic adaptation between sites with differing climates. Lower dispersal distances in parasitoids may be more likely to allow local genetic adaptation, and also leave more isolated fig and pollinator populations free from parasites.

19

Coevolution between bacterial endosymbionts and their psyllid hosts of the *Cardiaspina* genus (Hemiptera: Psyllidae)

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Psyllids are sap-feeding insects belonging to the hemipteran suborder Sternorrhyncha. The critically endangered Cumberland Plain Woodland of Western Sydney has recently experienced massive infestations by a psyllid belonging to the genus *Cardiaspina* (Hemiptera: Psyllidae). The psyllid attacks only one host plant in the region, *Eucalyptus moluccana* (Grey Box). *Cardiaspina* psyllids have not been reported previously from Grey Box, making our study the first description of this host-herbivore relationship. The first aim of our study was to determine the placement of this *Cardiaspina* sp. within the genus using genetic markers.

Psyllids are also known for their intimate interactions with bacterial endosymbionts. Many bacterial taxa have evolved obligate endosymbiotic associations with animal hosts, often characterised by vertical transmission of bacteria to host offspring. *Candidatus* Carsonella ruddii is the primary obligate endosymbiont of psyllids, while *Arsenophonus* is a genus of bacteria described in a range of arthropods and can be either vertically or horizontally transmitted. This study aimed to test the phylogenetic congruence of both bacteria with the phylogeny of their psyllid hosts, by using mitochondrial and nuclear host genes and at least two bacterial genes.

When looking at a number of species, we found that the Grey Box psyllid is very closely related to *Cardiaspina* species that feed on other eucalypt species in the Box group, supporting strong conservation of host plant associations. In fact, very low levels of genetic differentiation suggest that they could all be one *Cardiaspina* species, and this finding creates the need for a more comprehensive phylogenetic analysis of this genus. *Candidatus* Carsonella ruddii has been found with 100% prevalence in all species of the *Cardiaspina* genus tested and appears to have a phylogeny congruent to that of its host supporting the idea of long-term cospeciation with the host. Unexpectedly, *Arsenophonus* also has 100% prevalence in almost all *Cardiaspina* species screened. This makes the genus *Cardiaspina* an ideal system in which to compare phylogenetic congruence with the insects of a vertically transmitted primary symbiont and a secondary symbiont shown in other insect groups to be transmitted both vertically and horizontally. For the first time, we have tested such coevolutionary relationships at the lowest taxonomic level (between species) in the Psylloidea superfamily.

20

Functional genotypes are associated with commensal *Escherichia coli* strain abundance within host individuals and populations

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The selective pressures that determine genotype abundance and distribution frequently vary between ecological levels. Thus, it is often unclear whether the same functional genotypes will become abundant at different levels and how selection acting at

these different scales are linked. We examined whether particular functional genotypes, defined by the presence or absence of 34 genes, of commensal *E. coli* strains were associated with within-host abundance and/or host population abundance in a wild population of 54 adult mountain brushtail possums (*Trichosurus cunninghami)*. Our results revealed that there was a positive correlation between a strain's relative abundance within individuals and the strain's abundance in the host population. We also found that strain abundance at both ecological levels was predicted by the same group of functional genes (*agn43, focH,* micH47*, iroN, ygiL*, *ompT*, kspmT2 and K1) that had associated patterns of occurrence. We propose that direct selection on the same functional genes at both levels may in part be responsible for the observed correlation between the ecological levels.

21

A new molecular sexing technique for butterflies – does *Wolbachia* really feminise genetic males in *Eurema*?

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Wolbachia pipientis is an obligate, intracellular Alphaproteobacterium, related to *Rickettsia*. It is the most common endosymbiont of insects and 40% of all arthropods are estimated to be infected with *Wolbachia.* The transmission of *Wolbachia* is mainly vertical by maternal inheritance, while its manipulation of host reproductive systems can increase the number of infected females in host populations. For example, *Wolbachia* can bias host sex ratios and modify host sex determination through cytoplasmic incompatibility, parthenogenesis, male-killing or feminisation. Feminisation is the least reported phenotype, mostly detected in terrestrial crustaceans. It also occurs in three insect species, including two closely related butterfly species in the genus *Eurema.* Feminisation cause genetic males to develop into phenotypic females. In butterflies, the detection of the W chromatin body in females has so far been the main evidence for feminisation. However, this method is not reliable for all Lepidoptera and a molecular method based on sex chromosomal markers may be better. We developed a quantitative PCR (qPCR) assay, comparing relative gene dose ratios of Z-chromosomal genes with an autosomal gene, to differentiate directly between true genotypic females and *Wolbachia* feminised males. This test correctly genotyped the sex of butterflies that lacked the feminising *Wolbachia* strain *w*Fem. However, all *wFem* infected females that were negative in the W chromatin body assay (thus considered feminised males) also had female gene dose ratios. These results conflict with the current model of feminisation of males in *Eurema* and suggest re-assessment of the role of *Wolbachia* as feminising agent in this host.

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Sex, slime and mitochondria

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While sexually-reproducing organisms each contribute nuclear DNA to their offspring, mitochondrial DNA is generally transmitted by a single parent. This is true for species with sexes, where mitochondria are typically inherited from the female, and in single-celled species that lack defined sexes and instead have mating types. The current explanation for uniparental inheritance is the genomic conflict theory, which hypothesises that uniparental inheritance has evolved to mitigate conflict between the mitochondrial and nuclear genomes. If mitochondria from both parents were allowed to mix, it could select for fast replicating, but energetically inefficient, 'selfish' mitochondria, which could spread and severely lower cell fitness. A recent empirical study in mice has indicated that the mixing of different, but normal, mitochondria lineages (heteroplasmy) could be sufficient to cause organism dysfunction. Could it be that cells avoid mixing mitochondrial haplotypes simply because it is costly? Here, I present a mathematical model that examines whether costs from mixing mitochondrial haplotypes could have led to the evolution of uniparental inheritance in an ancestrally-biparental population.

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Queensland fruit fly and the Lewontin-Birch introgression hypothesis

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Queensland Fruit Fly (Bactrocera tryoni - TRY) and Lesser Queensland Fruit Fly (B. neohumeralis - NEO) overlap in distribution over large areas of Queensland. The range of TRY also includes northern coastal NSW and has spread to inland NSW and to more Southern regions of Australia. In the 1960s, RC Lewontin and LC Birch put forward the hypothesis, based on the lack of detectable inviability in hybrids, that the Southern form of TRY might involve introgression from some NEO genes. Contemporary phenotypic intermediates in the wild have been shown to be genetically non-hybrid. However, genome sequencing data are consistent with at least one historical hybridisation event, although indicating that only around 1-2% of the genome is involved. This conclusion is complicated by the extreme similarity of the two genomes in virtually every genome region investigated.

Many questions are left unanswered. The chief of these relates to the question of how much hybridisation occurs in the areas of overlap, and how the two species can maintain their identity in the face of any such hybridisation. The species isolation mechanism (time of day for mating) is a strong barrier to hybridisation, although it can be breached in the laboratory, with TRY (dusk mating) essentially dominant to NEO (day mating). This suggests that NEO genes can be incorporated into TRY rather than vice versa. The observed direction of apparent introgression in Southern TRY is thus in agreement with the genetic observations on inheritance of mating-time.

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Maternal lineages best explain the associations of a semi-social marsupial in the presence of fine-scale genetic structure

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Kinship is a key factor that can influence the fitness benefits associated with social behaviors, through the operation of kin selection. A species' patterns of dispersal, and resulting fine-scale spatial genetic structure, can mediate kin selection by altering both the capacity for kin cooperation and the intensity of kin competition. We used proximity logger collars and multilocus genotypes to investigate how genetic relatedness influences the associations of mountain brushtail possums (*Trichosurus cunninghami*), in the context of fine-scale spatial genetic structure. We found that spatial proximity was an important factor influencing the nocturnal encounter rate. Further, proximity was associated with relatedness between individuals, a pattern that was stronger among females than males. After proximity was accounted for, we found that possums who shared a mitochondrial haplotype associated more often and for longer during nocturnal activity. By comparison, autosomal nuclear relatedness metrics did not explain associations. This is likely to represent, in part, mother-offspring associations and suggests that kin recognition may occur through familial cues. Females also associated for longer than did males, which may be attributed to a combination of kin preference and differences between the sexes in genetic structuring. Thus, this study demonstrates the way in which social behaviors may be shaped by how kin selection and fine-scale spatial genetic structure interact.

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Population structure of the rare, clonal *Senecio macrocarpus* in the grasslands of Victoria.

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The Natural Temperate Grassland of the Victorian Volcanic Plain currently occupies less than 1% of its prior range prior. Consequently, many of the plant species endemic to the Volcanic Plain are in decline due to habitat loss. The hexaploid *Senecio macrocarpus* F. Muell. ex Belcher (Asteraceae), a perennial forb, is no exception and has been part of grassland reestablishment efforts in Victoria, Australia. In order to inform conservation efforts for *S. macrocarpus*, the population structure of *S. macrocarpus* was investigated with thirteen neutral microsatellite markers and maternal lineages (haplotypes) were identified from two chloroplast regions, psbJ-petA and trnL-rpL32. *Senecio squarrosus* is an uncommon species similar to *S. macrocarpus*,which occupies the same geographical area. This co-occurring hexaploid species was included in the study to elucidate any potential gene flow between the two species. A total of 529 *S. macrocarpus* and 28 *S. squarrosus* individuals over 20 populations were sampled. Genotyping identified a total of 104 multi locus genotypes from 557 total individuals. The most common clone was found 108 times across 10 different populations. Global F_{st} could not be estimated due to extreme variation between loci, either having a severe excess or deficit of heterozygosity. F_{st} within loci was estimated between 0.08 – 0.49. Both PCoA and individual assignment results indicate that *S. macrocarpus*maintains the highest level of genetic diversity in the largest population located in South Australia. All other populations show similarity to the South Australian population via genetic distance and individual assignment of genetic clusters. Neutral markers show some *S. macrocarpus* individuals are nearly identical to the *S. squarrosus* individuals. Indicating recent speciation and common parent lineages. Chloroplast markers revealed few differences between populations of *S. macrocarpus* but were further removed from*S. squarrosus* individuals. The results suggest that *S. macrocarpus* may reproduce mainly via apomixis and this is the first evidence that supports apomixis in the tribe Senecioneae. This is being investigated further as apomixis within *S. macrocarpus* complicates the long-term viability and conservation of the species.

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A SNP-based approach to determining pedigrees and assessing genetic diversity in the Tasmanian devil insurance population.

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The Tasmanian devil is threatened with extinction due to a rare form of transmissible cancer, devil facial tumour disease (DFTD). In less than 20 years DFTD has wiped out up to 85 % of the devil population leading to the establishment of an insurance population of devils. The success of this insurance program depends on maintaining as much wild diversity as possible. Accurate pedigree information is also vital to the success of the program to ensure that inbreeding does not occur within captivity. The use of free-range enclosures means that in many cases neither dam nor sire is known. Traditional markers used in the devil have often lacked the allelic diversity required to resolve these complicated pedigrees. We have developed a set of single nucleotide polymorphic (SNP) markers which have greater discrimination than traditional markers in determining familial relations among devils. As some of these markers are placed in functional regions of the genome we are also able to use the assay to assess adaptive genetic diversity in the captive breeding context. We are incorporating this genetic data into the captive breeding program to increase the chance of survival of the species and enhance the success of future reintroductions to the wild.

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Population genetics of the invasive northern Pacific seastar

Mark Richardson, Craig DH Sherman

The northern Pacific seastar, *Asterias amurensis*, is a benthic marine predator, which has established several large invasive populations in Australian waters since its introduction to Hobart, Tasmania approximately 20 years ago. Recently, it has expanded its invasive range into the Tidal River estuary, Wilson's Promontory Marine National Park. Given the relatively recent nature of these introductions, Australian *A. amurensis* populations provide an exciting model to study contemporary evolutionary processes. In this talk, I will provide a summary of our research to date, where we address; the source, diversity and connectivity between invasive populations, whether these populations have undergone rapid evolutionary change and if they have the capacity for thermal adaptation and further range expansion. An understanding of *A. amurensis* population dynamics and evolutionary responses to novel environmental conditions, will not only inform management practices, but further our knowledge of the genetic basis of important processes in invasion ecology.

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Population viability and major histocompatibility complex (MHC) genetic diversity of two dolphin populations in Western Australia

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Genetic diversity is considered essential for populations to adapt to a changing environment. Measures of genetic diversity to evaluate how isolated, inbred and viable a population is, are typically based on various neutral markers, such as microsatellites or mitochondrial DNA control regions. However, genetic diversity to guide conservation management is better reflected by coding regions of functionally important genetic loci, such as the major histocompatibility complex (MHC) genes. In this study we assessed population viability and MHC diversity of two bottlenose dolphin (*Tursiops* cf. *aduncus*) populations in Western Australia. From demographic data, the larger Shark Bay population appears to be stable, whereas the smaller Bunbury population was forecast to decline. Furthermore, we found the more viable Shark Bay population to be more genetically diverse for at least one (MHC II, DQB, exon 2) of the three MHC loci that we investigated. Our findings are consistent with the hypothesis that large, viable populations typically display greater genetic diversity compared to smaller, less viable populations. A larger population, such as the Shark Bay dolphin population, is thus potentially more robust to natural or human-induced changes to coastal ecosystems it inhabits across Australasia.

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Selective replication of mitochondria at the edge of an expanding invasion

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Invasive species introductions provide an ideal opportunity to study current evolution. Although the process of invasion has been shown to involve both random and adaptive genetic changes, it is rare to capture these changes while they are occurring. Further, few examples of selection on the mitochondrial genome in wild populations exist. Here we show that, in a recent invasion, there were rapid directed changes in mitochondrial DNA genotype proportions, with two genotypes found in heteroplasmic states. We considered processes of admixture, selection and random drift at both the population level and in the gametogenic tissue. We show that the observed changes are best explained by selective replication of mitochondria carrying newly-arisen genotypes at the advancing front. Our finding of current selection on mitochondrial DNA, which is often postulated as a constraint for phylogenetic analyses, is rarely seen in action and is important to understanding adaption in invasive populations.

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Sequence variation within the mitochondrial DNA affects patterns of gene expression in the mitochondrial transcriptome

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Mitochondria are key components of cellular metabolic processing, providing most of the cellular energy required for survival. The small set of genes located within the mitochondria has recently been the subject of much attention by evolutionary biologists, as a groundswell of studies have documented that allelic variance within the mitochondrial DNA (mtDNA) often confers modifications to the phenotype. Mitochondria have been shown to play an active role in the process of ageing, and mitochondrial allelic variance has been linked to this process. Recent studies suggest that some of this allelic variance is even male-specific in its effects. Here, we use the fruitfly *Drosophila melanogaster* as a model to advance our understanding of the link between the mitochondrial genotype and phenotype, in young and old flies of each sex. We sequenced full mitochondrial genomes of 13 lines, and examined mtDNA-specific transcriptional profiles of 9 (out of 13) key mitochondrial genes expressed alongside an isogenic nuclear background. We found mitochondrial haplotypic effects on patterns of gene expression within the mitochondrial transcriptome. These effects were in part mediated by both the age and sex of the flies. Furthermore, we identified certain mtDNA SNPs associated with large effects on gene expression. Our results indicate that mtDNA-mediated effects on phenotypic expression are highly dynamic, across the sexes and across ontogeny, and putatively regulated via differential expression among the core set of mtDNA protein-coding genes.

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The Identification and Characterisation of Immune Genes in the Milk Transcriptome of the Tasmanian devil (*Sarcophilus harrisii*)

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Tasmanian devil (*Sarcophilus harrisii*) joeys, like other marsupials, are born underdeveloped and immunologically naive, unable to mount an adaptive immune response. In marsupials, the milk is vital, not only for nutrition but also for providing passive immunity. In the Tasmanian devil, the milk constituents important for post-natal development and immune protection, have not been previously investigated. The aim of our study was to identify and characterise the genes expressed in the Tasmanian devil milk, with a focus on immune gene expression. A transcriptome was sequenced using Tasmanian devil milk, which was obtained during lactation at day 121. The transcriptome was assembled and annotated using Trinity and Trinotate. A total of 233 660 transcripts were expressed in the milk transcriptome, which included approximately 25 000 immune gene transcripts. The top 200 most highly expressed transcripts were dominated by milk protein genes and ribosomal genes, but also included immune genes such as cytokines, major histocompatibility complex, chemokines, and lysozyme. This study provides the first insight into the components of Tasmanian devil milk, and in particular, the proteins important for immune protection of devil young.

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Studying immunogenetic diversity to aid the conservation of threatened species.

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Maintaining the genetic diversity of threatened populations is essential to preserving species' adaptive potential. We can use studies of immunogenetic diversity in natural populations to learn how evolutionary processes affect key genomic diversity. This data allows us to refine management methods to maximise diversity, with the aim of maximising the long-term genetic health of the population. We are using measures of immunogenetic and genomic diversity, alongside pedigree-based mean kinship methods, to aid in the conservation of Tasmanian devil. Devil populations in the wild are gravely threatened by a contagious cancer, so in 2006 an "insurance population" was established across zoos and reserves to protect the species from extinction. Our work examines how genetic diversity of the insurance population is being preserved, and tests whether current approaches can be improved. Although this work is in the early stages, we demonstrate how the latest sequencing, analytical and computational methods allow us to test the role of evolutionary processes on maintaining immunogenetic diversity and fitness. These results will aid not only Tasmanian devil conservation, but also provide a greater understanding of the assumptions underlying conservation management strategies for all species.

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Characterisation of Toll-like receptors in two bottlenecked species, Tasmanian devil and Koala

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Toll-like receptors (TLRs) are evolutionary conserved in all animal species and they play key roles in recognising and binding a wide range of pathogens. At least twelve TLRs have been observed in mammals and they can be divided into viral and non-viral TLRs according to their ligand recognition. The aim of this study was to characterize the levels of genetic diversity at TLRs in two bottlenecked Australian native marsupial species, the Tasmanian devil (*Sarcophilus harrisii*), and the koala (*Phascolarctos cinereus*). In this study, we have identified ten TLRs (TLR2-10 and TLR13) in the devil and nine (TLR2-10) in the koala. TLR13 ortholog couldn't be found in the koala sequence transcriptome. The TLRs protein sequence similarity with human is on an average of 60% in the devil and 64.17% in the koala. The devils TLR4, TLR5, TLR7, TLR8, TLR9, TLR10 and TLR13 are monomorphic and TLR2, TLR3 and TLR6 are harbouring only two alleles in the 25 study samples. With the nine koala TLRs, TLR10 is monomorphic and 2 to 6 alleles have been observed varied at other TLRs in the 20 koalas from the NSW population. Future studies will focus on TLRs diversity and disease status in these species.

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Variation at innate immunity genes in Hawaiian honeycreepers

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Publish consent withheld

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Expression of the microRNA, mir-31, is reduced in leukocytes of dogs with atopic dermatitis

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Canine atopic dermatitis (AD) is a complex genetically-linked immunological hypersensitivity which has similar clinical signs and pathological features to human AD, and involves immune dysregulation and skin barrier impairment. Accumulating evidence supports the involvement of specific microRNA (miRNA) in regulating the immune system, and in autoimmune and other immune-dysfunction diseases. Few studies to date report the role of miRNA in human AD and asthma and no information is available for canine AD. Considering the homology between dog and human miRNA sequences, canine AD may serve as a useful animal model to study the role of miRNA in human AD. To explore the immune-regulatory mechanisms in canine AD, expression patterns of miRNA from leukocytes of atopic dogs (n=7) were compared to controls (n=6), utilising the Affymetrix GeneChip miRNA 2.0 array, which contains probes from 133 species, including 291 *Canis familiaris* miRNAs probes (cfa-miR). Fifteen differentially expressed cfa-miRs were identified; some with previous links to immune dysfunction diseases in humans. The cfa-miR-31 was present at lower levels in atopic dogs, suggesting a potential dysregulation that may relate to T-regulatory cells. Further analysis, utilising the comparative aspect of the array will be presented. This study suggests that miRNAs may be altered in atopic dogs, may provide biomarkers to assist with diagnosis, and are potential therapeutic targets for AD.

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Characterisation of the Tasmanian devil immunome and identification of SNPs within devil immune genes

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The world's largest remaining marsupial carnivore, the Tasmanian devil, is being driven to extinction by the spread of a disease known as the devil facial tumour disease (DFTD). This disease has wiped out over 80% of the devil population since its emergence in 1996. In order to investigate the immunology of the disease and for the development of therapeutic agents against DFTD, a thorough understanding of the devil immune system is required. In 2011 two genome sequences of the Tasmania devil were released. This has provided us with raw data required to study the devil immune system in depth. I have investigated the devil immunome, focusing on cytokines, chemokines and genes of the innate immune system. The majority of these genes are conserved with direct orthologues in humans and opossum, though lineage specific duplications are present in chemokines, interferons and several interleukins. Using re-sequencing data from nine devils I have identified SNPs within devil immune genes. While the polymorphism of devil genes in low, immune genes containing non-synonymous SNPs are present. Maintenance of this variation will be critical for the devil breeding program.

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Cathelicidins in the Tasmanian devil (*Sarcophilus harrisii*)

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Antimicrobial peptides are a primitive component of the innate immune system. Cathelicidins are a predominant family within mammals, contributing to host immunity through antimicrobial and immunomodulatory functions. They have been studied extensively in eutherian mammals but marsupials are relatively unexplored. Marsupials give birth to immunologically naïve young which are protected from infection by cathelicidins in the pouch. This unique reproductive physiology has encouraged lineage specific expansion of the cathelicidin gene family, resulting in numerous diverse peptides.

The Tasmanian devil (*Sarcophilus harrisii*) is under threat from a contagious cancer, devil facial tumour disease (DFTD). Human and bovine cathelicidins exhibit anti-tumour activity against a number of cancers. Studies in the tammar wallaby have revealed the potency of marsupial cathelicidins against drug resistant bacteria. As such, the Tasmanian devil genome provides new avenues in the search for cathelicidins with therapeutic potential to treat DFTD and resistant pathogens.

We identified six cathelicidins in the Tasmanian devil genome. Transcriptome BLAST searches show that cathelicidins are expressed in the spleen, lymph node, testis, milk and devil facial tumour. A multiple sequence alignment reveals that Tasmanian devil cathelicidins are highly variable within this species, and amongst other marsupial (tammar wallaby 19-70% similarity) and eutherian (human 34-49% similarity) cathelicidins. The sequence alignment was used to construct a neighbour joining phylogenetic tree. As expected, Tasmanian devil cathelicidins cluster with other marsupials and are distantly related to eutherians. Two Tasmanian devil peptides were orthologous to a tammar wallaby and grey short-tailed opossum cathelicidin, demonstrating the existence of ancestral cathelicidins.

Two Tasmanian devil putative mature peptides were synthesised and tested against a range of fungal pathogens. One peptide was capable of killing *Candida krusei*, *Candida parapsilosis* and was 3 and 6 times more effective against *Cryptococcus neoformans* and *Cryptococcus gattii* respectively, than the antifungal drug fluconazole. In future studies, all six cathelicidins will be tested against a range of resistant bacterial pathogens and DFTD cells. This study highlights the potential of marsupials such as the Tasmanian devil to provide new drugs to treat human and animal disease.

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Novel Defensin Peptides of the Tasmanian Devil *(Sarcophilus harrisii)*

Elizabeth Jones, Yuanyuan Cheng, Denis O'Meally, Katherine Belov

The Tasmanian devil is threatened with extinction due to the emergence of a contagious cancer called Devil Facial Tumour Disease (DFTD). Tasmanian devils under 18 months old do not get DFTD. It is possible that genes of the innate immune system may play a role in protecting juveniles and therefore the characterization of these genes is important. Defensins are a small antimicrobial peptide family that displays a wide range of antimicrobial and immunoregulatory functions in a diverse range of species. Known as one of the natural antibiotics of the body this gene family displays extraordinary sequence and bioactivity diversity, reflecting the selection pressures and specific microbial challenges that individual species have faced over millennia. With the recent sequencing of the Tasmanian devil genome it is now possible to characterize the defensin gene family. Using genome and transcriptome mining and hidden markov models we have identified 34 beta defensins and 5 alpha defensins in the Tasmanian devil genome. Each of these genes have similar characteristics to defensins found in other mammals, including a six cysteine motif in the mature peptide, cationic charge (between 1+ to 11+) and high proportion of hydrophobic residues (>30 %). These genes show conservation across mammalian species, as well as species specific gene expansions. Preliminary analysis suggests at least one defensin is widely expressed in the heart, kidney, liver, lung, uterus, blood, lymph node and in primary tumour tissue. Several other defensins show tissue specific expression in the testis and brain. This work provides the first steps for understanding the role of these peptides in the Tasmanian devil and DFTD.

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Asexual queen succession system in termites: evolution and mechanism

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The evolution and maintenance of sexual reproduction is believed to involve important tradeoffs. The queens of social insects are faced with a dilemma over the costs and benefits of sexual and asexual reproduction. Asexual reproduction by a queen doubles her contribution to the gene pool. However, overuse of asexual reproduction reduces the genetic diversity of the offspring and thus the ability of the colony to adapt to environmental stress. Recent research suggests that queens of some termite species can solve this tradeoff by the conditional use of sexual and asexual reproduction, whereby queens produce the next generation of queens by parthenogenesis but use sexual reproduction to produce other colony members. This reproductive system, so called AQS (Asexual Queen Succession), has been found in *Reticulitermes speratus*, *R. virginicus R. lucifugus* and in some higher termites, indicating that it has evolved multiple times independently in termites. In the AQS species, queens produce parthenogenetic offspring under the presence of kings by closing micropyles (sperm-gates, i.e., tiny openings for sperm entry) of their eggs. I discuss possible physiological mechanism and genetic background underlying the asexual queen succession system in the AQS species. In addition, AQS system and consequent sex-asymmetric inbreeding provide ideal opportunity to test kin selection in diploid organisms, although a strong test of the theory has proven difficult in diploid social insects because they lack relatedness asymmetry unlike Hymenoptera.

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How Robust is Species Detection using Environmental DNA?

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Answers to some of the most important questions in wildlife science, conservation and management require reliable data on species' presence, abundance, and distribution. DNA, extracted from hair, feaces, and other environmental sources (e.g. soil, water) is a valuable source of this information that does not require handling, capturing, or even observing individual animals. DNA-based detection methods promise a number of advances over traditional detection methods, based on visual identification of specimens using diagnostic morphological criteria. It is now widely considered that DNA-based methods of environmental monitoring can be undertaken with relatively high throughput and at low cost per sample, delivering substantial sensitivity benefits over traditional methods. However, many studies are undertaken without parallel development of robust quantitative frameworks that allow the probability of detection to be considered in order to derive clearly interpretable outcomes. For example, determining whether an invasive species is present or absent requires a probability estimate that encompasses both the sampling methodology and the DNA technique itself. The development and application of frameworks that deal explicitly with these probabilities will be essential if the promise of DNA surveillance as a standardised tool to address applied ecological questions is to be realised.

I will present an overview of DNA detection in the context of wildlife management using examples from my own research on invasive species. Particular focus will be on the development of a probabilistic framework for the design, implementation and interpretation of detection of environmental DNA (eDNA) methods to detect aquatic invasive species how this is critical for defensible decision-making.

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Sex, genomics and epigenetics

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Although sex determining pathways are highly conserved between vertebrates, the first step shows remarkable variation. Mammals, including humans, have a conserved X chromosome, and a degenerate Y that contains the male-dominant *SRY* gene that evolved from the X-borne *SOX3*. The Y is hard to sequence because it is full of repetitive sequence. Birds present the reverse situation, a highly conserved Z chromosome common to both sexes and a female-specific, degenerate W. Sex appears to be determined by differential dosage of a Z-borne gene *DMRT1*. Reptiles and fish show a huge variety of sex determining mechanisms. Some are genetic, including XY systems (male heterogamety) and ZW systems (female heterogamety), some highly differentiated like the human XY and chicken ZW, and some cytologically homomorphic. Sequencing projects reveal that these systems use a variety of different genetic triggers, but, remarkably, the same genes (including *SOX3* and *DMRT1*) have independently evolved sex determining functions in several lineages. Many reptiles lack sex chromosomes, determining sex via incubation temperature. Some species, like the dragon lizard and a flatfish, do both, having chromosomal sex determination at moderate temperature and a sex-switch override at extremes. These systems may prove to be the most informative, yielding novel sex determining genes or, more remarkably, orthologues of the same genes, and providing insights into how they interact with the environment via epigenetic pathways.

1. Graves, J.A.M., 2014. Chromosome Research 22: 45-57 Graves, J.A.M. 2014. Nature Genetics 46, 215–217 402. Graves, J.A.M. 2013. Developmental Dynamics 242: 354-359

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Gene expression associated with the recent evolution of viviparity

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The transition to viviparity (live-bearing) from oviparity (egg-laying) requires profound morphological and physiological changes in both the mother and embryo, and these changes must be associated with significant changes in gene expression. The Australian skinks, *Lerista bougainvilli* and *Saiphos equalis* offer rare opportunities to uncover the genetic mechanisms of viviparity because live birth evolved recently, perhaps within the past 100,000 years. These species therefore have the potential to reveal the genetic changes associated with the evolutionary transition to viviparity "in action". I will present the results of a gene expression study analyzing almost all genes expressed in the uterus of both species during pregnancy and non-pregnancy. Furthermore, I compare expression both in the embryonic and maternal membranes of the placenta of *L. bougainvilli* throughout multiple stages of pregnancy. In particular, I will focus on suites of genes associated with the major physiological changes required to maintain pregnancy, including genes associated with nutrient provisioning, placental morphology, and the maternal immune system. Finally, I will compare these genetic changes to another viviparous lizard as a starting point to assessing the convergence of gene expression mechanisms across viviparous reptiles.

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Persistent use of sperm stored over winter in multiply-mated female garter snakes

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In some species, sperm is stored within the female reproductive tract for months to years, and yet remains viable to fertilize eggs and produce offspring. Female red-sided garter snakes store sperm for over seven months of winter dormancy. In previous work we demonstrated that these stored sperm account for an average of 25% paternity in a litter when the female mates with a male at spring emergence. Here we tested whether last-male sperm precedence was prevalent when a female mates with two males during the spring. On average, paternity was shared equally among the first (P1 proportion of paternity of the first male to mate) and second male (P2) to mate, and stored sperm (Pss), but the variance in paternity was high. Thus, last male sperm precedence may diminish when a female has more than two mates. Male size did not affect paternity, but as the interval between matings increased, P1 increased at the expense of Pss. Interestingly, as the second male's copulation duration increased, P1 also increased at the expense of P2. This result suggests that female-influence over sperm and/or copulatory plug transfer during matings may also affect which male fathers her offspring. Finally, all females were spring "virgins", consequently sperm stored from autumn matings remain competitive even when faced with two rivals in sperm competition and is likely the driver of the evolution of sperm longevity.

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Angiogenic genes in the skink uterus and the evolution of live birth

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The potent angiogenic factor VEGF111 is a rare splice variant of vascular endothelial growth factor (VEGF) found previously only in DNA-damaged human cells. VEGF111 was recently discovered in the pregnant uterus of viviparous (live-bearing) members of the bimodally reproductive three-toed skinks (*Saiphos equalis*), providing a possible link between the evolution of live birth and cancer susceptibility. In tandem with experiments in laboratory mice and cultured cells, we are now exploring the expression of angiogenic factors including VEGF in Australian skinks. Unexpectedly, we have found VEGF111 expressed in oviparous sister taxa to *S. equalis*, suggesting that the role of VEGF111 in viviparity is more complicated than previously thought. We are now localising the expression of this gene within the pregnant uterus of *S. equalis* to further elucidate its role in uterine angiogenesis and the evolution of live birth.

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The evolution and conservation of the Major Histocompatibility Complex Class III region in *Pogona vitticeps*.

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The Major Histocompatibility Complex (MHC) plays an essential role in the immune response of vertebrates. Genes within the MHC region are traditionally classified into three classes (Class I, II and III) based on the organisation of the human MHC, where the gene dense Class III region separates the Class I and II regions encoding for genes involved in antigen presentation. The Class III regions of mammals, aves, amphibia and teleost fishes have been previously investigated, with major differences in Class III gene content noted between aves and mammals. However, to date, there is no published data investigating this region in reptiles, which hold a key phylogenetic position in the genome evolution of vertebrates. This study aims to provide insight into the Class III region in a reptile, the Australian central bearded dragon, *Pogona vitticeps* (Agamidae) and to develop a comparative map in order to investigate the evolution and conservation of this region throughout vertebrates. Through analysis of the recently assembled *P. vitticeps* genome and physical mapping of Class III genes, we have revealed the Class III region of *P. vitticeps* contains at least 38 human Class III orthologues distributed over nine sequence scaffolds and resides on the long arm of chromosome 2. The *P. vitticeps* Class III region does share some conserved synteny with the human Class III region; with the presence of some genes (for example *SAPCD1*, *VWA7*,*LSM2* and *VARS*) clustered together in both species. However, some rearrangements have occurred. Class III-containing bacterial artificial chromosome (BAC) clones were end sequenced in an attempt to join the sequence scaffolds and gain a better assembly of the region. Analysis of BAC-end sequencing data has revealed the presence of non-Class III genes within the P. *vitticeps* Class III region, resulting in what appears to be a greatly extended Class III region within this agamid. This expanded Class III region, is in stark contrast to the severely restricted Class III region of aves (comprising of a single gene, *C4*) and the well conserved gene content of the Class III region observed between frog and mammals.

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Sex chromosome markers reveal a rapid transition from genotypic to temperature dependent sex determination in the bearded dragon

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Modes of sex determination in reptiles have undergone rapid turnover throughout their evolution. In some species, sex is determined genotypically by the presence of sex chromosomes (GSD). Alternatively the environment (usually temperature)

may be the primary driving force over male and female development (TSD). The recent discovery of transitional modes of reptile sex determination suggests that there is not a strict dichotomy between GSD and TSD. In the Central Bearded Dragon (*Pogona vitticeps*), exposure to high temperatures during embryonic development can override genotypic sex determination and cause male-to-female sex reversal despite the presence of well-characterised ZW sex chromosomes. Here we provide the first evidence that temperature sex-reversal occurs in the wild and that sex-reversed individuals can successfully reproduce. Through laboratory manipulations we show that sex-reversed ZZ females produce clutches of offspring with extreme male-bias (97.5% male) at low incubation temperatures; whereas high incubation temperatures produce female-biased clutches via sex reversal (82% female), thus achieving a classic TSD response curve. When the environmental conditions for sex-reversal occur and persist, the transition between modes could be quickly reinforced as the proportion of ZZ individuals increases in the population and drive the W chromosome towards its eventual loss.

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Diversifying selection at the Major Histocompatibility Complex class II beta in the New Zealand endemic Hochstetter's frog, *Leiopelma hochstetteri*

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The New Zealand native frogs, family Leiopelmatidae, are among the most archaic in the world and all four species are among the top 100 evolutionary distinct and globally endangered (EDGE) amphibians. *Leiopelma hochstetteri* (Hochstetter's frog) is a small, semi-aquatic frog with numerous, fragmented populations scattered across the northern North Island. We aimed to characterize diversity of the Major Histocompatibility Complex (MHC) class II beta in *L. hochstetteri* to gauge the immunogenetic health of five sampled populations, comparing neutral microsatellite (STR) markers. We characterized the MHC class II DAB gene from a spleen transcriptome and used cloning and sequencing to investigate diversity. Populations showed higher differentiation at the DAB locus than at microsatellite markers, suggesting the action of diversifying selection whereby selective forces have varied across *L. hochstetteri* populations. This has led to unique DAB variation in each population and enhancing total allelic diversity, with 74 unique alleles observed in 121 animals. We also observed very low DAB diversity in the Otawa population. This population may be of greater extinction risk from future disease challenges given its limited range of MHC alleles.

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Genetic controls over neuron dendrite arbor shape: convergence on microtubules

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Neuron dendrite branching is carefully controlled to build a complex nervous system. Using *Drosophila* sensory neurons as a model, we previously described a network of class-specific and regional transcription factors that define dendrite arbor shape. We have now investigated the genetic pathways controlled by these factors. Our analysis reveals a transcriptional-effector system for dendrite complexity based on the selection and orientation of microtubule nucleation sites. This mechanism has surprising parallels to the nucleation of the mitotic spindle in dividing cells. We also have developed time-lapse microscopy of branching dendrites *in vivo* followed by automated computer vision analysis. With this we carried out a live-imaging based genetic screen. Isolated from this screen, we find that the atypical myosin (MyosinVI) creates actin tracks within the dendritic growth cone that guide microtubule growth into nascent branches. These two new findings converge to emphasize how the nucleation and orientation of dendrite microtubule growth regulates branching.

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Genetic Control of Cellular Zinc Distribution

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Zinc is an abundant and essential dietary nutrient required as an enzymatic or structural cofactor for many proteins. As such, the cellular distribution of zinc ions must be tightly regulated to ensure adequate supply to each cellular organelle while avoiding potential toxicity due to competition with other essential metal ions Transport of zinc ions between and within cells is mediated by members of the Zip (SLC39) and ZnT (SLC30) families of transmembrane domain proteins. The genome of the vinegar fly *Drosophila melanogaster* encodes ten Zip proteins and seven ZnT families, with clear homologues of each of the twenty-four mammalian Zip and ZnT proteins. The tools available for functional genetic analysis in *Drosophila* make it an excellent system in which to investigate the in vivo roles of each of these zinc transporters and how they interact at the cellular and systemic levels.

Previously, we have carried out a systematic analysis of *Zip* and *ZnT* gene function in the fly[1] and identified a cellular zinc toxicity phenotype which we then used to detect more subtle zinc transport activities in the developing fly eye[2]. Here, we extend this work, presenting additional zinc toxicity and zinc deficiency phenotypes which represent mislocalization of zinc in specific cellular organelles. This work will be supported by a detailed investigation of the in vivo subcellular localization of each zinc transporter, quantification of cellular zinc distribution using zinc fluorophores and an examination of how each of the

transporters impacts on the zinc dyshomeostasis phenotypes. Preliminary findings from a pilot genetic modifier screen aimed at identifying additional zinc homeostasis genes will also be presented.

1. Lye JC, Richards CD, Dechen K, Paterson D, de Jonge MD, Howard DL, Warr CG, Burke R. J Exp Biol. 2012 Sep 15;215(Pt 18):3254-65.
2. Lye JC, Richards CD, Dechen K, Warr CG, Burke R. J Biol Inorg Chem. 2013 Mar;18(3):323-32.

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Gene co-citation networks associated with worker sterility in honey bees

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The evolution of reproductive self-sacrifice is well understood from kin theory, yet our understanding of how actual genes influence the expression of reproductive altruism is only beginning to take shape. In this study we use microarray meta-data and a co-citation analysis to describe what gene interactions might regulate a worker's response to ovary suppressing queen pheromone. We reconstruct a total of nine gene networks that vary in size and gene composition, but that are enriched for genes of reproductive function. The networks identify, for the first time, which candidate microarray genes are of functional importance, as evidenced by their degree of connectivity to other genes within each of the inferred networks. Our study identifies single genes of interest related to oogenesis, including eggless, and further implicates multi-gene pathways related to insulin, ecdysteroid, and dopamine signaling as potentially important to reproductive decision making in honey bees. The networks generated here are provisional but do offer a new multi-gene framework for understanding how honey bees regulate personal reproduction within their highly social breeding system.

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Protein with tau-like repeats regulates neuronal aging and lifespan in *C. elegans*

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Protein with tau-like repeats (PTL-1) is the sole MAP2/MAP4/tau homolog in *Caenorhabditis elegans* (*C. elegans*). Dysregulation of tau is a pathological hallmark of neurodegenerative diseases such as Alzheimer's disease. Therefore, reducing tau levels has been suggested as a therapeutic strategy. We used PTL-1 in *C. elegans* to model the biological functions of a tau-like protein without the complication of functional redundancy. We have shown that PTL-1 maintains the age-related structural integrity of neurons, suggesting that excessive reduction in the levels of a tau-like protein is detrimental. Our data also demonstrates that the regulation of neuronal ageing by PTL-1 occurs via a cell-autonomous mechanism. We transgenically re-expressed PTL-1 in a null mutant background using a pan-neuronal promoter to show that PTL-1 functions in neurons to maintain structural integrity. We next expressed PTL-1 in touch neurons and showed rescue of the neuronal ageing phenotype of *ptl-1* mutant animals in these neurons but not in another neuronal subset, the ventral nerve cord GABAergic neurons. Knockdown of PTL-1 specifically in touch neurons also resulted in premature neuronal ageing in these neurons but not in GABAergic neurons, further supporting the conclusion that PTL-1 functions in a cell-autonomous manner. Intriguingly, our data also demonstrates that *ptl-1* mutants are short-lived. Expression of PTL-1 in touch neurons alone was unable to rescue the shortened lifespan observed in null mutants, but pan-neuronal re-expression of PTL-1 restored wild-type longevity, indicating that premature neuronal aging and organismal ageing can be decoupled. Overall, our findings suggest that some of the effects of tau pathology may result from the loss of physiological tau function, and not solely from a toxic gain-of-function due to accumulation of tau.

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Functional Characterisation of Voltage Gated Chloride Channel Proteins in Drosophila

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The membrane-bound proteins of the voltage gated chloride channel (CLC) family perform crucial roles in stabilising membrane potentials, transepithelial transport, cell volume regulation and acidification of intracellular organelles. Mammals have nine CLCs clustering into three subgroups and disruption of some members result in four notable human diseases; Dent's disease, Bartter's syndrome, osteopetrosis and congenital muscle myotonia. The *Drosophila* genome encodes three CLCs, corresponding to each of the mammalian CLC subgroups.

This study focuses on the functional characterization of the fly CLC-b (hCLC-6-7) and CLC-c (hCLC-3-5). Null mutations have been generated in both these genes. *CLC-b*$^{-}$ homozygotes are adult viable but have impaired locomotory activity, a median longevity less than half that of heterozygotes and a pronounced sensitivity to elevated dietary zinc levels. Histological analysis of the *CLC-b*$^{-}$ mutant central nervous system will be presented, investigating whether *Drosophila* lacking CLC-b display signs of Lysosomal Storage Disorders. In contrast, *CLC-c*$^{-}$ homozygotes cease to develop beyond 2nd instar larvae. Mosaic analysis indicated that cells homozygous for the *CLC-c* mutation have an increased LysoTracker signal prior to being replaced by surrounding heterozygous cells 3-5 days after clone generation. This implies an increase in lysosome production, typically seen

in the initiation of autophagy, in the absence of CLC-c. A detailed analysis of the cellular defects caused by loss of CLC-c prior to cell death will be presented.

This research will use the power and flexibility of *Drosophila* genetics to elucidate the *in vivo* function of this critical class of Chloride Channel genes and will in future allow us to model the effect of human pathogenic mutations in the fly.

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Dihydrolipoamide dehydrogenase (DLD) as a potential therapeutic target for Alzheimer's disease

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Altered energy metabolism is associated with Alzheimer's disease (AD) and is proposed to influence disease progression. Amyloid beta (Aβ) plaque formation, a major contributor to AD pathology as well as a marker of disease progression is frequently concurrent with distorted brain metabolism. Unfortunately in AD pathogenesis, it is still difficult to distinguish cause from consequence. For example, the decrease in energy metabolism associated with AD may be interpreted either as a consequence of, or response to, factors associated with the disease such as elevated levels of oxidative stress. Dihydrolipoamide dehydrogenase (DLD) is a core metabolic enzyme associated with four important mitochondrial enzyme complexes. Interestingly, *dld* gene variants are genetically linked to late-onset Alzheimer's disease (AD); and reduced activity of DLD-containing enzyme complexes has been observed in AD patients. To understand how energy metabolism influences AD progression, we suppressed the *dld-1* genein worms expressing the human Aβ peptide and also decreased the activity of the DLD enzyme by exposure to the chemical inhibitor, 2-methoxyindole-5-carboxylic acid (MICA). As previously reported, we see that expression of human Aβ in the worm model of AD is associated with decreased lifespan, enhanced paralysis, reduced acetylcholine neurotransmission, hypersensitivity to serotonin, perturbation of chemotaxis and increased Aβ oligomerization. Suppression of either the *dld-1* gene or the activity of its encoded enzyme not only increased lifespan but also alleviated the symptoms associated with expression of human Aβ. Suppression of the *dld-1* gene also results in a decrease in the abundance of toxic Aβ oligomers. These protective effects of *dld-1* suppression seem to be associated with calcium homeostasis as they could be reversed by exposure to a calcium ionophore.

1. Sun et al. Down-regulation of energy metabolism in Alzheimer's disease is a protective response of neurons to the microenvironment.J Alz Dis, 2012, 28: 389-402
2. Brown AM et al. Association of the dihydrolipoamide dehydrogenase gene with Alzheimer's disease in an Ashkenazi Jewish population. Am J Med Gen, 2004, 131B: 60-66
3. Stacpoole PW. The puruvate dehydrogenase complex as a therapeutic target for age-related disease. Aging Cell, 2012, 11: 371-377
4. Vlassenko AG et al. spatial correlation betw een brain aerobic glycolysis and amyloid beta (Aβ)deposition. PNAS, 2010, 107: 17763-17767

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Examining the genetic and environmental influences on nicotinic acetylcholine receptor trafficking.

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Nicotinic Acetylcholine Receptors (nAChRs) are pentameric ionotropic channels that mediate fast synaptic transmission and are evolutionarily conserved across taxonomies, ranging from *C. elegans* to humans. The Dα6 nAChR subunit is known to be the target of the insecticide spinosad in *D. melanogaster*. Loss of Dα6 function confers high level resistance to this insecticide. We have tagged the *Drosophila* Dα6nAChR subunit with fluorescent markers to study nAChR trafficking and localization *in vivo* in *Drosophila*. The tagged receptor is functional *in vivo* as tagged Dα6 subunits are able to complement a *Dα6 null* mutation by restoring susceptibility to spinosad. Creating this system where nAChRs can be tracked allows us to examine how nAChR trafficking is affected by genetic and environmental changes. We exposed *Drosophila* larvae expressing the fluorescent Dα6 nAChR subunit to a sub lethal concentration of spinosad to determine its impact on trafficking. Larvae raised on food spiked with spinosad showed decreased levels of *Dα6* expression in the central nervous system compared to larvae raised on control food. These data suggest that trafficking may be induced to remove nAChRs from the membrane, allowing the fly to adapt to exposure to toxins.

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LandGenReport - a landscape genetic tool to analyse the effect of landscape features on population structure using genetic data

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The ever fasting development of genetic techniques such as second generation sequencing offers unique opportunities for ecological researchers to study the spatial structure of populations and how they are influenced by landscape features. The creation of such kind of data namely samples of genotyped individuals with known spatial locations, is not paralleled by the application of landscape genetic methods to analyse them. Though a standard approach to study the effect of landscape features using resistance matrices combined with a least-cost approach and compare these with genetic distances is developed, we see a lack of publications applying this approach. Our explanation for such a lack of publications is partly due to the required expertise which encompasses the integration and data exchange between three specialised type of software packages (population genetic, geographic information systems and statistical) to analyse the data. We present a newly developed R package that incorporates all necessary steps into a single framework and discuss the performance of the approach under different landscape scenarios. Next to sample size and number of used markers it turns out that the geometry of the landscape and sample sites is important for the performance of the approach. Finally we present how a simulation approach can be used to test the performance in a given landscape scenario before the actual study is undertaken.

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Genetic management of harvest and restocking in Australian eastern king prawns

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The eastern king prawn, Penaeus (Melicertus) plebejus, is widely distributed along the east coast of Australia. It exhibits great mobility and the species distribution transcends management jurisdictions of the three eastern states. The species is targeted for stock enhancement, which requires managers to avoid genetic loss in aquaculture, or using inappropriate stock in restocking. Highly variable mitochondrial control region (mtCR) was used to examine the reproductive performance of wild-caught female broodstock in the production of hatchery-bred cohorts for restocking. Our data showed that mtCR can be a useful tool for tracking lineages and provided clear genetic evidence that unequal contribution and under-producing females can be common even in wild-caught broodstock, therefore highlighting the importance of monitoring the genetic composition of hatchery cohorts prior to release for stock enhancement. Also, using kinship verified by hatchery observations and mtCR haplotypes, we showed that microsatellite null alleles are very common. We compared several methods of microsatellite allele estimation; pedigree-free and pedigree-based methods provided comparable results.

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Understanding genetic variation across a heterogeneous landscape in an invasive species

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Perhaps no other continent has a darker history in terms of the introduction of foreign species than Australia, with the classics – rabbits, pigs, goats, cats, and cane toads – having devastating ecological and economic impacts. However, invasive species also provide an evolutionary experiment that potentially allows us to understand range expansion dynamics, rapid evolutionary change and adaptation to novel environments. The European common starling, *Sturnus vulgaris*, is a successful invasive bird that has been established in Australia for 40 generations. It is primarily found across the eastern and southern areas of Australia but found within a wide range of environmental conditions. Using Genotyping By Sequencing (GBS), we determined the SNP genotype of 540 common starlings from 24 locations across the starlings' environmental range in Australia. This allowed the identification of regional patterns of genetic structure and connectivity within a heterogeneous landscape and allowed us to relate environmental and landscape features to genetic patterns. By understanding the response of invasive species to their non-native range we will gain greater insight into the interaction of species and their environment and better understand how invasions may proceed. Such knowledge could help guide the allocation of sparse resources available for managing invasive species.

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Genetically Engineering Underdominace for Species Conservation Applications in Hawai'i

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In theory, genetic modifications that are linked to underdominance (heterozygotes are less fit than either homozygote) can be introduced and maintained by natural selection in a wild population, even if they come at some fitness cost. This is stable in the sense that the effector gene is maintained indefinitely without any further intervention; however, in a different sense the system is completely reversible back to a wild-type state if desired (underdominance results in an evolutionary bi-stable switch). There is also the added beneficial property of geographic stability—the genetic modifications "stick where you put them." Early attempts at engineering underdominant systems with translocations (in the 1970s and '80s) were not successful. We have

revisited the problem and engineered single-locus underdominance, with gene expression knock-down/rescue of haploinsufficient ribosomal proteins, in the highly tractable model organism *Drosophila melanogaster* as proof of principle. This system has a very robust fitness configuration and is designed to be portable to a wide range of species. Currently we are focused on a species conservation application with non-native *Culex quinquefasciatus* mosquitoes that vector avian malaria (*Plasmodium relictum*), which is expanding its range in Hawai'i and is a major threat to the survival of several endemic Hawaiian honeycreeper bird species.

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Species from faeces: predator scat metabarcoding in Tasmania

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Predator-prey dynamics have altered dramatically in Tasmania over the last two decades. The Tasmanian devil (*Sarcophilus harrisii*) has suffered a devastating disease, while there is evidence that eastern quoll (*Dasyurus viverrinus*) numbers have decreased and feral cat (*Felis catus*) numbers have increased as devils have declined. The red fox (*Vulpes vulpes*), which is a significant predator of native wildlife, has also been reported since the late 1990s.

DNA from predator scats constitutes a unique ecological resource and could provide critical information about predation by native versus introduced predators in Tasmania. In a metabarcoding approach, we have used Illumina paired-end sequencing to amplify 130-180 bp fragments of four mitochondrial genes (12S, 16S, COI and ND2) from 35 Tasmanian predator scats. In addition, four genomic DNA mixtures and 12 scats of known origin from captive animals (in some cases with known diet) were used to validate the approach. Primers were selected to primarily target vertebrate DNA, with a focus on native mammals, reptiles and birds. Over 15 million sequence reads were obtained: these were assigned to taxonomic groups with reference to public resources and a custom DNA sequence database of Tasmanian terrestrial vertebrates. We demonstrate that we can successfully detect predator and prey DNA from scats of unknown age collected in field conditions and stored for up to 5 years. An improved understanding of the impact of introduced predators on native species will contribute to wildlife management plans in Tasmania and elsewhere.

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The South American seagrass *Zostera chiliensis*, endangered or invasive?

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6. *Facultad Ciencias del Mar, Universidad Católica del Norte, Coquimbo, Chile*

The seagrass *Zostera chilensis* is classified as an endangered species with only three known populations occurring along the coast of central Chile. Recent molecular work has revealed no distinction between *Z. chiliensis* and the Australian seagrass *Z. nigricaulis*, suggesting a very recent introduction to Chile. Successful amplification of seagrass samples from Chile using species-specific microsatellite markers developed for *Z. nigricaulis* support the idea that Chilean populations are derived from Australia. Using a combination of molecular data, historical shipping records, fragment viability data and oceanic modeling, we explore the possibility of a trans-oceanic dispersal event spanning several thousands of kilometers, versus a human mediated introduction event via shipping. Levels of genotypic diversity and patterns of connectivity among the three isolated populations along the Chilean coast are also explored. The results from this study have important implications for understand how potentially rare (but important) trans-oceanic dispersal events can lead to establishment of new populations.

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Blending molecular genetics with pedigree data to breed endangered species

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4. *Australian Centre for Wildlife Genomics, Australian Museum, Sydney, NSW, Australia*

Applying traditional species management methods to zoo populations with unknown pedigrees, or to species housed in group management scenarios has always been problematic. With the development of the new species management software, PMx, a number of the more challenging areas have been resolved. However for those species with a high number of unknown pedigrees long-term management is still difficult. Molecular genetics is evolving rapidly with vastly increased volumes of data becoming increasingly inexpensive to obtain for many non-model species. The application of this data means that we are now in a position to generate and apply data as one of the newer tools in the management of small populations. A successful partnership has been developed between the Zoo and Aquarium Association, San Diego Zoo Global and the Royal Zoological Society of Scotland, who are strong advocates of the use of genetic data to inform species management, and the Australian Centre for Wildlife Genomics at the Australian Museum, who have expertise in applying genetic techniques for real world applications for non-model animal species.

This talk will outline some of the more successful applications of microsatellite data with pedigree data to improve the breeding of critically endangered species, in particular the orange-bellied parrot, honeyeaters and scimitar-horned Oryx. Issues surrounding the use of relatedness estimators for small, closed populations will be discussed, and how we can better manage both zoo-based populations and small isolated wild populations long-term.

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Eye gene transcriptomes of diving water beetles: a contrast of surface and subterranean photic niches

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The entry of animals into aquifers constitutes a form of niche creation, and two tribes of closely related Australian diving beetles (Dytiscidae) have independently invaded such closed groundwater systems from surface waters on multiple occasions. The subterranean lineages display convergent phenotypes common to aphotic cave habitats (eye loss and pale integumentation), and this group provides ideal comparative opportunities to explore the 'regressive evolution' of vision from a molecular perspective. Here we present data from the sequenced transcriptomes of two surface and three subterranean species, and focus on 45-candidate protein coding genes related to phototransduction and eye pigmentation. Forty-three candidate genes were found to be transcribed in surface and/or subterranean species. Four of the candidate genes were expressed in all surface species, but were absent from all subterranean transcriptomes, namely UV opsin, a non-visual ciliary opsin, a visual arrestin *Arr1* and the myosin III gene *ninaC*. These gene products play interrelated roles in phototransduction cascades. One subterranean species transcribed the visual long wavelength opsin and the major visual arrestin *Arr2*, albeit in an aphotic environment. Both distance and likelihood tests of selection on these transcripts show no significant differences compared to surface relatives and evidence of purifying selection acting on these two genes. Overall, the results provide evidence for parallel loss of eye gene function in subterranean species, but also show that the vast majority of 'eye genes' retain their expression in aphotic environments. We discuss the possibility of pleiotropic roles, as opposed to incipient stages of pseudogene development, relative to the natural and evolutionary history of these beetles.

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A role for apoptosis in worker sterility: gene expression in the plastic ovaries of the honey bee

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Worker sterility is a defining characteristic of social insects. Studies into a mutant 'anarchistic' strain of honey bee (in which the normally sterile workers activate their ovaries and lay eggs) identified a short list of candidate genes for worker sterility. Interestingly, these genes are implicated in the mTOR signalling pathway which controls cell growth and proliferation. The mTOR pathway is both pro-apoptotic and anti-apoptotic, prompting us to investigate the role of apoptosis in worker ovary plasticity. By quantifying the expression of the genes of interest in the ovaries of workers with non-activated and activated ovaries we found *Anarchy* (GB13621), a peroxisomal membrane protein, predicted the ovary state of workers with close to 90% accuracy. *Anarchy* suppresses the activation of the worker's ovary by responding directly to the presence of the queen and is specific to the regulation of reproductive state in the worker caste as we found no differential expression in queens. Using RNA interference, we knocked down the expression of *Anarchy* in wildtype workers and found evidence that it interacts with a key gene in the apoptosis regulatory pathway.

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Expressing the diamondback moth ABC transporter C2 in transgenic *Drosophila* causes susceptibility to Bt insecticidal toxin

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The diamondback moth, *Plutella xylostella*, is a serious agricultural pest of *Brassica* crops worldwide and can be extremely difficult to control. Populations commonly evolve resistance to insecticides used against them, including biological based Bt toxins produced by the bacteria *Bacillus thuringiensis*. Hundreds of different Bt toxins have been described, and many are highly specific to targeted pests yet benign to non-target organisms[1]. For example, Cry1Ac kills lepidopteran moth pests, but not flies (e.g. Drosophila), or beneficial insects such as parasitoids.

The precise mode of action of Bt toxins remains controversial[2]. Two well supported models, the 'Classic Model' and 'Sequential Binding Model', both result in midgut pore formation yet differ in the types of toxin receptors required. Although many Bt midgut receptors have been proposed in the literature (alkaline phosphatase[3], aminopeptidases[4], cadherin[5]), mutations in the membrane bound ABC transporter C2, have recently been associated with resistance to Bt Cry1A toxins in multiple Lepidoptera[678].

As *Drosophila* is not susceptible to Cry1Ac, we used this model system to investigate whether the diamondback moth ABCC2 protein acts as a functional toxin receptor. The moth ABCC2 gene was fused with a GFP reporter gene, cloned into the pUAST vector, and transgenic Drosophila lines then generated. Using the GAL4/UAS system, we observed successful expression of the ABCC2-GFP construct, through GFP localization to cell membranes. When fed with artificial diet containing Cry1Ac toxin, larvae expressing ABCC2 in the midgut showed 100% mortality, while expression in salivary glands had no affect on survival. We aim to further use this system to help understand Bt toxin mode of action, through adding support to either the Classic Model or Sequential Binding Model.

1. Crickmore, N., Zeigler, D. R., Feitelson, J., Schnepf, E., Van Rie, J., Lereclus, D., Baum, J., and Dean, D. H. (1998) Revision of the nomenclature for the Bacillus thuringiensis pesticidal crystal proteins, Microbiology and Molecular Biology Reviews 62, 807-+.
2. Vachon, V., Laprade, R., and Schwartz, J. L. (2012) Current models of the mode of action of Bacillus thuringiensis insecticidal crystal proteins: A critical review, Journal of Invertebrate Pathology 111, 1-12.
3. Jurat-Fuentes, J. L., and Adang, M. J. (2004) Characterization of a Cry1Ac-receptor alkaline phosphatase in susceptible and resistant Heliothis virescens larvae, European Journal of Biochemistry 271, 3127-3135.
4. Knight, P. J., Knowles, B. H., and Ellar, D. J. (1995) Molecular cloning of an insect aminopeptidase N that serves as a receptor for Bacillus thuringiensisI Cry1Ac toxin., The Journal of Biological Chemistry 270, 17765-17770.
5. Nagamatsu, Y., Toda, S., Koike, T., Miyoshi, Y., Shigematsu, S., and Kogure, M. (1998) Cloning, sequencing, and expression of the Bombyx mori receptor for Bacillus thuringiensis insecticidal CryIA(a) toxin, Bioscience Biotechnology and Biochemistry 62, 727-734.
6. Atsumi, S., Miyamoto, K., Yamamoto, K., Narukawa, J., Kawai, S., Sezutsu, H., Kobayashi, I., Uchino, K., Tamura, T., Mita, K., Kadono-Okuda, K., Wada, S., Kanda, K., Goldsmith, M. R., and Noda, H. (2012) Single amino acid mutation in an ATP-binding cassette transporter gene causes resistance to Bt toxin Cry1Ab in the silkworm, Bombyx mori, Proceedings of the National Academy of Sciences of the United States of America 109, E1591-E1598.
7. Baxter, S. W., Badenes-Perez, F. R., Morrison, A., Vogel, H., Crickmore, N., Kain, W., Wang, P., Heckel, D. G., and Jiggins, C. D. (2011) Parallel Evolution of Bacillus thuringiensis Toxin Resistance in Lepidoptera, Genetics 189, 675-U814.
8. Gahan, L. J., Pauchet, Y., Vogel, H., and Heckel, D. G. (2010) An ABC Transporter Mutation Is Correlated with Insect Resistance to Bacillus thuringiensis Cry1Ac Toxin, Plos Genetics 6.

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Characterizing resistance and the potential for adaptation to a new diamide insecticide in D. *melanogaster*

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Insecticide exposure can act as a powerful selective pressure, resulting in a strong adaption response. D. *melanogaster*, while not directly targeted by insecticides, is frequently exposed due to its nature as a human commensal. There is evidence for recent, rapid adaptation at loci associated with insecticide resistance in D. *melanogaster* - especially to older compounds with widespread use [1].

The anthrallic diamides belong to a class of recently released insecticides with a unique mode of action. Here we have used the Drosophila Genomic Reference Panel (DGRP) to carry out Genome Wide Association (GWA) mapping of the genetic basis of resistance to the anthrallic diamide, Chlorantraliliprole. Not only does the GWA analysis allow us to study the genetic architecture of resistance, but as the DGRP are naïve to Chlorantraniliprole, it allows us to predict variants that may be subject to selection upon further exposure. These predictions could potentially be tested by using natural populations selected for resistance.

1. [1] Garud, N. R., Messer, P. W., Buzbas, E. O. & Petrov, D. A. Soft selective sweeps are the primary mode of recent adaptation in Drosophila melanogaster. arXiv preprint arXiv:1303.0906 (2013).

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Comparative, quantitative transcriptomes in sympatric fruit fly species reproductively isolated by time of mating

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The sibling tephritid fruit flies *B. tryoni* and *B. neohumeralis* are sympatric species that provide a unique Australian model for the genetics of speciation. The species are genetically extremely similar but are reproductively isolated by time of mating, a trait which is genetically controlled and sensitive to light intensity. A previous, candidate gene approach revealed that *cryptochrome*, which encodes a light sensitive protein with input into the circadian clock, is differentially expressed in brain and antennae (An et al., 2004). We have now prepared replicated quantitative transcriptomes of brain and antennae, sampled at morning and night, from the sibling speicies. The raw reads were assembled *de novo* using Trinity, with a separate assembly for each tissue, but combining the two species due to the extreme similarity of the genomes, thus enabling a direct comparison of read counts for each transcript. ANOVA was used to test the difference in gene expression between (i) time of day, (ii) the two species and (iii) the interaction ie where the time-of-day pattern of expression is different between *B. tryoni* and *B. neohumeralis*. Data will be presented from the brain analysis revealing candidate genes that are differentially expressed between the species. However, due to the large number of statistical analyses, very few time-of-day differences and no interaction differences reach significance.

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Expression patterns and transcriptome analysis of sex determination genes in *Bactrocera* fruit flies

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Bactrocera tryoni (Froggatt) (Diptera: Tephritidae) is Australia's most destructive horticultural pest. Suppression methods, including the release of irradiated, sterile individuals (Sterile Insect Technique, SIT) or individuals infected by a common insect symbiont, *Wolbachia* (α-Proteobacteria) (Incompatible Insect Technique, IIT), are pest management strategies that will become increasingly useful with the introduction of a male-only strain.

To develop a male-only strain of *B. tryoni*, an understanding of the genes involved in the sex determination pathway, and when they are activated, is necessary. The pathway has been well studied in *Drosophila melanogaster* (Meigen) (Diptera: Drosophilidae), and this has provided a starting point for characterising genes involved in sex determination in other Diptera. However, the initial signal in male *Bactrocera* is communicated by the Y-located dominant male determiner, *M*, which is absent in *Drosophila* species. The mode of action of *M*, and its direct targets have yet to be characterised in fruit flies or in any other Y-determined insect.

We utilised a Y-located molecular marker in a related species, *Bactrocera jarvisi*, to separate male and female embryos at early stages of development. Quantitative PCR pinpointed the timing of important changes in transcript abundance of some sex determination genes, including *transformer*,*transformer-2* and *doublesex*. Sex-specific poly(A)$^+$ transcriptome sequencing, targeting two stages of development, was undertaken and the transcriptomes assembled *de novo*. Fifteen sex-determination gene homologues and two cellularisation gene homologues of *Drosophila melanogaster* (Diptera: Drosophilidae) were newly identified in *B. jarvisi*: *extra-macrochaetae* (*emc*) displayed a zygotic transcription profile contrary to the maternal expression in *D. melanogaster*; *sisterless A* (*sisA*) expression occurred very early; *slow as molasses* (*slam*) and *null*otranscripts increased 80- and 17-fold respectively over time. These data contribute fundamental information to sex-determination research, and provide candidates for the sourcing of gene promoters for transgenic pest-management strategies.

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The *Drosophila melanogaster* phospholipid flippase dATP8B is required for odorant receptor function

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Insects rely on olfaction for virtually all aspects of their existence, thus this chemosensory system has evolved into an exceptionally discriminative network capable of perceiving thousands of odours. In the model organism *Drosophila melanogaster*, detection of volatile cues is achieved by three families of receptor proteins, the odorant receptor (OR), gustatory receptor (GR), and ionotropic-like receptor (IRs) families, which are expressed in specific functional classes of olfactory receptor neurons (ORNs). Odorant ligands binding to these proteins initiate signal transduction, however, the underlying mechanisms of olfactory signalling are not well defined. In a search for new olfactory genes, we undertook a large scale EMS-induced mutant screen using electrophysiology to test for olfactory defects. We found a mutant with a pronounced reduction in the responses of ORNs expressing ORs, whilst GR- and IR- expressing ORNs were unaffected. Deficiency mapping and whole

genome re-sequencing identified the causative gene, *dATP8B*. RNAi analyses showed that OR-expressing neurons require dATP8B for olfactory function. Immunohistochemistry analyses indicate that dATP8B localises to the site of signal transduction in the dendritic membrane of OR-expressing ORNs. OR localisation and dendritic morphology appear normal in the *dATP8B* mutant. As *dATP8B* encodes a P4-type ATPase, a family of proteins thought to be essential for maintaining the natural asymmetry of phospholipids in bilayered membranes, our findings suggest a requirement of phospholipid asymmetry for OR signalling.

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Expression patterns of selected digestive enzyme genes in the hepatopancreas of redclaw (*Cherax quadricarinatus*) fed two different carbohydrate sources

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Despite the rich diversity of freshwater crayfish species, current world aquaculture production is largely based on only a small number of species. Redclaw, is a relatively recent entrant into this industry, but has been recognised as a candidate species with significant potential because of some key physiological traits attractive for culture. To date however, the industry has yet to develop specific commercial formulated diets for this species. Traditional standard dose-response trials have evaluated effects of different feed ingredients and their combinations but there is still only limited information on this animal's genetic make-up in relation to digestive metabolism. Here, we examined expression profiles of expressed sequenced tags (ESTs) from hepatopancreatic transcriptomes in redclaw individuals fed either starch or soluble cellulose in their diets at the 20 per cent inclusion level. Individuals were fed with either a starch (RD, reference diet) or soluble cellulose (TD, test diet) diet for one week and then RNAseq libraries were constructed from the hepatopancreas and sequenced using the Ion Proton sequencing platform with data annotated using the NCBI database. Analysis of ESTs from both test groups revealed that most digestive enzyme genes expressed in redclaw were involved in carbohydrate metabolism and the diet with soluble cellulose (TD) showed higher expression levels of carbohydrate metabolism genes. In particular, genes that encoded enzymes involved in hydrolysis of lignocellulosic material showed the highest expression levels. In contrast, TD resulted in lower expression of alpha amylase compared with the RD in redclaw. Identification of a vast array of redclaw digestive enzyme genes and presence of various isoforms suggest that redclaw have an innate genetic capacity to utilise a wide range of carbohydrate substrates of different structural complexity. Thus, there is a potential to incorporate complex plant polysaccharides into their formulated diets in order to reduce total feed costs.

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Epigenetic inheritance and the legacy of parental obesity

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Intrauterine nutrition can program the metabolism of offspring, creating stable changes in physiology that may have significant health consequences later in life. We have previously found, using a mouse model of natural-onset obesity and type 2 diabetes, that offspring exposed to maternal obesity *in utero* exhibit a latent predisposition for metabolic disease in adulthood that is associated with widespread epigenetic changes. Our most recent work demonstrates that a latent metabolic phenotype is also conferred by being born of an obese *father*. While offspring can avoid overt disease by consuming a healthy diet, most worrying is the finding that the latent phenotype can persist through several generations. These findings and their implications will be discussed.

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The (genetic) book of dead; why deep-sequencing trace, degraded & ancient DNA is both interesting and useful.

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The advent of the 'next' generation of DNA sequencing platforms has had a profound influence on our ability to sequence DNA. Arguably, the field that has gained most from these advances is ancient DNA (aDNA); whereas once scraping out a few hundred bases of mtDNA was an achievement, now whole genomes are sequenced off aDNA templates. This presentation will, using AustralAsia examples, showcase how NGS technologies have advanced our understanding of archaeology and paleontology, and discuss how lessons learnt from working with degraded DNA is now contributing more widely into the fields of forensics, conservation biology and medical research.

The Trace and Environmental DNA (TrEnD) laboratory has, in recent years, used NGS metabarcoding (amplicon sequencing) to investigate the applicability of the approach to characterise a variety of complex (multi-species) substrates including herbal medicines, sediment, excavated bulk-bone and faecal/gut samples. While NGS technologies are a powerful tool, our data suggests that careful consideration of work practices including; template input, contamination control, library generation and data analysis, is necessary to obtain data sets free of artifacts.

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Improving phylogenetic analyses of large and small datasets

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Recent years have seen dramatic increases in the amount of data we use in phylogenetics, combined with huge improvements in the methods we use to analyse that data. But there is still a long way to go. More data brings more challenges, and even the best available methods can be improved. In this talk I will briefly review a handful of exciting advances in phylogenetics. These will include new ways to model molecular evolution on datasets large and small, as well as improvements in how we assess the adequacy of our phylogenetic analyses. At the end, I'll map out some of the biggest remaining challenges, and how we might be able to solve them.

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Comparing pacemaker models of genome evolution in mammals

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The molecular clock hypothesis posits that DNA substitutions accumulate at a constant rate through time, and that this evolutionary rate is homogeneous among lineages. However, this assumption is usually violated in practice, with lineages evolving at different rates. The causes of rate variation can be divided into lineage effects and residual effects. Lineage effects include the factors that cause a universal change across a genome, such as a change in underlying mutation rate or a change in generation time. In contrast, residual effects are heterogeneous across the genome and include factors such as selection.

The Universal Pacemaker (UPM) Model of genome evolution ascribes all important rate variation to lineage effects. According to this model, all genes share the same pattern of among-lineage rate variation. At the other end of the spectrum, the Degenerate Multiple Pacemaker (DPM) model proposes that each gene has a distinct pattern of among-lineage rate variation. Between these extremes is the Multiple Pacemaker (MPM) model, whereby genes are clustered into distinct pacemakers.

We developed a computational framework to test among these pacemaker models of genome evolution. We used it to analyse 426 genes from a broad taxonomic range of mammal species. Our results favour the MPM with 3 to 18 pacemakers, with poor support for the UPM and the DPM. We also investigated whether the pacemakers are associated with gene function and family. Although the exact number of pacemakers will vary among data sets, we suggest that genomic rates of evolution are governed by several discrete pacemakers, implying that the MPM should generally have higher support than the UPM and the DPM.

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Invasion history of Black rats (*Rattus rattus*) in Australia - insights from mitochondrial and nuclear phylogeography

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Black rats (Rattus rattus) are human kinds greatest pest, having invaded all inhabited continents and being responsible for massive good production loses, the transmission of human and animal diseases and many extinction events. Black rats are genetically and systemically diverse with numerous instances of independent human-mediated range expansions, including into Australia after European contact. We analysed the patterns of genetic diversity in black rats across their global range to establish the invasion history of black rats in Australia. Two major mitochondrial lineages are present in Australia with but with differing geographic distribution patterns. We estimated the minimum number of populations that contributed black rats from the mitochondrial data. Analysis of microsatellite allele frequencies within Australia suggests that populations are geographically structured but that mitochondrial ancestry does not predict population membership. Comparison with populations from Asia, the original range of the black rat group, strongly suggests mitochondrial lineage capture prior to global range expansion, a result that is confirmed with genomic scale data. We discuss the implications of lineage capture for the invasive properties of black rats.

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Testing methods for inferring population history from individual genome-scale sequences

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Knowledge of past population sizes provides important insights into the evolutionary history of a species. Several methods have been developed to infer the change in demographic history through time using genetic sequences, such as the Bayesian skyline and skyride methods. Most of these demographic reconstruction methods use sequence data from only a small number of loci. In 2011, Li and Durbin developed the pairwise sequential Markovian chain (PSMC) method, which uses genome-scale diploid sequences to infer the demographic history of an organism. Results from this method show that the European and Chinese populations experienced a severe bottleneck about 10 - 60 kyr ago, distinct from the milder bottleneck experienced by African populations. We tested the robustness and resolution of the PSMC method with regard to variable parameter values, such as mutation rate, demographic history, and sequence lengths.

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Estimating the evolutionary timescale of flowering plants using complete chloroplast genome sequences

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The origins and evolution of flowering plants (Angiospermae/Magnoliophyta) have been major topics of research interest in phylogenetics. In particular, the evolutionary timescale of angiosperms has proven to be a source of considerable attention, although most studies have been based only on a small number of loci and/or taxa. However, the development of next-generation sequencing techniques in recent years has produced substantial amounts of genetic data, with many chloroplast genome sequences now being available.

The objective of this study is to estimate the evolutionary timescale of angiosperms by analysing whole chloroplast genome sequences using a Bayesian phylogenetic relaxed-clock approach. Our data set comprises published sequences from GenBank as well as novel data produced by collaborators at the Royal Botanic Gardens, Sydney. Combined with a number of fossil calibrations, our analysis provides the most comprehensive and reliable estimate of the timescale of angiosperm evolution to date.

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Phylogenetic regionalization: a new framework to support conservation biogeography

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A key question that remains unexplored in evolutionary biogeography is how much of the phylogeny for multiple taxonomic groups is present in a region? Here, we propose a multi-level framework to enhance our ability to identify areas of lineages congruence across multiple groups. The aims were to create a tool that identify unique phylogenetic regions across multiple taxonomic groups and also that helps to improve conservation prioritization of lineages and not taxonomic species only. In order to test our framework, we used species occurrence and phylogenies corresponding to five test groups: fish, frogs, Acacias, eucalypts and plant genera. The total number of analysed occurrence records is over 70,000 with more than 500 species which comprises about 700 genera. We applied the method in the largest Australian river system, Murray-Darling Basin region, because it has a high relevance for human colonisation and therefore conservation. We argue that such approaches are highly useful to improve conservation prioritization at broad geographical level which is essential to enhance our understanding of diversity distribution under climate change. The initial results successfully identified areas of concurrent centres of endemism as well as phylogenetic diversity that were classified as unique phylogenetic regions. Such areas were not previously identified as important by traditional diversity assessment methods. Hence, that information enabled us to propose for the first time a genetic-based regionalization for the MDB and therefore concept prove our framework.

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Phylogenetics and evolution of Australian Nasutitermitinae

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In Australia, Termites are keystone species and ecosystem engineers in many environments, particularly in arid areas. Australia contains a total of 260 termite species, representing 5 of the 7 generally recognized families worldwide. One important group in Australia is Nasutitermitinae. There are 6 representative genera with over 44 representative species in Australia (of over 200 species worldwide), which are found all across the Australian continent in various ecoregions including temperate forests, semi-deserts, savannah woodland and others. Australian Nasutitermitinae are particularly known for their variability in nest architecture and construction. Despite the ecological and economic importance of the Australian termite fauna, their systematics and evolution is poorly understood. We are constructing a comprehensive phylogeny of the Australian Nasutitermitinae based on mitochondrial and nuclear gene markers and will report our most recent results.

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Evolution of devil facial tumour disease chromosomes

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An usual transmissible cancer, known as devil facial tumour (DFT) disease, is threatening the Tasmanian devil with extinction in the wild. DFT disease appears to have arisen in a female devil toward the end of last century. It is the tumour itself that is the infectious agent, having since spread through much of the population when devils bite each other. DFT disease has caused a dramatic drop in devil numbers across much of Tasmania. The only other example of a transmissible tumour in the wild is canine transmissible venereal tumour (CTVT), which has been spreading through dogs for over 1000 years. The most important difference between DFT and CTVT is that CTVT in most cases does not kill its host the tumour and host are able to co-exist in the population. Is there a chance that DFT could evolve to reach this more desirable scenario?

The initial tumour appears to have resulted from a shattering and rejoining of two chromosomes, followed by the accumulation of other structural mutations, which resulted in the formation of several distinctive DFT marker chromosomes. Interestingly, these genomic regions are not only extensively rearranged in DFT but are also highly rearranged between different marsupial species, suggesting a potential link between tumour and evolutionary breakpoints. By cytogenetically mapping genes to DFT chromosomes isolated from individuals from different geographical locations, it has been possible to trace the evolution of this tumour as it is passes through the population. Until recently, structural mutations in DFTs were seemingly restricted to particular genomic regions, predominantly regions consisting of chromosome 4, 5 and X material but a more recent karyotypic strain of the DFT has been detected in a population where DFT disease prevalence is reduced and there has been a limited effect on population structure compared to other areas affected by DFT disease. This DFT strain has an additional large marker chromosome. The possible implications of this relatively major chromosomal change on the impact of DFT disease will be discussed.

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Polymorphisms in *Cyp2r1* and disruption of the vitamin D pathway associate with the *SuprMam1* breast cancer susceptibility locus in mice.

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High levels of vitamin D are hypothesized to reduce the risk of breast cancer. In order to be biologically active, dietary vitamin D must be converted to its biologically active form $1,25(OH)_2D_3$. Cyp2r1 is a major vitamin D hydroxylase that catalyzes the first step of this activation producing $25(OH)D_3$. *Cyp2r1* is located within *SuprMam1*, a mammary tumour susceptibility locus identified in the BALB/c-Trp53+/- mouse model of spontaneous breast cancer (Blackburn et al, Am J Path, 2007). We have examined the vitamin D pathway in SM09 congenic mice, which contain the BALB/c *SuprMam1* locus on a C57BL/6 background.

qPCR and western blotting for *Cyp2r1* in tissues from SM09 and control mice revealed a significant 2-3-fold reduction in *Cyp2r1* expression in mammary glands and liver (female but not male) of SM09 mice, however differences in plasma $25(OH)D_3$, calcium or phosphate levels were not found. Instead, 3-fold higher levels of plasma parathyroid hormone (PTH), a major vitamin D / calcium regulator, were present in female (but not male) mice carrying the BALB/c allele of the *SuprMam1* locus. Affymetrix expression profiling of mammary glands found differential expression of many genes of the vitamin D pathway, consistent with disruption of the pathway. Increasing dietary calcium or vitamin D returned PTH levels to normal in BALB/c and SM09 mice. We are currently characterizing several polymorphisms in the *Cyp2r1* promoter which may alter promoter function.

Thus, chronically elevated PTH levels due to an interaction between low calcium / vitamin D intake and reduced Cyp2r1 expression from the BALB/c allele of *Cyp2r1* may contribute to increased breast cancer susceptibility. The SM09 congenic mice may serve as a valuable model for studying the role of gene-environment interactions of the vitamin D pathway in cancer and other diseases.

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Identification of genomic alterations in oesophageal squamous cell cancer

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We collected tumour and matched normal DNA samples from 158 ESCC patients in the Chaoshan District of Guangdong Province, an area of high ESCC prevalence in China, and performed whole-genome sequencing (WGS>30X), whole-exome sequencing (WES>100X), and array comparative genomic hybridization (a-CGH) analyses.

We identified eight significantly mutated genes, of which six are well known tumour-associated genes (TP53, RB1, CDKN2A, PIK3CA, NOTCH1, NFE2L2), and two have not previously been described in ESCC (ADAM29 and FAM135B). Notably, FAM135B is identified as a novel cancer- implicated gene as assayed for its ability to promote malignancy of ESCC cells. Additionally, MIR548K, a microRNA encoded in the amplified 11q13.3-13.4 region, is characterized as a novel oncogene, and functional assays demonstrate that MIR548K enhances malignant phenotypes of ESCC cells. Moreover, we have found that several important histone regulator genes (MLL2 (also called KMT2D) , ASH1L, MLL3 (KMT2C) , SETD1B, CREBBP and EP300) are frequently altered in ESCC. Pathway assessment reveals that somatic aberrations are mainly involved in the Wnt, cell cycle and Notch pathways. Genomic analyses suggest that ESCC and head and neck squamous cell carcinoma share some common pathogenic mechanisms, and ESCC development is associated with alcohol drinking.

This study represents a comprehensive characterization of genomic alterations in ESCC, and provides insights into the genetic mechanism(s) of ESCC tumorigenesis. These findings enable us to determine further the biological and therapeutic significance of the newly discovered mutated and amplified genes, which may ultimately lead to the development of effective diagnostic and therapeutic approaches for ESCC.

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WWOX, the Chromosomal Fragile Site *FRA16D* Spanning Gene: its role in metabolism and contribution to cancer

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The *WWOX* gene spans the common chromosomal fragile site *FRA16D,* that is located within a massive (280kb) intron. The WWOX gene is very long, at 1.1Mb, which may contribute to the very low abundance of the full-length 1.4kb mRNA. Alternative splicing also accounts for a variety of aberrant transcripts, most of which are devoid of C-terminal sequences required for WWOX to act as an oxido-reductase. The mouse Wwox gene also spans a chromosomal fragile site implying some sort of functional relationship that confers a selective advantage. The encoded protein domains of WWOX are conserved through evolution (between humans and *Drosophila*) and include WW domains, an NAD binding site, short-chain dehydrogenase/reductase enzyme and nuclear and mitochondrial compartmentalization signals. This homology has enabled functional analyses in *Drosophila* that demonstrate roles for *WWOX* in ROS regulation and metabolism.

Indeed the human *WWOX* gene is also responsive to altered metabolism. Cancer cells typically exhibit altered metabolism (Warburg Effect). Many cancers exhibit *FRA16D* DNA instability that results in aberrant WWOX expression and is associated with poor prognosis for these cancers. It is therefore thought that aberrant WWOX expression contributes to the altered metabolism in cancer. In addition, others have found that a specific (low expression) allele of WWOX genotype contributes to cancer predisposition.

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Anthropogenic selection enhances cancer evolution in Tasmanian devil tumours

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The Tasmanian Devil Facial Tumour Disease (DFTD) provides a unique opportunity to elucidate the long-term effects of natural and anthropogenic selection on cancer evolution. Since first observed in 1996, this transmissible cancer has caused local population declines by >90%. So far, four chromosomal DFTD variants (strains) have been described and karyotypic analyses of 253 tumours showed higher levels of tetraploidy in the oldest strain. We propose that increased ploidy in the oldest strain may have evolved in response to effects of genomic decay observed in asexually reproducing organisms. In this study, we focus on the evolutionary response of DFTD to a disease suppression trial. Tumours collected from devils subjected to the removal programme showed accelerated temporal evolution of tetraploidy compared with tumours from other populations where no increase in tetraploid tumours were observed. As ploidy significantly reduces tumour growth rate, we suggest that the disease suppression trial resulted in selection favouring slower growing tumours mediated by an increased level of tetraploidy. Our study reveals that DFTD has the capacity to rapidly respond to novel selective regimes and that disease eradication may result in novel tumour adaptations, which may further imperil the long-term survival of the world's largest carnivorous marsupial.

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Sweet Taste Gene Expression And Obesity: Is There A Link?

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Sugar intake has been linked to weight gain and obesity[1]. The role of taste perception in the development of obesity is currently unclear. As taste is a major factor influencing food preference, we hypothesize that physiological differences in taste perception may play a role in ingestive behaviour, in particular leading to individual differences in the consumption of sugar. To address this hypothesis, we aimed to examine the relationship between sweet taste, the expression of sweet taste genes, and dietary intake in lean and obese individuals.

An observational study recruited lean (n=25) and obese (n=23) participants, collecting a range of anthropometric, dietary and sensory measures, as well as biological samples to assess sweet taste gene expression (oral and blood samples). Taqman gene expression assays were utilised to assess the sweet taste receptors (*TAS1R2* and *TAS1R3*), and associated secondary messenger molecules (*GNAT3*, *PLCβ2*, *TRPM5* and *ITPR3*).

Obese individuals had reduced expression of the sweet taste receptor *TAS1R3* in oral samples and reduced sweet taste sensitivity compared to lean. The reduced sweet taste and *TAS1R3* expression in obese was associated with a preference for sweeter foods and increased dietary intake of sugar. Furthermore, reduced *TAS1R3* expression in the obese was independent of a promoter variant (rs35744813) previously associated with altered sweet taste[2,3]. No association was observed between sweet taste or dietary intake and the other sweet taste genes analysed.

This finding supports the hypothesis that differences in taste physiology may exist between lean and obese individuals, influencing ingestive behaviour and the overconsumption of sugar. In a further study it will be important to measure the contribution of dietary exposure to differences in taste receptor expression, to identify whether an obesogenic taste phenotype can be reverse adapted.

1. Morenga, L.T., Mallard, S. & Mann, J. (2013) Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. BMJ, 346 e7492.
2. Fushan, A.A., Simons, C.T., Slack, J.P., Manichaikul, A. & Drayna, D. (2009) Allelic Polymorphism within the TAS1R3 Promoter Is Associated with Human Taste Sensitivity to Sucrose. Current Biology, 19 (15): 1288-1293.
3. Mennella, J.A., Finkbeiner, S. & Reed, D.R. (2012) The proof is in the pudding: children prefer lower fat but higher sugar than do mothers. Int J Obes, 36 (10): 1285-1291.

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RNA pathogenesis via Toll-like receptor-activated inflammation in expanded repeat neurodegenerative diseases

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Previously, we hypothesized that an RNA-based pathogenic pathway has a causal role in the dominantly inherited unstable expanded repeat neurodegenerative diseases. In support of this hypothesis we, and others, have characterized rCAG.rCUG100 repeat double-strand RNA (dsRNA) as a previously unidentified agent capable of causing pathogenesis in a Drosophila model of neurodegenerative disease. Dicer, Toll, and autophagy pathways have distinct roles in this Drosophila dsRNA pathology. Dicer dependence is accompanied by cleavage of rCAG.rCUG100 repeat dsRNA down to r(CAG)7 21-mers. Among the "molecular hallmarks" of this pathway that have been identified in Drosophila, some [i.e., r(CAG)7 and elevated tumor necrosis factor] correlate with observations in affected people (e.g., Huntington's disease and amyotrophic lateral sclerosis) or in related animal models (i.e., autophagy). The Toll pathway is activated in the presence of repeat-containing dsRNA and toxicity is also dependent on this pathway. How might the endogenously expressed dsRNA mediate Toll-dependent toxicity in neuronal cells? Endogenous RNAs are normally shielded from Toll pathway activation as part of the mechanism to distinguish "self" from "non-self" RNAs. This typically involves post-transcriptional modification of the RNA. Therefore, it is likely that rCAG.rCUG100 repeat dsRNA has a characteristic property that interferes with or evades this normal mechanism of shielding. We predict that repeat expansion leads to an alteration in RNA structure and/or form that perturbs RNA modification, causing the unshielded repeat RNA (in the form of its Dicer-cleaved products) to be recognized by Toll-like receptors (TLRs), with consequent activation of the Toll pathway leading to loss of cell function and then ultimately cell death. We hypothesize that the proximal cause of expanded repeat neurodegenerative diseases is the TLR recognition (and resultant innate inflammatory response) of repeat RNA as "non-self" due to their paucity of "self" modification.

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Estimating evolutionary timescales using genomic data

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Evolutionary timescales can be estimated from DNA sequence data using the molecular clock, a statistical model that describes the behaviour of evolutionary rates among organisms. Although originally based on the assumption of rate constancy among lineages, molecular clocks now include 'relaxed' variants that are able to accommodate heterogeneous rates. These have provided useful insights into evolutionary rates and timescales across the Tree of Life.

Genome-scale data offer exciting opportunities for improving our understanding of molecular evolution and refining our estimates of evolutionary timescales. However, they also bring considerable computational and analytical challenges. I describe some of the approaches that have been used to estimate evolutionary timescales from genome-scale data. I focus on two recent examples, one concerning the diversification of birds and the other concerning the evolutionary dynamics of potato blight fungus. I also speculate on the future of molecular clocks, describing the key limitations and highlighting some of the most promising research directions.

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A fish out of water: understanding the evolution of land-dwelling fish using contemporary analogues of a critical step in vertebrate evolution.

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Adaptation to life on land was among the most important events in vertebrate evolutionary history. During the late Devonian, approximately 350 million years ago, fish left the water and colonised the land. Up until now, this extraordinary transition has been inferred from the fossil record. Yet, fossil evidence is fragmentary, mostly morphological and consequently limits the inference of adaptation. In particular, fossils cannot identify the genetic changes, ecological shifts or behaviours that underlie adaptation. There are however, a number of contemporary examples of fish that have made or are making a similar transition to life on land. Within the family Blenniidae there are fish from small islands throughout the Indian and Pacific Oceans that essentially represent each evolutionary step involved in the transition to land. Within this remarkable system, there are genera that are almost exclusively terrestrial, spending the vast majority of their time out of water. That is, they do not voluntarily return to water. Sister to these "land fish" are genera that are amphibious, spending part of their time on land and part of their time in the water. Ancestral to all of these are a variety of genera that retain their aquatic phenotype. Collectively, these fish provide a unique and unrivaled opportunity to investigate the genetics and behaviour of a land invasion from aquatic origins in vertebrates. Here using a combination of phylogenetics, ancestral character state reconstructions and field based behavioural observations of these fish I will explore the answers to the following questions: 1. what drive fish to make a transition to land and 2. is the invasion of land by fish and unusual or frequent evolutionary event? These results will help reveal the ecological, behavioural and evolutionary processes that might have been involved with the initial invasion of land by fish in the Devonian.

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Estimating the evolutionary timescale for Dictyoptera

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Origins of Australian freshwater fishes

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The world's fish fauna is dominated by two groups, Ostariophysi (9622 species) and Acanthopterygii (16207 species), which combined account for about 81 per cent of all fishes. Most freshwater fish faunas are dominated by ostariophysans, consisting of minnows (Cypriniformes), characins (Characiformes) and catfishes (Siluriformes). In contrast, the worlds marine habitats are dominated by acanthopterygian fishes. The freshwater fish fauna of the Australian continent (which includes New Guinea) is exceptional in being dominated by acanthopterygian fishes rather than ostariophysans. Indeed, Australia has the only freshwater representatives for many acanthopterygian families. This makes the continental fish fauna unlike any other apart from the island of Madagascar. While Australia's extraordinary mammal fauna receives a lot of attention, our fish fauna is no less distinctive. While most Australian freshwater fishes ultimately have marine origins, most families have been present in Australian freshwaters for at least 40-80 million years. In this presentation I will review the phylogenetic information that examines the number of marine – freshwater transitions for various Australian freshwater groups and provide approximate estimates for the timing of these invasions.

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Evaluation of the Generalised Mixed Yule-Coalescent method for species delimitation across multiple mitochondrial genes

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The process of species discovery and delimitation can be aided by the application of computational methods to genetic data. Molecular methods for species delimitation have the potential to speed up the progress of traditional taxonomy, particularly in cases where cryptic species are believed to be present.

Among the molecular methods for species delimitation, those that use a phylogenetic approach have been shown to outperform techniques based on pairwise genetic distances. At present, phylogenetic species delimitation is usually based on a single locus. This can be problematic because trees inferred from different genes can be mutually incompatible, even when there is complete linkage.

The Generalised Mixed Yule-Coalescent (GMYC) is a widely used approach to computational species delimitation. The GMYC treats the phylogenetic tree as the product of a mixture of speciation (between species) and coalescent (within species)

processes. Using maximum likelihood, the method estimates the point in time that separates these two processes, yielding an estimate of the number of distinct evolutionary units or putative 'species' in the data set. Although the GMYC appears to exhibit robust performance on a variety of datasets, its sensitivity to the choice of genetic marker has not been evaluated.

Using a case study based on cetaceans (whales, dolphins, and porpoises), we examined the behavior of the GMYC when applied to gene trees inferred from each of the major protein-coding and ribosomal RNA genes in the mitochondrial genome. Our results indicate that the results of species delimitation can vary across different mitochondrial markers. Further development of the GMYC method should consider how the procedure can be extended to accommodate multilocus data while retaining its theoretical and computational advantages.

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Mixture models of nucleotide sequence evolution, and the evolution of yeast genomes

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Molecular phylogenetic studies of homologous sequences of nucleotides often assume that the evolutionary process was globally stationary, reversible, and homogeneous (SRH), and that the data can be modeled accurately using one or several site-specific, time-reversible rate matrices. However, a growing body of data suggests that evolution under globally SRH conditions is an exception, rather than a norm. To address this issue, we introduce a family of mixture models that considers heterogeneity in the substitution process across lineages (HAL) and across sites (HAS). We also introduce an algorithm for searching model space and identifying a model of evolution that is less likely to over- or under-parameterize the model. The merits of our algorithms are illustrated with an analysis of 42,337 2nd codon sites extracted from a concatenation of 106 alignments of orthologs encoded by the nuclear genomes of eight species of yeast. The best HAL-HAS model provides a better fit between the tree and data than other models do, and the parameter estimates for this model indicate not only a complex ancestral sequence but also a complex evolutionary process.

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Can immunity against viral infection be inherited epigenetically in *Caenorhabditis elegans*?

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Antiviral RNA-mediated silencing (RNAi) acts as a powerful innate immunity defence in plants, invertebrates and mammals. In *C. elegans* RNAi is systemic, *i. e.* RNAi silencing signals can move between cells and tissues. Furthermore RNAi effects can be inherited transgenerationally and may last for many generations. Neither the biological relevance of systemic RNAi nor transgenerational RNAi are currently understood. Here we examined the role of both pathways to protect *C. elegans* from viral infection. We studied the Orsay virus, a positive strand RNA virus related to *Nodaviridiae*, and the first and only virus known to infect *C. elegans*. We found that genes required for systemic or transgenerational RNAi did not have a role in antiviral defence. Furthermore, we found that Orsay virus infection did not elicit a systemic RNAi response even when a target for RNAi was provided using transgenes. Finally, we show that viral siRNAs, the effectors of RNAi, are not inherited to a level that provides any significant resistance to viral infection in the next generation. We conclude that systemic or transgenerational RNAi does not play a role in the defence to Orsay virus infection. Furthermore, our data suggest that there is a qualitative difference between experimental RNAi and antiviral RNAi. Our data are consistent with a model of systemic and transgenerational RNAi that requires a nuclear or germline component which is lacking in RNA virus infection.

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Assembling the methylome of an oilseed *Brassica*

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Superimposed on the underlying DNA sequence of plants and animals is a series of epigenetic marks that can provide considerable agility in terms of modulating gene expression, ontology, susceptibility to disease and response to the environment. Understanding the epigenetic processes corresponding with these functions may unlock hidden traits of agronomic importance. In oilseed *Brassica*s, reproductive development underpins yield quality of the final harvestable seed product. To understand the associated molecular epigenetic interactions we are developing the methylome of *Brassica rapa* oilseed line R-o-18, genome size ~300 Mbp.

Following resequencing and pseudochromosome construction of the R-o-18 genome, we performed multiple technical and biological whole genome bisulphite sequencing (WGBS) runs on Illumina MiSeq and HiSeq platforms. To determine the reproducibility of the WGBS technique we ran technical replicates differing at the library preparation and/or sequencing run stage. Raw data processing and read alignment was performed and optimised using both proprietary and open source software. Methylation calling using Bismark and BSMap were compared for read coverage and percent methylation in the CG, CHG and CHH contexts common in plants. Mean GC content of converted clean paired reads is approximately 10 % lower than unconverted DNA, and unique alignments of cleaned Illumina MiSeq WGBS data per run exceeds 5.2 million with a mean length of approximately 115 bp giving approximately 600 Mbp of unique alignments per run.

The distribution of methylation in different functional regions was assessed for 5' upstream, intra-exonic, intra-intronic and exon/intron boundaries. Methylation of selected key genes in distinct tissues were analysed in depth. Results from the WGBS comparisons and the methylome development will be presented. Our work is contributing towards the development of a framework underpinning new crop management strategies both in terms of information-led agronomy and in harnessing epigenetic variation in crop breeding.

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DNA methylation: silencing sex chromosomes in amniote vertebrates.

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Cytosine methylation is an epigenetic modification that plays a role in regulation of gene transcription. Methylation, particularly at promoter CpG-islands, can lead to stable silencing of the associated gene. In mammals, DNA methylation has several well characterized regulatory functions, including genomic imprinting and the chromosome-wide epigenetic silencing of the X chromosome (called X-chromosome inactivation; XCI). XCI is part of a dosage compensation system in therian (eutherian and marsupial) mammals that results in almost equal average transcriptional output from the X chromosome between the sexes in eutherian and marsupial mammals. In contrast, platypus (males have 5 Xs and 5 Ys: females 5 pairs of Xs) and chicken (with a ZW female: ZZ male sex chromosome system), dosage compensation appears less efficient, where average Z/X transcriptional output is higher in the homogametic sex than in the heterogametic sex. Nevertheless, we recently demonstrated that alleles on one X/Z is also silenced in the homogametic sex of these species.

DNA methylation is a late and stabilizing step in maintaining transcriptional silence of the X in eutherian mammals, but there are limited detailed data about DNA methylation in marsupials, monotreme and birds. Here we present the first genome wide approach to study DNA methylation in non-eutherian representatives from three amniote vertebrate lineages, each with independently evolved dosage compensation systems. We examine differential CpG methylation between males and females in distantly related taxa to identify the importance of methylation in amnoite vertebrate dosage compensation.

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The dynamic DNA methylation cycle in honey bee development and reproduction

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In honey bees (*Apis mellifera*) the epigenetic mark of DNA methylation is central to the developmental regulation of caste differentiation, but may also be involved in additional biological functions. We examined the whole genome methylation profiles of three stages of the haploid honey bee genome: unfertilised eggs, the adult drones that develop from these eggs, and the sperm produced by these drones. Each methylome exhibited distinct patterns of methylation, with 381 significantly differentially methylated genes (DMGs) between eggs and sperm. Extensive differential germline methylation suggests parent-specific epigenetic marking in the gametes, an observation consistent with predictions of parental imprinting in eusocial insects. However, it is also possible that this finding arose in part because methylation and demethylation during embryogenesis increases the number of genes that are methylated in eggs, and is nothing to do with parental imprinting. To further address the question of paternal imprinting in honey bees we performed reciprocal crosses between honey bee subspecies. These crosses revealed a strong parent-of-origin effect for ovary size in offspring workers. We are now exploring methylation in specific tissues such as ovaries of active and non-actively reproducing workers, and in the eggs of highly reproductive worker honey bees to determine if methylation is involved in reproductive behaviour in honey bees.

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SUMV-1 antagonizes the activity of synthetic Multivulva genes in *Caenorhabditis elegans*

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Chromatin regulators contribute to the developmental control of gene expression. In the nematode *Caenorhabditis elegans*, the roles of chromatin regulation in development have been explored in several contexts, including vulval differentiation. The synthetic multivulva (synMuv) genes are regulators of vulval development in *C. elegans* and the proteins encoded by these genes include components of several histone modification and chromatin remodelling complexes. By inhibiting ectopic expression of the epidermal growth factor (LIN-3) in the nematode hypodermis, the synMuv genes prevent inappropriate vulval induction. In a forward genetic screen for modifiers of the expression of a hypodermal reporter gene, we identified a mutation that results in increased expression of the reporter. This mutation also suppresses ectopic vulval induction in synMuv mutants and we have consequently named the affected gene suppressor of synthetic multivulva-1 (*sumv-1*). We show that SUMV-1 is required in the hypodermis for the synMuv phenotype and that loss of *sumv-1* function suppresses ectopic expression of *lin-3* in synMuv mutant animals. In yeast two-hybrid assays SUMV-1 physically interacts with SUMV-2, and reduction of *sumv-2* function also suppresses the synMuv phenotype. We identified similarities between SUMV-1 and SUMV-2 and mammalian proteins KAT8 NSL2 and KAT8 NSL3, respectively, which are components of the KAT8/MOF histone acetyltransferase complex. Reduction of function of *mys-2*, which encodes the enzymatic component of the KAT8/MOF complex, also suppresses the synMuv phenotype, and MYS-2 physically interacts with SUMV-2 in yeast two-hybrid assays. Together these observations suggest that SUMV-1 and SUMV-2 may function together with MYS-2 in a nematode KAT8/MOF-like complex to antagonise the activity of the synMuv genes.

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The transcriptional repressor CTBP-1 functions in the nervous system of *Caenorhabditis elegans* to regulate lifespan

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C-terminal binding proteins (CtBPs) are recruited by a variety of transcription factors to mediate gene repression. Nematode CTBP-1 has previously been shown to play a role in the regulation of lifespan; *Caenorhabditis elegans* strains carrying a deletion in the *ctbp-1* gene showed a 10-20% increase in mean and maximal lifespan compared with wild-type control strains. We set out to identify the tissues in which CTBP-1 functions to regulate lifespan in *C. elegans*. Our analysis of reporter genes shows that CTBP-1 is predominantly expressed in the nervous system with lower levels detectable in the hypodermis. Tissue-specific rescue experiments demonstrated that CTBP-1 functions in the nervous system to regulate lifespan. Previously, the lifespan extension in a *ctbp-1* mutant was attributed, at least in part, to the misregulation of a lipase gene, *lips-7*. We therefore focussed on *lips-7* and found that re-expressing CTBP-1 in the nervous system significantly reduced *lips-7* transcription. In addition, we studied another *ctbp-1* mutant allele that also displayed a long-lived phenotype. In this case, *lips-7* expression was unaffected. This observation argues that, while *lips-7* may play a role in lifespan, its de-repression is not essential for the extension of lifespan phenotype. We show that a prominent site of LIPS-7 expression is the hypodermis, a site of fat storage in *C. elegans*. Interestingly, we did not observe co-localisation of CTBP-1 and *lips-7* transcription in the nervous system, indicating that CTBP-1 may be acting indirectly in a cell non-autonomous manner. In summary, our data confirm that CTBP-1 is involved in the regulation of *lips-7* transcription but suggest that it may perform additional roles in the nervous system that contribute to the regulation of longevity.

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Homeodomain interacting protein kinase (HPK-1) is required in the soma for robust germline proliferation in *C. elegans*

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hpk-1 encodes the sole C. elegans member of a family of evolutionarily conserved protein kinases called the homeodomain interacting protein kinases (HIPKs). Mammalian homologues of HPK-1 have been implicated in control of numerous cellular processes including cell survival and proliferation. A *C. elegans* strain carrying a *hpk-1* mutation has previously been studied (Raich et al., 2003), but no obvious phenotypes were reported. We decided to use the *hpk-1* mutant strain and *hpk-1* RNAi to investigate the role of HPK-1 in the development and maintenance of the *C. elegans* germline.

A significant reduction in germline proliferation was observed in the strain carrying the *hpk-1* mutation. The phenotype was characterised by reduced brood size, reduced size of the mitotic region and a decrease in the number of proliferative cells. Knockdown of *hpk-1* by RNAi resulted in a comparable phenotype, confirming that HPK-1 is required for normal germline proliferation. Our results furthermore suggest that HPK-1 is not only required for the maintenance of the mitotic region in adult germlines but that it is also necessary for the establishment of the progenitor pool during development as the reduced proliferation phenotype was also observed at the L4 stage.

Interestingly, the brood size and number of proliferative cells were rescued in *hpk-1* mutants with HPK-1::mCherry-expressing transgenes from which no germline expression had been detected. In addition, knockdown of *hpk-1* in a soma-sensitive RNAi strain resulted in reduced proliferative cell number whereas knockdown in a germline-sensitive RNAi strain had no significant effect. These observations suggest a role for HPK-1 in soma-dependent control of germline proliferation.

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Identification of polycomb group genes and Phosphoinositide-3-Kinase as new regulators of wing disc eversion in *Drosophila*

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Epithelial to Mesenchymal Transition, the process whereby epithelial cells transition into motile mesenchymal cells is an important process in development, and is associated with cancer metastasis. In *Drosophila melanogaster* an EMT like event occurs during the eversion of the wing disc. Cells of the peripodial epithelium lose apico-basal polarity, cell-cell junctions break down and the cells take on an invasive, migratory phenotype. In an RNAi screen for EMT factors we identified the secreted axon guidance factor Netrin-A. We find that Netrin-A, and its paralog Netrin-B, promote DE-Cadherin breakdown in the peripodial epithelium, by downregulating its receptor Frazzled (Manhire-Heath *et al*, *Nat. Comms*. 2013, 4:2790). To find EMT factors that genetically interact with Netrins we rescreened the hits from the primary screen in a genetic background lacking *netrin-B.netrin-B* mutants are viable and fertile, but loss of *netrin-B* enhances the penetrance of eversion defects of other genes, such as *netrin-A*. The screen identified two gene candidates, the polycomb group (PcG) gene *sex combs extra* (*Sce*) and phosphoinositide-3-kinase (*Pi3K92E*). PcG genes are epigenetic regulators that target histones, and are well known for their ability to suppress homeotic genes. Pi3K92E catalyses the production of phosphatidylinositol-3,4,5 triphosphate (PIP3), which can recruit numerous signalling proteins and thereby regulate cell survival, cell migration and cell death. Knockdown of other PcG genes also caused eversion failure defects, consistent with these proteins functioning in complexes. Using an *in vitro* overnight culture assay we have also confirmed that RNAi to *Sce* and *Pi3K92E* disrupts the eversion process. Future work will characterise the role of PcG genes and Pi3K92E in controlling epithelial breakdown and acquisition of motility, and investigate the interaction between these genes and the Jun-Kinase and the Netrin/Frazzled pathways, which are known to regulate eversion.

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Genome-wide BAC-end sequencing of olive flounder (Paralichtys olivaceus) using two BAC libraries

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The olive flounder Paralichthys olivaceus, is one of the most economically important marine aquaculture species, with a selective breeding program to increase aquaculture production in Korea. However, a paucity of information is available regarding the olive flounder genome. Bacterial artificial chromosome (BAC) library is an important tool in genomic research. We constructed two BAC libraries, with large, high quality inserts and deep coverage, for P. olivaceus as a crucial part of the olive flounder genome project. The libraries were constructed in the EcoRI and HindIII sites of the vector CopyControl pCC1BAC. The two libraries contain a total of 66,816 BAC clones arrayed in 174 384-well microtiter plates and correspond to 14.18 haploid genome equivalents based on olive flounder genome size of 550Mb. A random sampling of 335 BACs indicated an average insert length of 118 kb with a range of 80 to 180 kb, and 1.5% of the BACs do not contain inserts. The combined libraries have a greater than 99% probability of containing any single-copy sequence. The BAC library has been arranged in three-dimentional pools allowing screening with various PCR-based markers. BAC colonies were screened with PCR-generated gene-specific probes for the DMRT1 and 2 positive clones covering this gene fully were identified. We generated a

total of 26,824 high-quality BAC-end sequences (BES), 96.5% of which were paired-BES. The average read length was 676 bp, representing 20.8 Mb of genomic DNA in total or 3.8% of the genome. This database of BAC-end sequences is useful for the assembly of the complete olive flounder genome sequence and is important for identification in functional genomics experiments.

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Comparative phylogeography of four aquatic species from the Murray-Darling Basin

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The Murray-Darling Basin (MDB) has a complex biogeographic history as it is surrounded by more independent river basins than any other Australian basin. As a result portions of the aquatic fauna have a mix of relationships to all surrounding regions, as well as an endemic component. Our project has three principal goals. 1) Is there an historical signature on biodiversity in the MDB and adjacent drainages that remains evident in the genetic structure of widespread species? 2) Are there concordant patterns of genetic structure across disparate aquatic and water-dependent organisms? 3) What are the impacts of dams on dispersal and degree of erosion of local genetic diversity of aquatic organisms? We chose four unrelated aquatic species that were widespread across the MDB, but that lacked known complications due to introgression or presence of cryptic species: the fish Australian Smelt (*Retropinna semoni*), river turtle (*Emydura macquarii*), yabby (*Cherax destructor*) and shrimp (*Macrobrachium australiense*). We are exploring patterns of genetic diversity using SNP variation from thousands of loci to address these three questions.

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Phosphoinositide-specific phospholipase C (PLC) functions as an effector molecule in the signal transduction process.

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Generally, PLC hydrolyzes PIP2 to generate inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). IP3 binds to the IP3-receptor in the membrane of endoplasmic reticulum (ER) and induces the release of Ca2+ into the cytoplasm, whereas DAG, along with Ca2+, activates protein kinase C (PKC). Thirteen PLC isozymes have thus far been cloned from mammalian species, and have been classified into six classes based on their structures and activation mechanisms; PLCδ (delta) (1, 3 & 4), β (beta)(1–4), γ (gamma) (1, 2), ε (epsilon), ζ (zeta), and the recently-discovered η (hepta) (1, 2). These PLC β s are regarded as the sole isoenzymes activated by chemoattractants in the leukocytes. Studies conducted with mice lacking PLCβ2 and PLCβ3 showed that these PLC pathways are critical to chemoattractant-mediated signal transduction and the regulation of protein kinases. Recently, the results of other studies have shown that bradykinin increases IL-6 production in synovial fibroblasts via the bradykinin β2 receptor, PLCβ3, PKCδ, and NF-κB signaling pathways. We cloned a partial fragment of the PLCB3 (878 bp) gene from the brain cDNA of the olive flounder (Paralichthys olivaceus) by designing degenerative primers based on highly conserved domains of PLCB genes, after multiple alignments using ClustalW with full-length cDNAs from previously reported mammalian. In this study, we describe the molecular cloning and sequencing analysis of the olive flounder PLCB3 gene (PoPLCB3), as well as the up-regulation of expression of this gene after lipopolysaccharide (LPS), poly I:C, scuticocilliate and bacterial infection, respectively.The cDNA for olive flounder PLCB3 (PoPLCB3) encodes for a polypeptide of 1,246 amino acids in length containing a well-conserved PH domain, catalytic X and Y domains, a C2 domain. From the sequence information of the BAC library, we assembled a contig containing the whole flounder PLCB3 cDNA sequences, and determined the exon/intron structure of the gene spanning >100,000 bp DNA. Phylogenic analysis and sequence comparison of PoPLCB3 with other PLC isozymes showed a close relationship with the PLCB3 isozyme. Tissue-specific mRNA of PoPLCB3 was expressed predominantly in the kidney, heart and brain tissues. PoPLCB3 gene expression was compared with that of the inflammatory cytokines IL-1β and TNF-α in infected spleen and kidney tissues via real-time RT-PCR assays following stimulation with LPS, poly I:C, scuticocilliate and bacterial infection, respectively.

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Investigating the Role of Homeodomain-Interacting Protein Kinase in *Caenorhabditis elegans*

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Proteins of the Homeodomain-Interacting Protein Kinase (HIPK) family regulate an array of processes in mammalian systems, such as the DNA damage response, cellular proliferation and apoptosis (D'Orazi et al., 2002, Hofmann et al., 2002). Members of this protein family are serine/threonine kinases that are predominantly localised to the nucleus. The nematode *Caenorhabditis elegans* expresses a single HIPK protein, called HPK-1, which is broadly expressed and, like its mammalian counterparts, is localised to nuclear speckles, suggesting that it may be involved in analogous cellular processes. We have previously shown that HPK-1 is required for the promotion of germline proliferation during nematode development and into adulthood (Berber et al., 2013).

Interestingly, HPK-1 was also reported as one of the proteins necessary for the extended lifespan of worm strains carrying a mutation in the gene encoding the insulin receptor *daf-2* (Samuelson et al., 2007). To expand on this observation, phenotypic analyses have been conducted on a worm strain carrying a deletion mutation within *hpk-1*, focusing on the tissue-specificity and molecular mechanisms by which HPK-1 may regulate various cellular processes, including ageing. The role of HPK-1 in stress responses is also being investigated, as observations of animals carrying a fosmid-based fluorescent reporter indicated that HPK-1 is induced under conditions of heat stress (Berber et al., 2013).

BERBER, S., LLAMOSAS, E., THAIVALAPPIL, P., BOAG, P. R., CROSSLEY, M. & NICHOLAS, H. R. 2013. Homeodomain interacting protein kinase (HPK-1) is required in the soma for robust germline proliferation in C. elegans. *Dev Dyn*.

D'ORAZI, G., CECCHINELLI, B., BRUNO, T., MANNI, I., HIGASHIMOTO, Y., SAITO, S. I., GOSTISSA, M., COEN, S., MARCHETTI, A., DEL SAL, G., PIAGGIO, G., FANCIULLI, M., APPELLA, E. & SODDU, S. 2002. Homeodomain-interacting protein kinase-2 phosphorylates p53 at Ser 46 and mediates apoptosis. *Nat Cell Biol,* 4**,** 11-19.

HOFMANN, T. G., MOLLER, A., SIRMA, H., ZENTGRAF, H., TAYA, Y., DROGE, W., WILL, H. & SCHMITZ, M. L. 2002. Regulation of p53 activity by its interaction with homeodomain-interacting protein kinase-2. *Nat Cell Biol,* 4**,** 1-10.

SAMUELSON, A. V., CARR, C. E. & RUVKUN, G. 2007. Gene activities that mediate increased life span of C. elegans insulin-like signaling mutants. *Genes Dev,* 21**,** 2976-94.

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Monitoring freshwater fish communities using eDNA metabarcoding.

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Monitoring species distributions and biodiversity is the fundamental basis for ecological studies and biodiversity management. Obtaining such detailed information is often difficult due to the limitations of current monitoring tools that are often time-consuming and expensive. Also, monitoring can be especially problematic from a management perspective since most environmental agencies focus their efforts on species present at low densities, such as recently introduced or spreading Invasive Alien Species (IAS) and rare or threatened native species. Recent advances in extracting environmental DNA (eDNA), DNA barcoding and sequencing technologies now make it possible to analyse whole species communities by extracting and analysing eDNA fragments (eDNA metabarcoding). Here we propose an eDNA-based methodology that can potentially be used for the monitoring of entire fish communities. By carefully selecting the target fragments, PCR primers and PCR blocking primers we expect to be able to develop a highly sensitive, accurate and reliable eDNA metabarcoding tool. The developed methodology is expected to significantly improve monitoring surveys and will be tested in the field to evaluate the effects of a spreading population of invasive fish on the native fish community. The ultimate goal of this research would be to deliver an eDNA metabarcoding tool that can be used to improve the monitoring and management of invasive and rare or threatened native species.

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Parameterisation of eDNA Detection Probabilities for the Identification of Rare Aquatic Species

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The use of environmental DNA (eDNA) is a relatively new indirect method of species detection. It offers several advantages over traditional detection techniques such as its sensitivity and that it does not require capture, handling or direct observation of the species of interest. Despite the rising interest on utilising eDNA and its potential application to many areas of wildlife research, there are a number of factors that can impact upon the limits of eDNA detection and these are still relatively unknown for most species. These factors include the persistence of eDNA in water, the impacts of environmental conditions (e.g. temperature, UV light, PH, flow rates), and where and when to sample. The practical applicability and adoption of eDNA as a standard tool in monitoring requires a quantitative framework that provides a probability of detection, and must also be robust and cost-effective in comparison with traditional wildlife detection methods. In this poster presentation, I will present our preliminary findings comparing *in-situ* eDNA detection of the Oriental weatherloach (*Misgurnus anguillicaudatus*) with standard fish sampling methods. I will also outline the various approaches we will take to improve detection probabilities for rare species using eDNA.

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Early T-cell gene response to activation in Bullmastiffs

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Proliferation is fundamental to T-cell differentiation, homeostasis and immune response. Initiation of proliferation following receptor mediated stimuli requires a temporally programmed gene response. The immediate-early response genes in T-cell activation engage the cell cycle machinery and promote subsequent gene activation events. These genes are of interest in understanding immune regulation and the molecular basis of lymphoma. The present study was undertaken to characterize the early T-cell gene response in Bullmastiffs, a breed that has an increased incidence of lymphoma. Gene expression profiles were charaterized using canine gene expression microarrays and quantitative reverse transcription PCR (qRT-PCR), and paired samples from eleven Bullmastiff dogs. Significant functional annotation clusters were identified following stimulation with a low mitogenic dose of phytohemagluttinin (PHA) (5µg/ml), including the Toll-like receptor signalling pathway and phosphorylation pathways. Using strict statistical criteria, 13 individual genes were found to be differentially expressed, nine of which have ontologies that relate to cancer development, or proliferation and cell cycle control. These included, prostaglandin-endoperoxide synthase 2 (PTGS2/COX2), early growth response 1 (EGR1), growth arrest and DNA damage-inducible gene (GADD45B), phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1), V-FOS FBJ murine osteosarcoma viral oncogene homolog (FOS), early growth response 2 (EGR2), hemogen (HEMGN), polo-like kinase 2 (PLK2) and polo-like kinase 3 (PLK3). Differential gene expression was re-examined using qRT-PCR, which confirmed the expression patterns seen in these genes. PTGS2 and EGR1 showed the highest levels of response in these dogs. Both of these genes are involved in cell cycle regulation and are known to be associated with cancer development in mice and humans. This study provides a comprehensive analysis of the early T-cell gene response to activation in Bullmastiffs. Key genes identified in this analysis may provide quantitative markers of T-cell response and with further study may point to underlying mechanisms of lymphoma susceptibility in this breed.

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Expansion of olfactory receptor gene family in Australia lizard Pogona vitticeps

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Detection of environmental odor chemicals is important to help animals detect prey and discriminate palatable and noxious foods, to identify and select mates and in communication more generally. This physiological function is mediated by a group of proteins called olfactory receptors (OR). The genes encoding the olfactory receptors (OR genes) constitute the largest gene superfamily in vertebrates. By data mining, we have identified 168 OR genes from the deeply sequenced genome of Australia lizard *Pogona vitticeps*. These OR genes can be classified into 17 families. Three of the genes belong to 'fish-like' class I OR genes while the remaining 164 genes belong to mammalian-like class II OR genes which detect airborne odorants. Compared to the insectivorous *Anolis carolinensis*, the OR gene family of the omnivorous *Pogona vitticeps* is expanded, particularly, families 9, 11 and 14. The *Pogona* OR genes are distributed on 40 scaffolds in the Pogona genome, with 108 of them are organized into gene clusters. OR genes showing a high level of similarity tend to be located together, suggesting that local duplication is a mechanism of OR family expansion. We compared the amino acid sequences of *Pogona* ORs to those in mouse with known odorant specificities. OR gene in family 52 showed homology to mouse Olfr690, a receptor gene for n-aliphatic aldehydes/alcohol – a fatty smell. This fish-like class I OR gene in *Pogona* may have important roles in detecting aromatic food. The expansion of OR genes is likely associated with the more diverse range of foods eaten by this species, including insects, fruits and flowers.

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T-DNA Insertion Lines with Altered Root System Architecture in *Arabidopsis thaliana*

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Root system architecture (RSA) development is a complicated process that is pivotal to processes as fundamental as the acquisition of water and nutrients, stress tolerance and anchorage in the soil. This poster presents preliminary results of a search for genes that contribute to root system architecture development in *Arabidopsis thaliana*. With the aid of reverse genetics, microscopic studies, molecular genetic techniques, and computer-aided phenotyping, we have identified genes—functioning in cytoskeleton polymerization/depolymerization, gravitropism, and in unknown processes—that contribute to root system architecture. The study will expand our understanding of how root systems grow and develop in plants, potentially facilitating the improvement of crop species.

Keywords: *Arabidopsis thaliana*, Root System Architecture (RSA), Reverse Genetics, Phenotyping, Molecular Genetics

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ArrayMaker: effortless genotyping-by-sequencing from whole genome sequence alignments

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Whole genome sequencing has revolutionized the study of genetics, and we are challenged with the task of extracting information from immense datasets in a simple, efficient and timely manner. Given the rapid decline in sequencing costs, genotyping-by-sequencing is now accessible to even small genetics projects, yet the bioinformatics and computing resources involved can be daunting if not prohibitive for some laboratories.

Here we present ArrayMaker, a user-friendly tool which extracts accurate single nucleotide polymorphism (SNP) genotypes from whole genome sequence alignments. SNP genotypes are output in a standard format compatible with both association analysis software and datasets genotyped on commercial array platforms. The application is initiated by a single Linux command. It requires no installation and has a single dependency, SAMtools[1].

ArrayMaker enables geneticists with any level of computing expertise to quickly and easily genotype aligned samples at any desired list of markers, facilitating genome wide association analysis, fine mapping, candidate variant assessment, data sharing and compatibility of data sourced from multiple technologies.

1. Li, H. et al. (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics, 25, 2078-2079.

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Further evidence of a lack of interaction between *APOE* and late-life blood pressure in predicting cognitive decline: The PATH Through Life Study

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The apolipoprotien E(*APOE*) **e4* allele[1,2] and hypertension[3,4] are two of the most prevalent genetic and environmental risk factors associated with cognitive decline in latter life.Genetic and environmental risk factors, however, do not act in isolation and interactions between these risk factors may modify the rate of cognitive decline[5]. The present analysis examines whether the *APOE *e4* allelemoderates the association between late-life high blood pressure and cognition in later life. We tested whether and interaction between the blood pressure variables hypertension or mean arterial pressure and *APOE* genotype was associated with greater cognitive decline in early old age. Cognitive function was assed at three time points over a period of 8 years in 1,741 cognitively normal community-dwelling adults aged 60-64 years at baseline. Using multilevel models it was found that *APOE* genotype did moderate the association between late-life high blood pressure on some cognitive tests, however, the inclusion of the interaction term either did not significantly improve model fit or explain any additional variation in the models. These results suggest that*APOE* genotype does not moderate the association between late-life blood pressure and cognition in latter life.

1. Small BJ, Rosnick CB, Fratiglioni L, Bäckman L (2004) Apolipoprotein E and Cognitive Performance: A Meta-Analysis. Psychology and Aging 19, 592–600
2. Wisdom NM, Callahan JL, Hawkins KA (2011) The effects of apolipoprotein E on non-impaired cognitive functioning: A meta-analysis. Neurobiology of aging 32, 63–74
3. Novak V, Hajjar I (2010) The relationship between blood pressure and cognitive function. Nat Rev Cardiol 7, 686–698
4. Gasecki D, Kwarciany M, Nyka W, Narkiewicz K (2013) Hypertension, brain damage and cognitive decline. Curr Hypertens Rep 15, 547–558
5. Payton A (2009) The Impact of Genetic Research on our Understanding of Normal Cognitive Ageing: 1995 to 2009. Neuropsychol Rev 19, 451–477

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Epigenetic analysis of devil facial tumour chromosomes

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A lethal contagious disease, Devil Facial Tumour Disease (DFTD) is ravaging the population of Tasmanian devils, *Sarcophilus harrisii*. The cells of this cancerous disease themselves act as the infectious agent, and are spread through the devil's social biting interactions. Although the devil's immune systems should reject these foreign infectious cancer cells through processes such as cell self-recognition by MHC detection, DFTD has evolved to evade such immune responses and is able to freely infect devils. This is believed to be, in part, driven by aberrant epigenetic changes within DFTD cells. Given its ability to propagate, DFTD is able to, and has been, evolving since its emergence last century. One such feature of its evolution is its ongoing DNA demethylation. However, it is not known if these demethylation changes occur globally across the genome or are more locally targeted; this could have varying implications for the evolution of DFTD and the stability of the cell line. Using immunostaining and immunofluorescence microscopy, in tandem with gene mapping techniques, this project will examine what regions of the genome are targeted by DNA demethylation, and will further extend this study to various other epigenetic markers, such as certain histone modifications, to identify ongoing epigenetic changes within DFTD cells and whether they are globally or locally targeted.

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Varroa, viral vectors and virulence: the evolution of honeybee diseases.

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Sometime in the 20th century, the *Varroa* mite switched hosts from the Eastern to the Western honeybee, with disastrous consequences. *Varroa's* spread into American and European bee populations was accompanied by outbreaks of viral disease, which often lead to colony collapse. Intriguingly, there are no viruses unique to *Varroa*-infested colonies. Instead, the mite's presence appears to drive the evolution of higher levels of virulence in ordinarily benign pathogens, which *Varroa* carries between bees as it feeds on their haemolymph. These observations match the theoretical prediction that the emergence of an arthropod vector should select for higher levels of virulence in a parasite, by reducing the parasite's dependence on the host for transmission.

Varroa has not yet arrived in Australia, but the majority of its associated viruses are present in benign form. Whether the arrival of the mite will foment outbreaks of virulent diseases remains an open question of considerable interest. Moreover, the theoretical prediction that vector-based transmission selects for higher levels of virulence lacks solid empirical support. Australia's status as the last remaining large *Varroa*-free landmass affords a unique opportunity to answer these questions. We are currently using a serial injection procedure to manually transfer benign Australian virus strains between bees. We hope to imitate the selection pressures exerted by vector-based transmission and track the resulting evolution of virulence, to better understand how the introduction of a vector can change the viral landscape of Australian honey bees.

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The cross-species use of high-density SNP genotyping arrays for kinship analysis in threatened equid species.

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Single nucleotide polymorphisms (SNPs) are the most widespread form of sequence variation encountered in genomes, and they hold great promise for elucidating the genetic characteristics mammalian populations (Morin et al, 2004). However, the difficulty and expense associated with SNP discovery in non-model species has hampered their wider implementation in conservation projects. Rapid advances in sequencing and genotyping technologies means large numbers of SNPs can now be interrogated simultaneously for relatively low cost in numerous model and domestic organisms, including the domestic horse (Equus caballus), using whole-genome genotyping arrays (Steemers et al, 2006). The application of SNP genotyping platforms across species boundaries presents a simple route to marker discovery in taxa sharing a recent evolutionary history. In this way genome-wide markers may be readily ascertained in non-model species, and high-quality genotyping data may be generated cheaply and efficiently in many individuals (Ogden et al, 2012). Samples from the order Perissodactyla (n=106) have been genotyped on the EquineSNP50 and SNP70 Beadchips. We identified 3028 domestic horse SNP sites (4.1% of sites interrogated) genotyping as polymorphic within at least one non-horse Perissodactyla species. A case study investigating the power of these newly discovered SNPs to predict kinship and parentage was undertaken in two endangered equid species. Przewalski's horse (Equus przewalskii) and Persian onager (Equus hemionus onager) samples with known ancestry were genotyped to evaluate the correlation between marker and pedigree-based estimates of relationship. A pruned set of informative SNPs revealed a strong correlation between molecular and genealogical kinship for Persian onager (r =0.85-0.96). More variable correlations were reported for Przewalski's horse (r =0.50-0.92). Simulations suggest as few as 50 highly polymorphic SNPs will provide sufficient power for paternity assignment with 95% confidence in both species. These results indicate a relatively small panel of SNP markers could be used to clarify pedigree relationships and parentage, providing a valuable resource for zoological institutions managing captive populations.

1. Morin, PA, Luikart, G, Wayne, RK, and the SNP workshop group 2004, 'SNPs in ecology, evolution, and conservation', Trends in Ecology and Evolution, vol.19, no.4.
2. Steemers, FJ, Chang, W, Lee, G, Barker, DL, Shen, R, Gunderson, KL 2006, 'Whole genome genotyping with the single-base extension assay', Nature Methods, vol.3, no.1.
3. Ogden, R, Baird, J, Senn, H, McEwing, R 2012, 'The use of cross-species genome-wide arrays to discover SNP markers for conservation genetics: a case study from Arabian and scimitar-horned oryx', Conservation Genetics Resources, vol.4.

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Detection of *de novo* mutations in parent to offspring trios in whole genome sequences of the domestic dog

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Genetic variations underpin the evolution of all living organisms and conversely the occurrence of Mendelian and complex genetic diseases. Through understanding of rate, type and origin of new mutations, interesting features of an organism's biology can be revealed. Many facets of the characterisation of *de novo* mutations in domestic dogs have yet to be explored. With the increased accessibility of next generation sequencing, assessing the whole genomes of parent to offspring trios for inherited (germline) mutations has become feasible. We will do this using 100 base paired-end Illumina HiSeq 2000 sequences with an expected genomic coverage of around 7.5 fold. The sequencing reads will be aligned to the most recent canine reference genome (CanFam3) using Burrows-Wheeler Aligner (BWA), SAMtools and the Genome Analysis Toolkit (GATK) in a best practice alignment protocol. After removal of PCR duplicates and local realignment around insertions and deletions we will call variants, undertake quality control of possible sequencing and alignment errors to exclude artifacts, categorize new mutation types for each trio set, phase haplotypes and validate the haplotype calls and mutation observations through polymerase chain reaction in the laboratory. At this time we are developing the analysis pipeline and refining it by testing various in-house and open source programs while additional samples are being gathered.

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Detection of cerebellar abiotrophy mutations in Australian Working Kelpie dogs using whole genome sequencing

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The Kelpie is an Australian derived dog breed developed for herding. Cerebellar abiotrophy (CA) is a movement disorder which results in early onset ataxia, and was first documented in Australian Kelpie (AK) and Australian Working Kelpie (AWK) dog breeds in 1989. The cerebellum controls movement and coordination and is affected in CA by a loss or failure of development of cerebellar Purkinje and granular cells, but with a degree of variation in severity. A genome-wide analysis study has mapped CA in working Kelpies to six associated regions across 5 chromosomes: 3, 22, 34, 35 and X, containing eight putative candidate genes. These results suggest that CA in the AWK is a Mendelian disorder with a complex genetic basis. Analysis of genome sequences from one CA affected and five unaffected Kelpies identified 12 protein-coding, and 12 regulatory single nucleotide polymorphisms (SNP) within these candidate genes. Confirmation of these variants and determination of allele frequencies in a larger number of cases and healthy control dogs is underway. Further work will involve sequencing more CA

affected working Kelpies employing the 100 base paired-end Illumina HiSeq 2000 sequencing, to identify additional SNP and potential insertion-deletion events.

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Sneaky queen bees selectively detect and infiltrate queenless colonies

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Insect societies are characterized by advanced cooperation, but at the same time the complexity of their colonies renders them susceptible to reproductive parasitism. Recently, a genetic study on the Brazilian stingless bee *Melipona scutellaris* showed that unrelated queens frequently invade and take over colonies in which the mother queen had died. In the present study, we investigated this phenomenon using microsatellite markers and radio frequency identification tags. We confirmed that alien queen take-overs are common within this species, and demonstrated that mated queens actively seek out colonies without a queen to reproduce in. Furthermore, we found that queens only penetrate their target colonies in the evening, when guarding efficiency is significantly reduced. We hypothesize that this strategy reduces the chance of the queens being attacked by entrance guards, thus maximizing their chance of successful infiltration.

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DNA Identification of Rhinoceros Horn

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Rhino numbers have dwindled over the past century; 3 of the 5 species are considered critically endangered, 1 species is vulnerable and 1 species is near threatened[1,8]. Much research has been conducted on the possible solutions in managing rhino populations. Poaching is found to be the biggest threat to rhinos and it has increased dramatically over the past decade due to the growing demand in Asia[8]. The numerous factors of poaching must be managed – this includes DNA forensics. The first part of the project will be to design a simple and reliable DNA test which can identify the species a rhino horn belongs to; this includes rhino horn products such as horn powder, dagger handles etc. A seized horn sample could then undergo a simple species ID test that will provide fast and reliable results[2]. Previous research has already identified regions of DNA that can be utilized to identify the species of the sample[6,7]. One of the Australian Museum's roles is to assist customs departments in the identification of confiscated items. This species ID test will give the museum a means to achieve this and will allow the persecutors of these seized horns to be charged accordingly as different charges may be handed down depending on what species of rhino horn has been seized. This test will also provide an insight into what species are being poached; management of poaching can consequently be improved.
The next aspect of the project will consist of individualization research; this involves identifying a rhino horn on an individual basis. This would permit a rhino horn to be matched up with the rhino specimen it was taken from[5]. It will also give an insight into the genetic diversity within and between rhino populations. Genetic diversity data allows informative decisions in the management of conservation units and breeding programs[3,4,11]. The project will involve designing improved microsatellites that can be added to the existing sets of microsatellites for rhinos[5,9,10]. These existing sets of markers are predominantly made up of di-nucleotide repeats which have a large stutter (~40%) making them quite unreliable. Improved microsatellites will include tri- and tetra-microsatellites which are much more reliable and hence will improve individualization analysis.

1. Amin, R., et al. (2006). "An overview of the conservation status of and threats to rhinoceros species in the wild." International Zoo Yearbook 40(1): 96-117.
2. Cooke, G., et al. (2012). "A rapid molecular method to detect the invasive golden apple snail Pomacea canaliculata (Lamarck, 1822)." Conservation Genetics Resources 4(3): 591-593.
3. de Groot, P. J. V. C., et al. (2011). "Conservation genetics of the black rhinoceros, Diceros bicornis bicornis, in Namibia." Conservation Genetics 12(3): 783-792.
4. Fernando, P., et al. (2006). "Genetic diversity, phylogeny and conservation of the Javan rhinoceros (Rhinoceros sondaicus)." Conservation Genetics 7(3): 439-448.
5. Harper, C. K., et al. (2013). "Extraction of nuclear DNA from rhinoceros horn and characterization of DNA profiling systems for white (Ceratotherium simum) and black (Diceros bicornis) rhinoceros." Forensic Science International: Genetics 7(4): 428-433.
6. Hsieh, H.-M., et al. (2003). "Species identification of rhinoceros horns using the cytochrome b gene." Forensic Science International 136(1): 1-11.
7. Linacre, A. and S. S. Tobe (2011). "An overview to the investigative approach to species testing in wildlife forensic science." Investigative genetics 2(1): 2.
8. Milliken, T. and J. Shaw (2012). The South Africa - Viet Nam Rhino Horn Trade Nexus: A deadly combination of institutional lapses, corrupt wildlife industry proffessionals and Asian crime syndicates. Johannesburg, South Africa, TRAFFIC.
9. Nielsen, L., et al. (2008). "Characterization of microsatellite loci in the black rhinoceros (Diceros bicornis) and white rhinoceros (Ceratotherium simum): their use for cross-species amplification and differentiation between the two species." Conservation Genetics 9(1): 239-242.
10. Scott, C. A. (2008). "Microsatellite variability in four contemporary rhinoceros species: implications for conservation."

11. Willerslev, E., et al. (2009). "Analysis of complete mitochondrial genomes from extinct and extant rhinoceroses reveals lack of phylogenetic resolution." BMC evolutionary biology 9(1): 95.

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Unexpected gene-flow patterns highlight importance of peripheral populations of the world's smallest penguin

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Little Penguins, the smallest member of the Penguin family, are endemic to Australia and New Zealand and are threatened by urbanisation and climate change. Human disturbance and the introduction of feral pests and invasive weeds have resulted in habitat reduction throughout the Little Penguin's range, restricting most extant populations to coastal islands free of predators and large human settlement. In Western Australia, the population at Penguin Island, in the Perth metropolitan region, is the northernmost extent of the Little Penguin distribution and hosts the largest colony of Little Penguins in WA. Predictions that rapid environmental and climatic changes will occur over the next century[2] coupled with expanding urbanisation in the region make it critical to gain an understanding of Little Penguin range limitations so that we are better able to predict future biodiversity outcomes. In this study we performed a fine-scale population genetic analysis of little penguins in Western Australia in order to understand the genetic status and conservation value of the Penguin Island population. Source populations located near Esperance WA were identified by multilocus and mtDNA genetic analyses supporting evidence that little penguins originated in the south[1,3] and small founder populations expanded northward to settle the Perth region. Contrary to our expectations genetic estimates of dispersal between Perth populations at the periphery of the little penguin distribution and south coast Western Australian populations near the core of little penguin distribution revealed biased dispersal with greater dispersal from Perth to the south. One plausible explanation is that gene flow is now restricted by oceanographic currents and could be affected by increasing sea surface temperatures and ENSO events. Furthermore, at Penguin Island, mortality and reproductive success appear to be influenced by ENSO events[4]. These results indicate that conservation of the Penguin Island colony depends largely on ensuring management practices address factors influenced by climate variability and connectivity with neighbouring penguin colonies.

1. Banks JC, Mitchell AD, Waas JR, Paterson AM (2002) An unexpected pattern of molecular divergence within the blue penguin (Eudyptula minor) complex. Notornis, 49, 29-38.
2. IPCC (2013) Climate Change 2013: The Physical Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA.
3. Peucker AJ, Dann P, Burridge CP (2009) Range-wide phylogeography of the Little Penguin (Eudyptula minor): evidence of long-distance dispersal. The Auk, 126, 397-408.
4. Cannell, Belinda L., Chambers, Lynda E., Wooller, Ron D., and Bradley, J. Stuart (2012). Poorer breeding by little penguins near Perth, Western Australia is correlated with above average sea surface temperatures and a stronger Leeuwin Current. Marine and Freshwater Research 63, 914–925.

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Genomic architecture and repeatability of rapid local adaptation

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In the light of recent and ongoing anthropogenic disturbance it becomes increasingly important to understand how species respond and adapt to environmental change. However, the genomic basis underlying rapid local adaptation still remains largely unclear. Recent advances in sequencing technology make it possible and affordable to preform large-scale analyses in non-model species. Invasive species are able to thrive in novel environments and are thus an excellent system to study rapid local adaptation. During this project we aim to gain insight into adaptive changes in genomic architecture and the repeatability of these changes. We will use the invasive plant annual ragweed (*Ambrosia artemisiifolia*) as a study system. This species is native to North America, but has been introduced to Europe and Australia. We have collected samples along a latitudinal gradient in North America, Europe and Australia. We have genotyped 384 individuals from Europe and North America using genotype-by-sequencing (GBS) and genotypes from Australia will be forthcoming. We have used these data to uncover the population structure and re-construct invasion history in the introduced range. Following this, we will use common gardens experiments and SNP genotyping to perform allele-trait and environment-allele associations. By examining the adaptive genomic diversity within and between native and introduced populations, we hope to improve our understanding of the genomic architecture of adaptation. Results from this study will enrich our comprehension of fundamental evolutionary processes, as well as providing a tool for predicting species responses to environmental change.

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Effects of Larval Competition on the Fitness of *Wolbachia*-Infected *Aedes aegypti* (Diptera : Culicidae)

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Wolbachia are intracellular endosymbionts that may spread throughout populations by manipulating the reproduction of their hosts. The principal vector of the dengue virus, the mosquito *Aedes aegypti*, is not naturally infected with *Wolbachia*, but it has been transinfected experimentally. A recent discovery that *Wolbachia* suppresses dengue virus replication in the mosquito has driven investigation into its application as a biological dengue control agent. Successful implementation of this strategy may eliminate the need for mosquito eradication to control the spread of the disease.

In order for *Wolbachia* to invade target populations and supress dengue transmission, infected mosquitoes must be competitive with the native inhabitants. *Wolbachia* impose fitness costs on the mosquito which decrease the likelihood of the infection reaching fixation in a population. Consequences of *Wolbachia* infection are well understood in the adult host. However, during field releases, *Wolbachia*-infected mosquitoes are likely to encounter fierce competition with wild-type mosquitoes in larval habitats. The effects of crowding and competition on *Wolbachia*-infected larvae are therefore important to understand so that the potential for *Wolbachia* to invade field populations can be assessed.

We tested for effects of two *Wolbachia* infections, *w*Mel and *w*MelPop, on the fitness of immature *Ae. aegypti* when developing under competitive conditions. Development of *Wolbachia*-infected larvae is delayed when competing in mixed cohorts with uninfected larvae. *w*MelPop-infected adults that develop slowly are smaller relative to uninfected, and have a shorter lifespan and a greater density of infection relative to faster developers. Adult females infected with either *Wolbachia* strain also have reduced fertility compared to uninfected females. These costs to fitness under larval competition may limit the potential for *Wolbachia* infections, particularly *w*MelPop, to invade areas where larval habitats are limiting. These results have implications for the use of *Wolbachia* as a biological control agent in areas where dengue is endemic.

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Bioinformatics Resource Australia – EMBL: Data Integration

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Bioinformatics capability is crucial to all life science research. To exploit the tools of bioinformatics, scientists need services that bring together data, software, hardware and expertise. As part of EMBL Australia's mandate to further Australian interests in global research, the BRAEMBL Data Integration Team has been established to assist Australian scientists integrate their molecular data into the global data infrastructure and to have better access to data, tools and services developed outside Australia.

The European Bioinformatics Institute's (EBI) data resources, in particular the data archives, face a number of problems when serving Australian users that relate to geographical separation (especially in network latency and challenging time zones). Beyond an interest in serving Australian scientists, the archives require the inclusion of Australian data to build and maintain comprehensive coverage across diverse research fields.

The Data Integration Team can assist Australian researchers with handling and collating experimental metadata; submission of array and NGS data to public repositories (including reads, assemblies and annotations); downstream analyses including the EBI Metagenomics Pipeline and the Ensembl Genebuild pipeline; batch submission of large datasets; and other EBI services as required. The aim of the BRAEMBL Data Integration team is to give researchers more time to focus on research, have data submitted to the highest community standards and in so doing, increase utility of these data to the international research community.

The European Bioinformatics Institute (EBI) is one of very few major centres in the world that provides data and services to support bioinformatics. The EBI is a part of the European Molecular Biology Laboratory (EMBL), and, with Australia's membership of EMBL and the creation of EMBL Australia, a natural collaborator in providing for the bioinformatics needs of Australia.

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Factors shaping disease-resistance-gene diversity in an Australian reptile

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It is important to understand wildlife disease and the mechanisms underlying disease resistance because disease is among the top five causes of species extinctions world-wide. The ability to combat disease is largely controlled by diversity at key disease-resistance-genes, such as those of the Major Histocompatibility Complex (MHC). Many genes within the MHC play a central role in pathogen recognition and are therefore one of the most important genes involved in disease resistance. Higher diversity at MHC genes equates to a greater range of pathogens that the immune system can identify and act upon. As a result, MHC genes are extremely valuable markers for population health and adaptive potential in conservation biology. However, knowledge on diversity at genes influencing disease resistance, and the mechanisms generating and maintaining this diversity, is conspicuously lacking in reptiles. As Australia has an extremely high diversity of reptiles this is a critical issue for wildlife conservation in this country. Given their role in the immune system, pathogen-mediated selection is considered to be the main selective force maintaining diversity at MHC genes. However, sexual selection can also play a role through non-random mating associated with external cues like pheromones or ornaments that signal MHC gene diversity. In some cases sexual selection may even have more influence on MHC gene diversity than pathogen-mediated selection. How diverse are dragon lizard MHC genes and which selective forces play a role in maintaining their diversity? We aim to address these gaps in the literature using an Australian dragon lizard and disease-resistance-genes of the MHC as a model system.

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Comparative analyses of Complement genes in Crocodilians

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Complement genes, a component of the innate immune response, consists of five major gene families which encode for distinct plasma proteins. These are involved in cell lysis and initiation of phagocytosis by opsonisation of pathogens and induction of the immune system to trigger processes leading to inflammation. Crocodilians are considered to be a good model to investigate the evolution and diversity of the immune system. One of the reasons is that these taxa appear to have an effective innate immune system allowing them to cope with a variety of pathogenic challenges due to common injuries sustained during aggressive displays as well as pathogen pressures in certain environments such as stagnant waterholes but usually they do not show signs of infection. Complement genes appear to be relatively conserved among higher vertebrates while that of fish and other poikilothermic vertebrates, which include crocodilians, have been suggested to be more diverse. However, there is limited knowledge of this system in crocodilians as a basis for definitive conclusions. To address this, we analysed and compared the Complement genes from genome sequence resources of three species of crocodilians (Australian saltwater crocodile, American alligator and Indian gharial). Overall, we re-annotated 13 genes and found a high degree of gene conservation when compared with other higher vertebrates. In addition, an unexpected level of polymorphism including non-synonymous substitutions was found among species. To further investigate these polymorphisms, some Complement genes are being surveyed in 20 species of crocodilians. This study will give some understanding as to whether such diversity may have expanded the innate capacity for immune recognition and response in crocodilians.

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Geneious R7: A bioinformatics platform for biologists

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Geneious R7 is an highly-cited bioinformatic software platform that allows researchers the command of industry-leading algorithms and applications for their genomic & protein sequence analyses via a single robust desktop program. Researchers can easily query, manage, share, and analyze all of their sequence data intuitively within a user-friendly interface. The R7 platform provides tools for next-generation sequence analysis, chromatogram assembly, sequence alignment, phylogenetics, primer design, molecular cloning and microsatellite analyses, and includes the ability to make custom workflows to automate analysis pipelines. It includes plugins for popular tree-builders such as PHYML, RAxML, FastTree, Garli and MrBayes, and NGS assembly algorithms Velvet, Bowtie2 and Tophat. Researchers can also create their own plugins to extend the software using a freely available plugin development kit. With Geneious, researchers spend less time learning difficult command-line interfaces and spend more time on solving deeper research questions.

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De novo assembly of circular genomes using Geneious R7.

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Circular chromosomes or genomes, such as viruses, bacteria, mitochondria and plasmids, are a common occurrence in nature. However, most de novo assemblers are unaware genomes can be circular and produce linear sequences with an arbitrarily defined start and end. This can result in repeated sections of sequence at the arbitrary start and end points, and an artificial drop in coverage in these regions which can affect downstream analyses. The Geneious de novo assembler is, to our knowledge, the only de novo assembler which produces circular contigs during the assembly process. This algorithm uses an overlap based approach to merge sequence reads and contigs together, where at each step the most similar contigs or sequences are merged. The circularize option allows similar sequences and contigs to circularize both *during* and at the end of the assembly process. In this study we test the Geneious de novo assembler on an Ion Torrent mitochondrial dataset from *Pathero leo persica (*Asiatic lion), and a whole genome shotgun dataset for *Pan troglodytes* (chimpanzee) produced from Illumina sequencing, and compare the results with those obtained from popular freely available de novo assemblers Velvet, MIRA and Spades. With both test datasets the Geneious de novo assembler was able to return a single circular contig representing the mitochondrial genome, a significant improvement on assemblies produced by the other assemblers. The circular contigs mapped with high concordance to the published genome and enabled the resolution of difficult to assemble repetitive regions.

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Characterisation and comparative analyses of the saltwater crocodile MHC

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The major histocompatibility complex (MHC) is a dynamic genome region with an essential role in the adaptive immunity of vertebrates, in particular self-recognition and protection against disease. The MHC is generally divided into classes I, II and III, which are subregions containing genes of similar function across many species, but different gene number and organisation. Although crocodilians occupy the evolutionary mid-point between mammals and birds, thus providing a unique evolutionary link between these groups, research on the MHC genomic region within this lineage has been relatively unexplored. To address this, we characterised the MHC region of the saltwater crocodile by screening, sequencing of BACs and then comparing the scaffolds from these with genome sequence data from the American alligator and Indian Gharial as well as other vertebrates. Six MHC regions from BACS spanning ~452 kb were identified as containing nine MHC class I, six MHC class II, four TAP and TRIM genes, and a single actin pseudogene. These MHC class I and class II genes were greater in length than their counterparts in the chicken *B* locus (2.5-11 times) suggesting that the compaction of avian MHC occurred after the crocodilian-avian split. Comparative analyses of the saltwater crocodile scaffolds showed separate regions for MHC class I and II and, when compared to two other genomes from the alligator and gharial, there were large syntenic areas among them (> 80% identity) with similar gene order. The close proximity of MHC class I and TAP in the saltwater crocodile suggests an ancestral structure of tetrapod MHC, while the linkage between MHC class I and TRIM39 observed in this species is consistent with newly-rearranged structure in human MHC. This organisation has not been observed in birds suggesting that rearrangement occurred after the divergence of crocodilians and birds from the common ancestor ~240 million years ago. These findings support instability of the saltwater crocodile MHC that differs from that expected in tetrapod ancestors.

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Functional Role of IMMP2L in Astrocytes and its Implication in Tourette Syndrome

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Gilles de la Tourette syndrome (TS) is a complex neuropsychiatric disorder characterized by the presence of multiple, involuntary motor and vocal tics that fluctuate in severity. The disorder is associated with a high prevalence of comorbid disorders including Autism Spectrum Disorder (ASD). Its high heritability and largely unknown genetic etiology has motivated intense research towards identifying the genetic basis of the disorder. Documentation of chromosomal abnormalities, linkage studies and candidate gene association studies have so far been able to implicate several disorder-associated genes with most evidence supporting *SLITRK1*, *IMMP2L*, *CNTNAP2*, *NLGN4X* and *HDC.* CNTNAP2, NLGN4X and SLITRK1 have been implicated in synaptic dysfunction due to the location of the proteins, which function at the neuronal synapse. IMMP2L is located on the mitochondrial inner membrane and has an incompletely characterized function. To date, little evidence has been found associating *IMMP2L* with a principal functional role in astrocytes.

The *LRRN3* gene, which encodes a leucine-rich repeat protein is transcribed anti-sense to the third intron of *IMMP2L*. Expression of *LRRN3* is enriched within the brain and there is evidence for the association of *LRRN3* with ASD. Consequently the functional relationship between the nested genes may have a significant implication on the understanding of TS and ASD.

Here we investigated the role of *IMMP2L* in mitochondrial function in human primary astrocytes and whether transcription through *IMMP2L* regulates *LRRN3* expression.

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Genome-wide SNP and population genetics of platypuses from across Australia

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Although the platypus, *Ornithorhynchus anatinus*, is regarded as a single species, there is morphological, behavioural and genetic variation across its geographical distribution. Recent mitochondrial and nuclear DNA studies have revealed at least three evolutionarily significant units within the species. However, it is unclear whether the genetic structures and differences between the documented evolutionary lineages have resulted in gene and/or genome-wide variations which could provide insight as to selection. Here we present a collaborative research project plan between two Australian universities and the University of Oxford to sequence the genome of platypuses from across Australia representing the documented evolutionary lineages. This population analysis study is one part of a large project which will address several other fundamental questions on monotreme genome biology and evolution. We aim to whole-genome sequence 40-50 animals from Queensland, New South Wales, Victoria and Tasmania, some of whom have already been sequenced. The datasets will be aligned and mapped for population genetic analyses and selection tests. Our data will provide valuable insights on population structure, history and genes that may have undergone selection.

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European and Asian contribution to the genetic diversity of South American chickens

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In recent years the debate regarding possible pre-Columbian introduction of chickens to South America has attracted much attention. A link between ancient Polynesian chickens and those found in a pre-Columbian archaeological site (El Arenal 1) and a modern population (the Mapuche Fowl) in Chile has been proposed based on a shared mtDNA lineage. However, this conclusion has been challenged because the specific lineage (haplotype E1) used to support the genetic connection is found worldwide, and is not specific to Polynesian chickens. Additional evidence of a contemporary Pacific mtDNA chicken profile in an early post-European Peruvian specimen has also been suggested to support pre-Columbian contact. To assess whether modern South American chickens contain ancient Polynesian and/or contemporary Pacific mtDNA profiles, we analysed the mtDNA control region of 229 village and Creole chickens from Chile, Peru, Colombia and Brazil. We compared the results with a dataset of approximately 3,600 sequences of ancient and modern specimens from across the world. Overall, we found that mtDNA profiles in modern South American chickens are related to those found in Asian and European populations, but neither modern Pacific nor ancient Polynesian mtDNA signatures were present in these populations. We will discuss different scenarios to explain these results together with some preliminary analyses of ancient chicken bones from the El Arenal 1 site.

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Ribosomal Protein Mutants Affect Female Fertility in Arabidopsis thaliana

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The eukaryotic cytoplasmic ribosome is essential for protein synthesis and is therefore vital for the development and survival of organisms. The ribosome is made up of 4 rRNAs and approximately 80 ribosomal proteins. Developmental phenotypes of ribosomal protein mutants in plants and animals suggest ribosomal proteins and the ribosome regulate developmental processes, although how this occurs is not known. In Arabidopsis thaliana, cytoplasmic ribosomal proteins are encoded by small gene families containing between two and five paralogs of the ribosomal protein gene. One such gene family, coding for RPL24, contains two ribosomal protein genes, RPL24A and RPL24B. Mutations in the RPL24B gene have been shown to affect female fertility in Arabidopsis by reducing the number of viable ovules. We show that the defective ovules present in the rpl24b mutant display arrested germ-line development. However, genetic analysis indicates that it is the genotype of somatic non-reproductive cells affecting fertility. We propose that RPL24 is involved in signalling between somatic and reproductive cells and that maintenance of this signalling is essential for fertility. Further understanding of the role of ribosomal proteins in fertility and identification of the inter-tissue signal that affects fertility may enable manipulation of plant sterility or induction of apomixis, which would be of major importance to agriculture.

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Population viability and major histocompatibility complex (MHC) genetic diversity of two dolphin populations in Western Australia

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Genetic diversity is considered essential for populations to adapt to a changing environment. Measures of genetic diversity to evaluate how isolated, inbred and viable a population is, are typically based on various neutral markers, such as microsatellites or mitochondrial DNA control regions. However, genetic diversity to guide conservation management is better reflected by coding regions of functionally important genetic loci, such as the major histocompatibility complex (MHC) genes. In this study we assessed population viability and MHC diversity of two bottlenose dolphin (*Tursiops cf. aduncus*) populations in Western Australia. From demographic data, the larger Shark Bay population appears to be stable, whereas the smaller Bunbury population was forecast to decline. Furthermore, we found the more viable Shark Bay population to be more genetically diverse for at least one (MHC II, DQB, exon 2) of the three MHC loci that we investigated. Our findings are consistent with the hypothesis that large, viable populations typically display greater genetic diversity compared to smaller, less viable populations. A larger population, such as the Shark Bay dolphin population, is thus potentially more robust to natural or human-induced changes to coastal ecosystems it inhabits across Australasia.

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Bioinformatics Infrastructure and Training Resources in Australia

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Over recent years, bioinformatics has developed into a key underpinning technology for many, if not most, fields of biological research. As such, it is more important than ever for research biologists to be aware of and have access to a broad range of bioinformatics resources. Often though, researchers are faced with two major obstacles in achieving this: namely a lack of expertise and knowledge in the application of bioinformatics, and subsequently the difficulty in accessing suitable high-end computing infrastructure on which to carry out bioinformatics analysis.

To address these challenges, a number of projects and initiatives have been launched in Australia to provide training, support and access to infrastructure and expertise in bioinformatics. These range from tutorials and systems to enable a researcher to carry out a full bioinformatics analysis by themselves, to groups who offer a complete hands-off analysis service. In this poster, I will describe some of the major resources available, what services they offer, and how researchers can access them.

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Gone with the wind? A systematic revision and biogeographic treatment of *Logania* R.Br. (Loganiaceae)

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The evolutionary relationships among taxa, and how their present geographic distributions came to be, are both prominent areas of biological research. Studies of these topics fall within the fields of systematics and biogeography. *Logania* R.Br. are morphologically variable plants that are near-endemic to Australia, with 37 species divided between two infrageneric sections, *L.* sect. *Logania* and *L.* sect. *Stomandra*. Preliminary studies have questioned the monophyly of *Logania*, and the genushas a disjunct distribution corresponding to a gap at the Nullarbor Plain. Therefore,*Logania* are favourable candidates to gain an insight into phylogenetic relationships and how these might intersect with Earth-history events.

We tested the monophyly of *Logania* and placed the genus in a broader evolutionary context using phylogenetic analyses of four molecular markers (*pet*D, *rps*16, *mat*K and *rbc*L). Two findings from these analyses rendered *Logania* non-monophyletic. We showed that *L. imbricata*, the only extant non-Australian *Logania*, belongs within *Geniostoma,* and that *L.* sect. *Stomandra* should be considered as a separate genus. Furthermore, we proposed two possible subgenera within *Logania sensu stricto*. Through biogeographical analyses, we found that disjunct distributions within *Logania* sect. *Stomandra* could have been caused by historical vicariant processes in the Nullarbor Plain region, but the distribution of *Logania* sect. *Logania* can only be explained by long-distance dispersal and establishment. As a result of this study, we have renamed *L. imbricata* to*Geniostoma imbricatum* and provided a formal taxonomic treatment re-classifying *L.* sect. *Stomandra* at genus level.

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Improving estimation of evolutionary timescales from multi-gene data sets using ClockstaR

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Molecular data can be used to estimate the evolutionary timescale of a group of taxa. This can be done using phylogenetic methods based on the molecular clock, a model that describes variation in evolutionary rates. The simplest model is the strict molecular clock, which posits that the rate is constant throughout the tree. This model has been rejected for many data sets, leading to the development of 'relaxed-clock' models that can account for rate variation among lineages.

The analysis of multi-gene data sets requires special consideration because the patterns of among-lineage rate variation can differ among genes. If this is the case, it is more appropriate to partition the genes according to their evolutionary patterns and to use a separate clock model for each group of genes. However, even in moderately-sized data sets, the number of possible partitioning schemes can be very large, so rigorous comparison of their statistical fit is computationally prohibitive.

We present ClockstaR, a method to select the optimal clock-model partitioning scheme. We show that using arbitrary partitioning schemes can result in misleading estimates of evolutionary timescales, a problem that can be avoided by carefully selecting the partitioning scheme with automated methods, such as ClockstaR.

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Genetic analysis of resistance to the insecticide indoxacarb in the diamondback moth (Plutella xylostella)

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The diamondback moth (DBM), Plutella xylostella, is a worldwide agricultural pest of crucifer crops. Populations of DBM have reportedly developed resistance to all major classes of insecticide and are often the first crop pest to develop resistance to new synthetic insecticides, making it not only a serious agricultural pest, but also an interesting model for the study of insecticide resistance mechanisms in arthropod pests. It is important to understand these mechanisms to aid in the development of integrated pest management and insecticide rotation programs.

DBM has become a serious pest of crucifer crops in Hawaii where it has developed a high frequency of resistance to indoxacarb, a synthetic oxadiazine insecticide. The genetic basis of resistance to indoxacarb is unknown. Using differential expression analysis of RNA sequence data, whole genome resequencing of resistant and susceptible individuals, as well as analysing genetic crosses, this project aims to provide a better understanding of the mechanism(s) of indoxacarb resistance in the highly-resistant Hawaiian 'Waipio' strain.