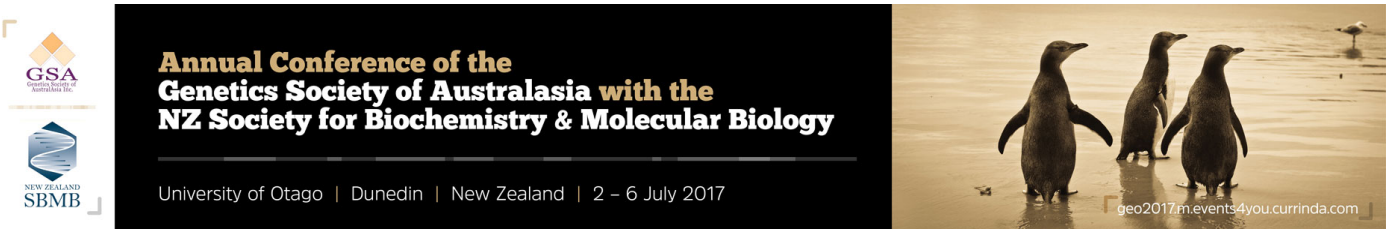


Abstract Book (excludes abstracts where authors have withheld consent to publish)

**id #621**

## Developmental genomics of sponges

**Maja Adamska**[1]

*1. Australian National University, Acton, ACT, Australia*

Sponges are likely to be the oldest of the extant animal lineages. In contrast to the eumetazoans (complex animals such as corals, insects and vertebrates), sponges have no nerves, muscles or gut. A decade ago, analysis of the first sequenced sponge genome (of the demosponge *Amphimedon*) demonstrated that development of the simple sponge body plan is governed by a limited number regulatory genes, suggesting a gradual assembly of the complex eumetazoan developmental toolkit.

However, sponges are a diverse phylum, composed of four distinct lineages: demosponges, hexactinellids, calcisponges and homoscleromorphs. We have sequenced genomes and transcriptomes of several calcisponges, including calcaroneans (*Sycon* and *Leucosolenia*) and calcineans (*Clathrina* and *Pericharax*), and a demosponge distantly related to *Amphimedon* (*Halisarca*). For some, we have also generated transcriptome datasets representing embryonic and postembryonic development and regeneration.

We have uncovered unexpected complexity and diversity of developmental toolkits among sponges, with calcisponge developmental regulatory gene families generally more complex than those in demosponges. Significant gene losses must have occurred independently in the calcisponge and demosponge lineages, followed by gene family expansions occurring independently in the calcaronean and calcinean lineages, and leading to spectacular developmental toolkit diversity among sponge species.

Gene expression analysis suggests deep conservation of body plan patterning and regeneration mechanisms between sponges and the eumetazoans. Strikingly, in the “expanded” gene families, sequence conservation correlates with similarity of gene expression profiles across the sponge species.

Overall, while sponges are clearly not “living fossils”, they provide a wonderful window into the evolutionary history of animal genomes.

**id #778**

## Predicting altered methylation patterns in early pre-eclampsia

**Suzan Almomani**[1], **Ian Morison**[1], **Noelyn Hung**[1], **Tania Slatter**[1], **Erin Macaulay**[1]

*1. Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand*

Pre-eclampsia is one of the most common adverse pregnancy conditions that complicates 5-10% of pregnancies worldwide, yet the underlying cause is unknown. The use of biomarkers to accurately identify women with an increased risk of developing pre-eclampsia would be a major step forward in antenatal care. Our lab previously performed genome-wide methylation sequencing to identify DNA methylation differences between dysfunctional pre-eclamptic placentas and matched healthy controls. Validation studies are currently being performed in an independent cohort to determine which of the identified methylation changes are representative features of pre-eclampsia. The early detection of methylation changes in pre-eclampsia would provide a feasible route to early diagnosis and potential intervention. We are currently determining whether our panel of candidate biomarkers for pre-eclampsia can be identified in the circulating cell-free placental DNA that comprises approximately 10% of a pregnant women’s blood plasma. We are performing deep sequencing using the MiSeq platform to examine the methylation of placental DNA in maternal plasma. We will measure the methylation of our candidate biomarkers in plasma samples from early gestation (15 and 20 weeks) and determine whether their differential methylation can be reliably profiled in maternal plasma from women who later developed pre-eclampsia. Ultimately, we seek to identify a DNA methylation signature of pre-eclampsia in maternal blood plasma that can be used clinically to predict women who are at risk of developing this threatening condition of pregnancy.

**id #762**

## Using DNA methylation to investigate a novel model for childhood acute lymphoblastic leukaemia

**Abdulmonem Alsaleh**[1], **Rob Weeks**[1], **Ian Morison**[1]

*1. Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand*

Childhood acute lymphoblastic leukaemia (ALL) is a disease that originates before birth from the proliferation of neoplastic lymphoid precursor cells in the bone marrow. Previously, we identified dense, biallelic methylation of the *TES* promoter as the most common molecular event in ALL being present in over 90% of B-ALL and 70% of T-ALL cases. We developed an array-based CpG region analysis pipeline (ABC.RAP) R package to analyse human DNA methylation array datasets. Exploring publicly available datasets, we found about 200 genes that are differentially methylated in ALL. The large number and consistency of the epigenetically modified genes raise the possibility that methylation-induced gene silencing in ALL may not be an acquired cancer-related phenomenon. We propose the presence of a distinct population of normal fetal lymphocytes that carry an epigenetic profile similar to that of ALL.

We developed an assay to detect methylated *TES* alleles using a deep targeted sequencing approach (MiSeq; Illumina). To date, we have examined blood samples from four neonates, and 10 cord blood samples. Remarkably, we detected the ALL-like methylation profile, i.e., *TES* methylated alleles, in one of the premature babies (3.2% of the CD19+ B cells of a four week-old, 28 week-gestation baby), and in three of

10 sequenced cord blood samples (1.3%, 0.16%, and 1.2% of CD19 negative cells). Importantly, methylation of *TES* has never been observed in normal adult blood. The next stage is to search for enrichment of the methylated *TES* alleles in purified stem cells, T cells and B cells.

**id #711**

## Genetic hitchhikers: what species are hiding in your sequencing data?

**Rachael Ashby[1, 2], Hayley Baird[1], Rudiger Brauning[1], Alan McCulloch[1], Chris Brown[3], Shannon Clarke[1], Neil Gemmell[2]**

*1. AgResearch, Invermay*

*2. Department of Anatomy, University of Otago, Dunedin*

*3. Department of Biochemistry, University of Otago, Dunedin*

High throughput sequencing is now routinely used for the generation of draft genomes, transcriptomes and high-density marker panels for non-model organisms. The demand for next generation sequencing (NGS) data in non-model species has also diversified the type of samples being sequenced, with wild samples and whole samples routinely collected, extracted and sequenced. The result is that vast quantities of data are being generated for species we know little about, often without any strong reference genome for comparison. Unfortunately, DNA extraction and NGS are not specific for the target organism; low levels of bacteria, virus and human genetic material are frequently identified and filtered through optimized bioinformatics pipelines. However in addition to these contaminants, genetic hitchhikers such as parasites, endophytes and commensal species can be sequenced along with the target species. These hitchhikers are often ignored or viewed as contamination, but we contend that this data could offer valuable insight into the target species and its environment. We support this view using data from the Greenshell™ Mussel, an endemic species of economic importance to the New Zealand aquaculture industry. We identify hitchhiker species present in the transcriptome and GBS data of this filter feeding species. We also show that these data, analysed appropriately, provide valuable insights into the mussel's biology. We argue that data from non-target organisms, once identified, should not always be discarded or ignored as contamination, and examine the potential applications for this data in other systems such as plant endophytes and insect parasites.

**id #630**

## Adaptive genomic divergence despite high gene flow in an Australian fish, golden perch (*Macquaria ambigua*)

**Catherine RM Attard[1], Chris J Brauer[1], Jonathan Sandoval-Castillo[1], Leanne K Faulks[1, 2], Peter Unmack[3], Dean M Gilligan[4], Luciano B Beheregaray[1]**

*1. Molecular Ecology Lab, Flinders University, Adelaide, SA, Australia*

*2. Sugadaira Montane Research Centre, University of Tsukuba, Nagano, Japan*

*3. Institute for Applied Ecology, University of Canberra, Canberra, ACT, Australia*

*4. Batemans Bay Fisheries Centre, New South Wales Department of Primary Industries, Batemans Bay, NSW, Australia*

**Background/Aims**

Populations that are adaptively divergent but maintain high gene flow may have greater resilience to environmental change because gene flow increases standing genetic variation and allows the spread of alleles that have already been tested elsewhere. We examined this possibility using golden perch (*Macquaria ambigua*) - a high dispersal, native freshwater fish of recreational importance - in the environmentally heterogeneous environment of the Murray-Darling Basin (MDB), Australia.

**Methods**

We developed a genome-wide SNP dataset using ddRAD for 171 golden perch in 13 sites across the MDB. We examined population structure, identified markers potentially under directional selection using genotype-environment association analyses for riverine flow, rainfall and temperature, and annotated identified candidates.

**Results**

We found high gene flow across the basin and three populations with low neutral differentiation. Genotype-environment association analyses detected adaptive divergence at, conservatively, 31 of the 3,139 filtered SNPs. These were predominately linked to an arid, environmentally extreme region with unstable rainfall, and candidate loci had functions involving fat storage, stress and molecular or tissue repair.

**Conclusions**

The high connectivity of golden perch in the MDB will likely allow adaptive traits in more extreme, hydroclimatically variable environments to spread and be selected in localities that are predicted to become environmentally unstable in future climates. Our study adds to growing evidence of adaptation in the face of gene flow, and highlights the importance of considering environmental disturbance and adaptive divergence in biodiversity management.

**Funding Sources**

ARC Future Fellowship (FT130101068) to L.B.B. and the associated Flinders University salary to C.R.M.A..

**id #782**

## Genotyping Brassicas: The need for a pangenome

**Jacqui Batley[1]**

*1. School of Plant Biology, University of Western Australia, Crawley, WA 6009, Australia*

The identification of genes underlying important quantitative trait loci is extremely challenging in complex genomes such as *Brassica napus* (canola, oilseed rape or rapeseed). Recent advances in next-generation sequencing (NGS) has enabled development of millions of SNPs. However, as an increasing number of genome sequences become available, there is a growing understanding that the genome of a single individual is insufficient to represent the gene diversity within a whole species. We have examined the SNP diversity within genes, and this allelic variation is an important source of phenotypic variation. However, we have observed significant gene presence absence variation and the impact of this variation on traits is only now being studied in detail. The sum of the genes for a species is termed the pangenome and the determination and characterisation of the pangenome is a requirement to understand variation within a species. We have developed Brassica pan genomes and using these the molecular analyses of candidate resistance genes using *B. napus* NGS data are presented, and the difficulties associated with identifying functional gene copies within the highly duplicated Brassica genome will be discussed.

**id #530**

## A genomewide investigation of susceptibility and resistance of bottlenose dolphins to cetacean morbillivirus

**Kimberley C Batley[2, 1], Jonathan Sandoval-castillo[2], Catherine CM Kemper[3], Catherine RM Attard[2, 1], Nikki Zanardo[2, 1], Ikuko Tomo[3], Luciano B Beheregaray[2], Luciana M Moller[2, 1]**

*1. Cetacean Ecology, Behaviour and Evolution Lab, Flinders University, Adelaide, SA, Australia*

*2. Molecular Ecology Laboratory, Flinders University, Adelaide, SA, Australia*

*3. South Australian Museum, Adelaide, SA, Australia*

**Background and aims**

Infectious agents have long been recognised as important factors influencing genetic variation associated with resistance and susceptibility to diseases. Cetacean morbillivirus (CeMV) is a highly contagious and deadly virus that is increasing in both geographic distribution and incidences. The largest mortality event in the Southern Hemisphere occurred in South Australia in 2013. It lasted approximately seven months and killed at least 41 southern Australian bottlenose dolphins (*Tursiops* sp.), predominately from the population in Gulf St Vincent. Here we aimed to identify candidate genes that may confer resistance or susceptibility to CeMV.

**Methods**

We conducted a genome-wide association study by generating double-digest restriction site-associated DNA (ddRAD) data for *case* (non-survivors) and *control* (putative survivors) bottlenose dolphins of this population. This included identifying single nucleotide polymorphisms (SNPs) with divergent allele frequencies between *case* and *control*, and then annotating the associated candidate genes.

**Results**

The dataset consisted of 262,892,901 filtered-sequence reads, from which 35,493 high quality SNPs were obtained. Association analyses found significant differentiation in allele frequencies amongst *case* and *control* dolphins at five SNPs. Annotation of these SNPs and associated flanking sequences resulted in two candidate genes (*MAPK8*, Mitogen-Activated Protein Kinase 8 and *INADL*, InaD-like protein annotation), which may be associated with stress response and CeMV immune response.

**Conclusions**

We identified two candidate genes that have strong evidence for association with resistance and susceptibility to CeMV. Biomarkers for these genes can now be developed to assess genetic variation in other cetacean populations affected by CeMV.

**id #513**

## Insecticide Metabolism - Control or Chaos?

**Philip Batterham[1], Felipe Martelli Soares da Silva[1], Wei-Kai Chen[1]**

*1. University of Melbourne, Parkville, VIC, Australia*

Insecticides are used globally to control insect pests that impact agriculture, human health and the welfare of domestic pets. The effectiveness of insecticides is diminished by the evolution of insecticide resistance, which often has a metabolic basis. In the literature there is an abundance of references to three phases of metabolism, mediated by metabolic enzymes such as Cytochrome P450s (Phase I) and Glutathione-s-tranferases (Phase II) and efflux proteins such as ABC transporters (Phase III). However there is not a single example of these enzymes and transporters forming a sequential detoxification pathway. At another level there are xenobiotic response pathways where, following induction, over a thousand genes change their levels of expression, but there is little evidence that these pathways are induced by insecticides or provide protection against them. Insect metabolic systems have been shaped for millennia by exposure to environmental toxins but, beyond some oft cited examples (nicotine and pyrethrum), the extent to which these toxins resemble current generation synthetic insecticides is not clear. Therefore we ask whether insecticide exposure is met with a controlled, evolved metabolic response or whether insect metabolism is thrown into chaos? We will discuss our work using genetic and metabolomic approaches to address this question in the model insect, *Drosophila melanogaster.*

**id #726**

## Australian rainbowfishes: a model system for ecological genomic studies of adaptation to climate change

**Luciano B Beheregaray[1], Louis Bernatchez[2], Jonathan Sandoval-Castillo[1], Chris Brauer[1], Catherine Attard[1], Peter Unmack[3], Steve Smith[4], Minami Sasaki[1], Katie Gates[1]**

*1. Flinders University, Adelaide, SA, Australia*

*2. Laval University, Quebec*

*3. University of Canberra, Canberra*

*4. University of Veterinary Medicine, Vienna*

Understanding whether natural populations will be able to adapt to rapid climatic change is a major research priority. We are using an evolutionarily young and ecologically important clade of eight rainbowfish species to understand adaptive resilience and to test predictions from the 'climatic variability hypothesis' for the major climatic regions of Australia. Here we present results from mechanistic transcriptomic studies in the lab and in the wild and from range-wide landscape genomic surveys for (i) a desert, (ii) a subtropical, and (iii) a temperate rainbowfish species (i.e. three major ecotypes). Our datasets include a whole *de novo* genome and three *de novo* transcriptomes (all annotated), transcriptomic (RNAseq) profiles for experimental and wild populations, phenotyping of adaptive traits, and genome-wide ddRAD SNPs for 51 populations / 1,020 individuals. Although the three species shared 80.9% of their 37,160 unigenes, climate change experiments revealed major differences in gene expression associated with future climates (only 2.1% of genes were shared between species). Transcriptomic profiles of wild populations along a latitudinal region indicate that candidate genes for high expression divergence (i.e. more variance among- compared to within-populations) correlate better with the environmental gradient than those showing high expression plasticity. Landscape genomics provided evidence for adaptive divergence associated with hydrological unpredictability (desert) and with major hydroclimatic gradients (temperate and subtropical), as well as for balancing selection in more variable environments (subtropical species). Rainbowfishes represent an ideal model system for clarifying climatic and geographic correlates of adaptation and for disentangling plastic from evolutionary responses to climate change.

Funding:ARC

**id #791**

## Drosophila to identify and unravel pathogenic mechanisms of human genetic diseases

**Hugo Bellen[1]**

*1. Baylor College of Medicine, Houston, TEXAS, United States*

High levels of Reactive Oxygen Species (ROS) cause neurodegeneration. We previously found that high levels of ROS lead to lipid droplets (LD) accumulation prior to neurodegeneration. These lipids are heavily peroxidated. We showed that increasing ROS in neurons alone will cause glial LD accumulation, suggesting that lipids are transferred from neurons to glia. We therefore explored the sources of energy and lipids that lead to LD accumulation in glia. We found that lactate is a critical source of energy for glial LD accumulation. Importantly, the lipids produced in neurons depend on fatty acid transport proteins and apolipoproteins for their transport and accumulation into glia. Surprisingly, human *APOE2* and *APOE3* alleles can functionally substitute for the fly apolipoprotein, *Glaz* in lipid transport. In contrast, *APOE4*, the most important Alzheimer's Disease susceptibility allele is severely impaired in lipid transport and acts as a loss-of-function mutation. We argue that *Apoe* is required for LD formation in glia and these LD play a protective role by scavenging ROS via peroxidated lipids.

**id #659**

## Genetic consequences of wildfire in an insular stand of the bird-pollinated, granite-outcrop endemic tree *Eucalyptus caesia*

**Nicole Bezemer**[1, 2]

*1. Centre of Excellence in Natural Resource Management, University of Western Australia, Albany, Western Australia, Australia*

*2. Botanic Gardens and Parks Authority, Kings Park and Botanic Garden, Perth, Western Australia, Australia*

In south-west Australia, granite outcrops support hyper-diverse plant communities and provide a refuge for fire-sensitive biota in an otherwise seasonally dry and fire-prone landscape. However, some granite plants require disturbances such as fire to trigger recruitment. Wildfires occur infrequently on granite outcrops, and the conservation status of many granite endemics makes experimental burns inappropriate. As such, opportunities to study the impact of fire on population genetics seldom arise. Following a wildfire in a small, isolated stand of the lignotuberous *Eucalyptus caesia* at Boyagin Reserve, I surveyed genetic diversity, recruitment and survival. The entire adult stand (n = 188) plus all seedlings located (n = 115) were mapped using a differential GPS and genotyped with 15 microsatellite loci. Despite temporary reduction of adults to 60 plants, there were no significant differences in adult heterozygosity pre- and post-fire. Conversely, there were marked differences in genetic variation between adults and seedlings, with reduced heterozygosity and increased fixation in the seedlings. Preliminary analyses do not support expectations of post-germination selection against homozygous progeny. Based on height measurements and survivorship, seedlings resulting from self-pollination could not be distinguished from outcrossed seedlings. Parentage analysis using CERVUS revealed mostly limited seed dispersal. By comparison, pollen movement was more extensive, yet restricted within the stand. Genetic mixing through wide pollen dispersal and occasional short-distance seed dispersal may buffer genetic decline in the study population. Alternatively, purging of deleterious alleles may preclude inbreeding depression. However, rare recruitment and high seedling mortality may diminish the genetic benefits of outcrossing or purging.

**id #622**

## Deciphering the role of DNA replication in human growth and development

**Louise Bicknell**[1]

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Mutations underlying Mendelian diseases provide a powerful resource to harness to understand cellular functioning and development. A particular focus of our research is on Meier-Gorlin syndrome (MGS), a rare autosomal recessive disorder characterised by extreme pre and postnatal growth retardation, as well as small ears, small kneecaps, breast agenesis and other mesenchymal abnormalities. Linkage analysis and exome sequencing led to our discovery of the first five disease genes. Three of these genes (ORC1, ORC4 and ORC6) encode subunits of the origin recognition complex (ORC), which bind across our genome at origins of DNA replication. Additional factors, including two further MGS genes, CDT1 and CDC6, interact with the ORC to recruit the MCM helicase complex. These origins are then considered licensed, to enable replication initiation during S phase. More recently, we have discovered mutations in an additional downstream member of the replication machinery, CDC45, underlies MGS. While mutations in CDC45 appear to be a relatively common cause of MGS, the majority of affected individuals also present with craniosynostosis (premature fusion of skull plates), phenotypically linking to abnormalities caused by disruption of another replication component, the CDC45-interacting protein RECQL4. The growth and mesenchymal developmental consequences of these “replication-opathies” are being explored using CRISPR-Cas9 editing in both stem cells and in a Xenopus laevis animal model.

**id #767**

## Understanding the genetic basis of mate choice in an iconic Australian species

**Parice A Brandies**[1], **Catherine E Grueber**[1, 2], **Jamie A Ivy**[2], **Carolyn J Hogg**[1, 3], **Katherine Belov**[1]

*1. School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia*

*2. San Diego Zoo Global, San Diego, CA, USA*

*3. Zoo and Aquarium Association Australasia, Mosman, NSW, Australia*

The koala (*Phascolarctos cinereus*) is an arboreal folivorous marsupial which forms the backbone of the Australian tourism industry. This iconic species is currently threatened by habitat destruction and removal, climatic induced changes, effects of urbanisation, and disease. While many studies have investigated the genetic basis of disease susceptibility in the koala, little is known about the role genetics may play in koala mate choice. Major Histocompatibility Complex (MHC) genes play an essential role in the adaptive immune response and have been found to influence mate choice in a wide range of organisms. Understanding whether this mate choice mechanism exists in the koala would assist captive breeding programs and help protect this vulnerable species. The aim of this study was to determine whether koalas exhibit MHC-dependent mate choice by genotyping a study population of koalas from San Diego Zoo at several MHC-linked microsatellite loci and correlating the genotypes with koala mate choice using detailed pairing records provided by the zoo. The results show that male koalas which are less heterozygous at MHC loci overall have a higher copulation success, however those that are heterozygous at MHCII DAB loci have a greater probability of producing offspring. Furthermore, females are more likely to breed with a male if they are more similar at MHCII DAB loci. These results demonstrate the complexity of the genetic basis of mate choice in koalas and may potentially aid future pairing recommendations in captive facilities.

**id #655**

## Bioinformatic discovery of noncoding RNA genes

**Christopher Brown[1], David Chyou[1], Ambarish Biswas[1], Patricia Baltazar[1], Thomas Nicholson[1], Bradley Croft[1], Peter Fineran[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Noncoding RNA (ncRNA) genes have been shown to have critical roles across all domains of life. We are interested in the structure and function of diverse ncRNA genes. These studies have included the computational discovery and accurate prediction of ncRNA genes, the RNAs they produce, the functions of these RNAs, and their evolution over short and long timeframes.

In a few model organisms many ncRNAs have been annotated on the genome sequence. However, for most genomes only a few types of RNAs (e.g. tRNA, rRNA) are automatically annotated by current pipelines (e.g. NCBI, JGI, Ensembl) with a few other classes inconsistently and poorly described. Major challenges in predicting ncRNA genes are that functional parts may be small and the evolutionary conservation is more difficult to detect than protein coding genes.

Most archaea and about half of bacteria use a recently discovered adaptive immune system that is ncRNA based. This CRISPR-Cas (Clustered regularly interspaced short palindromic repeats, and CRISPR associated proteins) system likely evolved as a defense against foreign genetic elements (notably viruses or bacteriophages). The CRISPR-Cas9 system has recently been repurposed as a precision genetic targeting tool in many organisms. We have developed a suite of tools to investigate the CRISPR adaptive immune system of prokaryotes (CRISPRSuite). The bioinformatic challenges of developing these tools and applying them to ~40,000 prokaryotic genomes will be described. Insights gained into the distribution, structure, function and and evolution of these systems will be presented.

**id #529**

## phylogenomics and species delimitation of pygmy perches: implications for biogeography, taxonomy and conservation

**Sean J Buckley[1], CR Attard[1], PJ Unmack[2], J Sandoval-Castillo[1], CJ Brauer[1], Luciano B Beheregaray[1]**

*1. Flinders University, Adelaide, SA, Australia*

*2. Institute for Applied Ecology, University of Canberra, Canberra, ACT, Australia*

**Background and aims**

Incongruences in the biogeography of aquatic taxa in southern Australia have existed due to the non-reciprocally monophyletic nature of the pygmy perches (Percichthyidae). In addition to their complex biogeographic history, pygmy perches also represent a threatened group with confusing taxonomy. However, the current understanding of their biogeography and phylogenetic relationships is based on limited genetic datasets.

**Methods**

We present the first study of the phylogenetic history of pygmy perches using genome-wide data. This work aims to reconstruct the phylogeny of pygmy perches and address biogeographic incongruences and cryptic diversification using a combination of phylogenomic approaches and a species delimitation framework. We also present a molecular clock and divergence time analysis, using the biogeographic barrier represented by the Nullarbor Plain as a calibration point.

**Results**

The overall topology and biogeographic patterns inferred by previous studies was supported with the genome-wide phylogeny obtained. It appears that the unique biogeographical patterns displayed by pygmy perches represent a biological reality, creating significant questions in the understanding of the biogeography of southern Australian freshwater organisms. Furthermore, the finding of previously unknown species identities demonstrate that the already threatened pygmy perches may be even more susceptible to extinction than previously thought. As such, these results have substantial implications for improving conservation legislation of pygmy perch lineages.

**Conclusions**

This study highlights the need for robust studies using genomic datasets to understand cryptic speciation and clarify taxonomy in pygmy perches, and for comparative analyses of other codistributed organisms to assess congruency of relevant biogeographical patterns.

**id #690**

## The devil's in the diet: a metabarcoding study of ecosystem changes.

**Catriona D Campbell[1], Elise Dewar[2], Anna J MacDonald[1], Elodie Modave[1], Bernd Gruber[1], Stephen D Sarre**

*1. University of Canberra, Bruce, ACT, Australia*

*2. Department of Primary Industries, Parks, Water and Environment, Hobart, Tasmania*

Tasmania supports a number of native Australian mammals whose mainland counterparts have severely declined or disappeared since European settlement such as the Eastern quoll (*Dasyurus viverinus*) and the Tasmanian bettong (*Bettongia gaimardi*). The top predator in Tasmania is the iconic Tasmanian devil (*Sarcophilus harrisii*) whose population has recently suffered a severe decline due to the arrival of devil facial tumour disease (DFTD). From the northeast DFTD has spread south-west covering more than half of the known distribution of the Tasmanian devil just ten years after it first appeared. There is evidence that the presence or absence of Tasmanian devils can affect the presence and absence of smaller predators such as the feral cat (*Felis catus*) and eastern quoll as well as the local prey species. The current and ongoing collapse of the Tasmanian devil population in the northeast of Tasmania and the unaffected populations in the northwest provides a unique opportunity to study ecosystem changes through the use of a non-invasive genetic technique using trace DNA. Applying this technique to mammalian predator scats collected over a six-year period, we were able to identify both predator and prey species and examine the change in frequency of devil scats across time and space. We then investigated changes in predator diets in response to changes in devil scat frequency and discuss the impact of DFTD across multiple trophic levels.

**id #733**

## Waking the sleeping dragon - molecular insights into the hibernation of the central bearded dragon

**Alexander Capraro[1], Denis O'Meally[2], Shafagh Waters[1], Paul Waters[1]**

*1. School of Biotechnology & Biomolecular Sciences, The University of New South Wales, Sydney, New South Wales, Australia*

*2. Centre for Animal Health Innovation, University of the Sunshine Coast, Sippy Downs, Queensland, Australia*

Hibernation is a seasonal physiological state utilized by animals to conserve energy during winter. Hibernation is complex, involving large changes in metabolism and cellular function. During hibernation, many stress responses must be modulated to tolerate physiological challenges, such as malnutrition, oxidative stress, and hypoxia that would otherwise be lethal. To elucidate the changes in gene expression responsible for this remarkable phenotype in the central bearded dragon (*Pogona vitticeps*), total transcriptomic profiles were generated using RNA sequencing for brain, heart and skeletal muscle at three time points: 1) late hibernation, 2) two days post-arousal, and 3) two months post-arousal. Hibernation was associated with enrichment of stress response pathways, including cell cycle arrest mediated by p53, and nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) signalling. Epigenetic regulatory pathways, including chromatin modification and microRNA-mediated gene silencing were enriched during hibernation. Genes responsible for post-translational modification processes, such as ubiquitination, and sumoylation were also upregulated. Neuroprotective strategies were observed in the brain, including downregulation of a N-methyl-D-aspartate receptor, and increased expression of tau-protein kinase genes. In heart, genes involved in cardiac hypertrophy, including cardiac-specific transcription factors and actin cytoskeleton proteins, were upregulated during hibernation. In skeletal muscle, there was evidence for protection against muscle atrophy, increased antioxidant capacity, and enhanced mitochondrial metabolism. This study provides exciting insights into the molecular mechanisms that govern hibernation in the central bearded dragon, as well as adaptive responses of the brain, heart and skeletal muscle.

**id #693**

## Developmentally important transcription factor *Fezf2* has a molecular and functional role in the mature brain

**Alison J Clare**[3, 1, 2], **Hollie E Wicky**[3, 1, 2], **Peter K Dearden**[3, 2], **Ruth M Empson**[4, 1], **Stephanie M Hughes**[3, 1, 2]

*1. Brain Health Research Centre, University of Otago, Dunedin, Otago, New Zealand*

*2. Genetics Otago, University of Otago, Dunedin, Otago, New Zealand*

*3. Department of Biochemistry, University of Otago, Dunedin, Otago, New Zealand*

*4. Department of Physiology, University of Otago, Dunedin, Otago, New Zealand*

Neuronal diversity is crucial for a functioning mature brain. Identifying the molecular factors that underpin this diversity is essential to our understanding of the brain in health and disease. *Forebrain embryonic zinc finger 2* (*Fezf2*) encodes a transcription factor essential to the specification of projection neuron fate in the developing cerebral cortex. As with many developmentally important transcription factors, *Fezf2* is expressed into adulthood, suggesting it could have importance for the maintenance of mature neurons. Despite the continued expression, a function for *Fezf2* in the mature brain has yet to be explored. In this work, we investigated a role for *Fezf2* in mature neurons using a lentiviral approach to conditionally knockdown the expression of *Fezf2* in the mature primary motor cortex (M1). RNA-seq analysis on this tissue revealed significant changes to the molecular profile of *Fezf2*-reduced M1 tissue, with 756 differentially expressed genes identified (FDR ≤ 0.05, LFC ≥ 0.2). Further term enrichment analysis indicated a common role for *Fezf2*-regulated genes in neuronal functions, including synaptic transmission and ion channel activity. We also saw significant overrepresentation of *Fezf2*-regulated genes implicated in sensory and motor-associated behaviour phenotypes. In a complementary study using *Drosophila*, we show that the conditional reduction of *Fezf2* homologue, *dfezl*, leads to significant changes in the sensorimotor behaviour of adult *Drosophila*, confirming a functional role. Our results demonstrate a regulatory role for *Fezf2* and contribute generally to the growing evidence that developmental transcription factors have a critical function in the mature brain.

**id #661**

## Identification and expression of pluripotency genes during ascidian whole body regeneration.

**Rebecca M. Clarke**[1], **Megan J. Wilson**[1]

*1. Developmental Biology and Genomics Lab, Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand*

Regeneration ability appears to inversely correlate with body and tissue complexity, consequently, adult stem cells in human organs can act to repair damage but have a limited ability to fully restore structure and function. A striking exception to this relationship is the invertebrate colonial tunicate *Botrylloides leachii.* Colonies consist of adults (zooids) sharing a common vascular system embedded in a gelatinous matrix (tunic). Minute fragments of this vasculature containing only a few hundred cells are capable of regenerating a whole functional adult organism within 8 days.

Successful whole body regeneration (WBR) requires either a long-lived cell population of pluripotent stem cells and/or cells to undergo dedifferentiation process. The two main theories are that they either arise from a small circulatory population of pluri- or multi-potent cells, or there is a 'stem cell' niche within the vascular lining that becomes activated when regeneration is induced.

This project aims to identify genes that are likely to play stemness roles *B. leachii* WBR and examine their expression pattern during WBR. We have recently sequenced the *B. leachii* genome and transcriptome providing a resource to identify conserved pluripotency genes for further studies. tBlastn searches of the assembled *B. leachii* genome will be done to identify orthologues of such genes as Sox2, *PIWI*, *Wnt* and *Foxd3*. Expression of putative stemness genes will be quantified using RT-qPCR across 5 stages of WBR. To determine the cell types express these pluripotency factors, *in situ*hybridisation on *B. leachii* regeneration fragments will be carried out.

**id #577**

## Ancient DNA clarifies the evolutionary history, taxonomy and distribution of crested penguins

**Theresa Cole**[1, 2], **Kieren Mitchell**[3], **Pere Bover Arbos**[3], **Jamie Wood**[2], **Janet Wilmshurst**[2, 4], **Paul Scofield**[5], **Alan Tennyson**[6], **Lara Shepherd**[6], **Jon Waters**[1]

*1. University of Otago, Lincoln, CANTERBURY, New Zealand*

*2. Landcare Research, Lincoln, New Zealand*

*3. Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide*

*4. School of Environment, The University of Auckland, Auckland, New Zealand*

*5. Canterbury Museum, Christchurch*

*6. Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand*

*Eudyptes spp.* represents a species complex of crested penguins inhabiting the temperate and sub-Antarctic regions of the Southern Ocean. Current conservation efforts, however, are hampered by limited systematic and taxonomic understanding of this species-rich genus. Moreover, undescribed sub-fossil material from the New Zealand Chatham Islands and mainland New Zealand suggests at least one species of crested penguin may have become extinct during the recent Holocene. Several studies have used genetic evidence to describe species-turnover events in recent New Zealand history, including those extinction-replacement of yellow-eyed penguins (*Megadyptes*) and little blue penguins (*Eudyptula*). We hypothesise that a similar pattern may have been observed in New Zealand crested penguins. This study uses ancient and modern DNA (whole

mitochondrial genomes and cytochrome oxidase 1) to (a) assess the systematic and evolutionary relationships between modern and extinct crested penguins, and (b) to explore the prehistorical distribution of *Eudyptes* taxa across the New Zealand region. An understanding of temporal shifts in crested penguin diversity is essential for prioritising conservation management strategies for these iconic taxa.

**id #671**

## Regulation and Evolution of Complex Developmental Gene Networks

**Andrew G Cridge[1], Elizabeth A Permina[2], Peter K Dearden[1]**

*1. University of Otago, Dunedin, New Zealand*

*2. Otago Genomics & Bioinformatics Facility, Dunedin, New Zealand*

Our aim is to understand how conserved genes change their role in the evolution of the insect segmentation network, how the network buffers change, and how that might constrain, or confer diversity of body plan.

We performed ChIP-seq with antibodies to early segmentation transcription factors *caudal*, *hunchback* and *orthodenticle* on embryonic tissue from *Drosophila melanogaster*, *Apis mellifera* and *Acyrthosiphon pisum*.

The ChIP-seq study identified multiple *cis*-regulatory motifs (CRMs) and gene targets for the three transcription factors in each of the insect species studied. Bioinformatic analysis allowed confident prediction of biologically important CRMs, as well as providing information on specific DNA sequences recognized by each of the transcription factors. In addition, we identified core ancestral genes regulated during embryo development responsible for embryonic patterning. We also identified genes that are regulated in only one species. These genes represent evolution of the network through a mechanisms that buffers change but still allow conserved segmentation output.

By continuing to study these genes we will learn how genes become co-opted into developmental networks, how such co-opted genes integrate with the rest of the network, and if these genes act to buffer regulatory changes in the transcription factors themselves. This also identifies the level of genetic robustness on which embryonic selection can act. This data has provided us, for the first time, with an understanding of how the targets of key transcription factors change over evolutionary time, effectively a measure of evolutionary change in a complex transcription factor network.

**id #754**

## Rapid response of a marine mammal species to holocene climate and habitat change

**Mark de Bruyn[1], Brenda L Hall[2], Lucas F Chauke[3], Carlo Baroni[4], Paul L Koch[5], A. Rus Hoelzel[6]**

*1. School of Life and Environmental Sciences, University of Sydney, Sydney, Australia*

*2. Climate Change Institute and Department of Earth Sciences, University of Maine, Orono, USA*

*3. Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa*

*4. Dipartimento di Scienze della Terra, Universita` di Pisa, Pisa, Italy*

*5. Department of Earth and Planetary Sciences, University of California, ,, USA*

*6. School of Biological and Biomedical Sciences, University of Durham, Durham, United Kingdom*

Environmental change drives demographic and evolutionary processes that determine diversity within and among species. Tracking these processes during periods of change reveals mechanisms for the establishment of populations and provides predictive data on response to potential future impacts, including those caused by anthropogenic climate change. Here we show how a highly mobile marine species responded to the gain and loss of new breeding habitat. Southern elephant seal, *Mirounga leonina*, remains were found along the Victoria Land Coast (VLC) in the Ross Sea, Antarctica, 2,500 km from the nearest extant breeding site on Macquarie Island (MQ). This habitat was released after retreat of the grounded ice sheet in the Ross Sea Embayment 7,500–8,000 cal years before present (YBP), and is within the range of modern foraging excursions from the MQ colony. Using ancient mtDNA, coalescent models and Approximate Bayesian Computation (ABC), we tracked the population dynamics of the now extinct VLC colony and the connectivity between this and extant breeding sites. We found a clear expansion signal in the VLC population ~8,000 YBP, followed by directional migration away from VLC and the loss of diversity at ~1,000 YBP, when sea ice is thought to have expanded. Our data suggest that VLC seals came initially from MQ and that some returned there once the VLC habitat was lost, ~7,000 years later. We track the founder-extinction dynamics of a population from inception to extinction in the context of Holocene climate change and present evidence that an unexpectedly diverse, differentiated breeding population was founded from a distant source population soon after habitat became emergent.

to be provided

**id #788**

## Sequencing the genomes of every kākāpō

**Andrew Digby[1], Bruce Robertson [2], Jason Howard [3], David Iorns[4]**

*1. Department of Conservation, Invercargill, SOUTHLAND, New Zealand*

*2. University of Otago, Dunedin, New Zealand*

*3. Duke University, Durham, USA*

*4. Genetic Rescue Foundation, California, USA*

The critically endangered kakapo is at risk of extinction due to threats such as infertility and disease which are believed to be results of inbreeding depression. Genetic management of the species is a central part of conservation action, but relatively low-resolution genetic tools are used. To address this, a project is under way to sequence the genomes of every living kākāpō - the first time this had been attempted for any species. The data will be made publicly available, and the resulting analyses will have many direct benefits for kākāpō conservation, including: a full pedigree for genetic management, identification of functional genes associated with low fertility and disease, and greater understanding of kākāpō population genetics. The data will also yield rich information of kākāpō evolutionary history, and will provide a test case for sharing and managing bioinformatic data from population genome datasets. An international collaboration that is funded by crowd-funding and private donations, the project is halfway towards its goal, with 82 genomes sequenced.

**id #540**

## Coming of age: the role of nuclear structure in zygotic genome activation

**Amy Dowdle[1], Jennifer Gerton[2], Justin O'Sullivan[3], Julia Horsfield[1]**

*1. Department of Pathology, University of Otago, Dunedin, New Zealand*

*2. Stowers Institute for Medical Research, Kansas City, Missouri, United States*

*3. Liggins Institute, University of Auckland, Auckland, New Zealand*

**Background:** In animals, initial embryonic development proceeds according to maternal instructions deposited in the egg prior to fertilisation in the form of mRNA and protein; the zygotic genome is transcriptionally silent during this time. At a certain point, the genome of the embryo becomes active during a phenomenon known as zygotic genome activation (ZGA): the "coming of age" of the embryo. The mechanisms of ZGA are as yet unclear. We hypothesise that it is the formation of a transcriptionally competent 3D genome that allows the embryo to initiate transcription and to take charge of its own development. Zebrafish are an excellent tool for asking such fundamental questions about developmental biology; in particular, the rapid development of zebrafish embryos allows for high throughput experiments that would be unfeasible in other model organisms.

**Methods:** We have performed ATAC-seq in zebrafish embryos across ZGA in an effort to investigate the accessibility of the chromatin. We also knocked down Rad21 (a subunit of the structural protein cohesin) to observe the role of cohesin in establishing or maintaining chromatin accessibility.

**Results:** We found that in both wild-type and rad21 knockdown conditions, chromatin accessibility increased slightly during ZGA and markedly post-ZGA. This increase in chromatin accessibility is supported by RNA-seq data from our lab as well as previous literature in other organisms, and alongside a ChIP-seq dataset for Rad21 suggests cohesin plays an important role during ZGA.

**id #753**

## One genome to rule them all: Genome plasticity in single strains of *Helicobacter Pylori*

**Jenny Draper[1, 2], L Hansen[3], S Abedrabbo[2], D Bernick[2], N Pourmand[2], N Kong[3], A Weiss[3], B Weimer[3], J Solnick[3], K Karplus[2], K Ottemann[2]**

*1. The Institute of Environmental Science & Research (ESR), Wellington, New Zealand*

*2. University of California at Santa Cruz, Santa Cruz, USA*

*3. University of California at Davis, Davis, USA*

Although it is well known that many bacterial genomes are highly variable, it is nonetheless traditional to refer to, analyse, and publish a single fixed genome for bacterial strains. In the process, inherent natural variation is artificially reduced ("only sequence from a single colony"), ignored ("just publish the consensus"), or placed in the "too-hard" basket ("analysis of raw read data is more robust"). Studies of bacterial genome evolution rarely survey existing diversity under normal laboratory conditions, instead focusing on changes occurring over a timecourse or in response to specific environmental pressures.

*Helicobacter pylori* is a highly studied bacterium due to its ability to cause ulcers and stomach cancer, with key experimental strains used worldwide. Although *H. pylori* is well known for having an extremely plastic genome with a high mutation and recombination rate, researchers rely heavily on single fixed reference genomes for these strains, and little is known about the degree of variation to expect in typical working stocks.

Here, I will discuss the variability seen in typical laboratory cultures of *H. pylori* strain SS1 and its parent strain PMSS1, as revealed by a combination of next-generation sequencing and traditional laboratory techniques. Within SS1 alone, the variation includes large inversions, nearly 50 SNPs at over 5% prevalence, movement of the transposon IS607, and dynamic copy-number variation of the cagA gene.

This work reveals that reliance on a single-colony genome or consensus assembly may be misleading, even at the level of a typical laboratory working stock.

**id #792**

## Adapt or perish: the function of CRISPR-Cas adaptive immune systems

**Peter C Fineran[1]**

*1. Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand*

Bacteria have evolved 'immune systems' as a result of their constant exposure to foreign mobile genetic elements, including bacteriophages and plasmids. For example, an estimated $10^{25}$ bacteriophage infections occur every second, which affect global nutrient cycles. Other mobile genetic elements can harbour antibiotic resistance or pathogenicity determinants, which influence bacterial evolution and our ability to treat infectious disease. To thwart these invaders, bacteria have many resistance strategies, including innate immunity, such as restriction-modification and abortive infection systems, and adaptive immunity provided by the CRISPR-Cas systems. Recently, there have been major advances in our understanding of these systems. They have also been exploited as molecular biology reagents and led to a new biotechnology revolution. I will present our recent research into the function of these fascinating adaptive immune systems in bacteria.

**id #536**

## Does animal personality explain harem access in the polygynous New Zealand sea lion (*phocarctos hookeri*)?

**Imogen Foote[1], Bruce C. Robertson[1], B. Louise Chilvers[2]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

*2. Massey University, Palmerston North, New Zealand*

Animal personality (consistent inter-individual behavioural differences) is a focus of ecological and evolutionary researchers due to its influence on individual fitness, and therefore the evolution of a species. Strong, single gene associations with personality traits occur, such as the dopamine receptor gene, DRD4, which has been associated with the bold-shy personality trait. Typical of otariids, the New Zealand sea lion (*Phocarctos hookeri*) breeds polygynously in high-density breeding colonies. Males physically compete for access to the breeding harem, resulting in a strong male reproductive success skew. Male size is thought to explain differences in male breeding success, but observations of similarly-sized adult males within and outside of the breeding harem suggest that size alone may not adequately explain male reproductive success. Here we propose that animal personality plays an important role in harem access and hence, male reproductive success. We hypothesize that if personality traits influence reproductive success, selection will act upon these traits to produce consistent genetic differences at personality-associated genes, and these genetic differences will be associated with a male's reproductive success. We screened genetic variation at personality-associated candidate genes (DRD4) in male NZ sea lions at the Sandy Bay breeding colony on the NZ subAntarctic Aucklands Islands. Using a proxy for male

personality (male access to the harem, which assumes males that access the harem are bolder personality types), we examined the association between male personality and genetic variants at the candidate genes. Our study will provide insight into the factors that influence reproductive success in otariids.

**id #588**

## Genomic analysis of hybridisation between the endemic kakī (black stilt), and the self-introduced poaka (pied stilt)

**Natalie J Forsdick[1], Richard Maloney[2], Tammy Steeves[3], Michael Knapp[1]**

*1. Anatomy, University of Otago, Dunedin, OTAGO, New Zealand*

*2. Department of Conservation, Wellington, NZ*

*3. School of Biological Sciences, University of Canterbury, Christchurch, NZ*

The Kakī (Black Stilt; *Himantopus novaezelandiae*) is a New Zealand endemic wading bird, restricted to the Upper Waitaki Basin in the central South Island. The Kakī is listed as nationally critical, due to habitat loss and modification, and predation. A captive breeding programme exists to improve breeding success and survival, with juveniles and sub-adults released to supplement the wild population. This, in combination with predator control, has resulted in the population increasing from ~23 individuals in 1981 to around 100 wild adults today. However, during the population decline throughout the 1900s, hybridisation has occurred with the closely-related Poaka (Pied Stilt; *Himantopus himantopus leucocephalus*). Hybridisation is of conservation concern when it involves rare native species, as it may negatively impact species recovery due to wasted reproductive potential, outbreeding depression, and may directly contribute to species extinction through genetic admixture. Previous genetic studies have used a small number of microsatellite and mitochondrial markers to determine the extent of introgression between Kakī and Poaka. With recent improvements in sequencing technologies, it is now possible to conduct a genome-wide investigation of the potential effects of introgression between these two species. This will involve de novo whole-genome assembly of modern Kakī to enable comparisons with Australian Pied Stilts, North Island Poaka, and historic/ancient Kakī samples to investigate the effects of a complex history of hybridisation. The results of this study will provide information for the Department of Conservation Kakī Recovery Programme, to ensure the survival and recovery of this critically endangered bird.

**id #744**

## genetics and conservation biology: a reexamination

**Ian R Franklin[1]**

*1. CSIRo, Retired, Belair, SOUTH AUSTRALIA, Australia*

In 1978, Michael Soule and Bruce Wilcox organised a meeting in San Diego which laid the foundations for many of the important issues in conservation biology. While genetic deterioration is perhaps not the the most important factor in species extinction, two principles have emerged, namely the need to maintain genetic variation for future genetic change, and to avoid loss in fitness arising from inbreeding depression. These two issues have been embodied in the so-called 50/500 rule. Despite much empirical and experimental observation, the genetics underlying the maintenance of genetic variation, and the nature of inbreeding depression, remain obscure. While both of these principles have been generally accepted, the conclusions that form the basis of this rule have been challenged. This talk revisits these issues in the light of our increased knowledge over the almost 40 years since the 1978 meeting.

**id #685**

## Volcanoes and earthquakes shed light on how disturbance shapes spatial patterns of genetic diversity

**Ceridwen Fraser[1]**

*1. Australian National University, Acton, ACT, Australia*

The processes underpinning spatial patterns of genetic diversity are not yet fully understood. Recent years have, however, witnessed an explosion of research using DNA to infer the evolutionary and biogeographic impacts of the Last Glacial Maximum (LGM, around 20,000 years ago). Hundreds of taxa have been found to show relatively high genetic diversity in 'refugial' regions unaffected by LGM ice, whereas populations in recolonised areas generally have lower genetic diversity. As an example, many terrestrial taxa have persisted on the Antarctic continent through Pleistocene glaciations, yet LGM ice cover was extreme. To test the hypothesis that Antarctic volcanoes could have nurtured hotspots of refugial diversity through ice ages, I have been combining genomic data with spatial environmental analyses to assess broad-scale diversity patterns. Results indicate that diversity generally declines with distance from geothermal regions, consistent with recent recolonisation of new ice-free terrain from volcanic refugia.

Intriguingly, such molecular signatures of past disturbance events can apparently endure for millennia, despite ongoing dispersal of organisms. This inertia has been suggested to result from early colonists rapidly reaching densities sufficient to block establishment by latecoming conspecific lineages. If these 'density-blocking' processes indeed underpin such structure, turnover should depend on subsequent disturbance events that 'clear the slate' and allow different lineages to establish. A new research programme will aim to directly test the role of disturbance in structuring spatial genetic patterns, using manipulative experiments as well as opportunities provided by large natural disturbances, e.g., earthquakes (including the November 2016 earthquakes in New Zealand).

**id #675**

## How the other half live: the evolution of repetitive DNA

**Austen R.D. Ganley[1], Huitong Zhou[2]**

*1. School of Biological Sciences, University of Auckland, Auckland*

*2. Gene-maker Laboratory, Lincoln University, Christchurch*

Repeats are *persona non grata* in the genomic world. They are often conflated with "junk" DNA and (pejoratively) labelled as "heterochromatic". We have active discrimination systems in place, most notably *repeatmasker*, to exclude repeats from the fruits of the genomic revolution. Yet, repeats are incredible diverse, abundant, and important contributors to genomes, particularly in eukaryotes. The approach of ignoring them, based on a path-of-least-resistance response to complications they pose, both creates problems and misses opportunities for biological insight. The "problems"

in dealing with repeats in genomic datasets are now largely overstated, and advances in sequencing technologies are providing ways to develop deep insights into their nature. To wit, the more we look, the more we find. Here I will talk about our work investigating the evolution of different types of repeats. I will propose a general framework for characterizing repeat evolution. This framework removes ambiguities that arise from previous models of repeat evolution, and enables a coherent description of the evolution of repeats to be incorporated into a biological understanding of their roles.

**id #668**

## The avoidance of random RNA interactions controls bacterial protein expression

**Sinan Ugur Umu[1], Anthony M Poole[1], Renwick CJ Dobson[1, 2], Paul Gardner[1]**

*1. School of Biological Sciences, Canterbury University, Christchurch, New Zealand*

*2. Department of Biochemistry & Molecular Biology, University of Melbourne, Parkville, Australia*

A critical assumption of gene expression analysis is that mRNA abundances broadly correlate with protein abundance, but these two are often imperfectly correlated. Some of the discrepancy can be accounted for by two important mRNA features: codon usage and mRNA secondary structure. We present a new global factor, called mRNA:ncRNA avoidance, and provide evidence that avoidance increases translational efficiency. We also demonstrate a strong selection for the avoidance of stochastic mRNA:ncRNA interactions across prokaryotes, and that these have a greater impact on protein abundance than mRNA structure or codon usage. By generating synonymously variant green fluorescent protein (GFP) mRNAs with different potential for mRNA:ncRNA interactions, we demonstrate that GFP levels correlate well with interaction avoidance. Therefore, taking stochastic mRNA:ncRNA interactions into account enables precise modulation of protein abundance.

**id #649**

## The tuatara genome — insights from the sole survivor of an ancient reptilian Order

**Neil Gemmell[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

The tuatara (*Sphenodon punctatus*) is an iconic and enigmatic terrestrial vertebrate, unique to New Zealand. Once widespread across the supercontinent of Gondwana, the tuatara, the only living member of an archaic reptilian order Rhynchocephalia (Sphenodontia) that last shared a common ancestor with other reptiles some 220-250 million years ago, is now only found on a small number of offshore Islands distributed around the coast of New Zealand. Through the efforts of a large international consortium, we have now sequenced, annotated, and analysed the 4.6-Gbp tuatara genome. In this presentation, I will highlight some of the challenges associated with sequencing this genome and the novel insights spanning genome architecture, sex determination, immunity, and homoeostasis that emerge from the genome of this important linchpin in vertebrate evolution. Last, the tuatara is a taonga, or special treasure, for Māori, and I will highlight the additional challenges, and rewards, of working in partnership with indigenous groups who have different cultural mindsets, albeit common goals.

**id #556**

## Predicting pandemics: the evolution and emergence of infectious disease

**Jemma Geoghegan[1]**

*1. Macquarie University, Sydney, NSW, Australia*

Emerging infectious diseases are often characterised by host-switching events in which a pathogen jumps from its original host to infect a novel species. With changes in land use and increased urbanisation, the frequency with which pathogens jump species barriers to emerge in new hosts is expected to rise. Nevertheless, most emerging infections in humans result in dead-end 'spillover' events in which a pathogen is transmitted from an animal reservoir to a human but is unable to achieve the sustained human-to-human transmission necessary for a full-blown epidemic. This talk will focus on my research into the evolutionary processes that might allow novel pathogens to adapt to new hosts; and the potential barriers to host adaptation. First, to better understand the determinants of host adaptation and emergence, I will present a model of key aspects of pathogen evolutionary dynamics at both intra- and inter-host scales. Second, using multivariate modelling and multimodel inference, I will identify those biological features of viruses that best determine inter-human transmissibility. Finally, I will present a comparative co-phylogenetic analysis, which aims to understand how viruses and their hosts co-evolve and reveal the nature of virus macroevolution.

**id #793**

## Dogs and Wolves in Time and Space

**Tom Gilbert[1]**

*1. University of Copenhagen, Copenhagen, COPENHAGEN, Denmark*

Despite the key position that dogs hold in the lives of many of us, and the extensive efforts of many previous scientific studies, a surprising amount remains to be learnt about our faithful friends. For example, considerable controversy exists over such basic questions as: When did we first domesticate the dog? Where was the domestication centre? Was there more than one? And perhaps most surprisingly, what was the dog even domesticated from? Given the extent to which we have both moved and shaped dog breeds in recent centuries, and taken a good stab at eradicating their wild relatives, it seems unlikely that analyses of modern genetic material alone will be able to solve these questions. As such deciphering dog domestication represents an exciting frontier on which palaeogenomic approaches stand to make enormous contribution, and indeed, in light of a vastly expanded reference dataset of contemporary genomic material, a number of intriguing findings are already coming to light.

**id #790**

## Me, my dog and maize: studying evolution and domestication at the population level using ancient DNA

**Tom Gilbert[1]**

*1. University of Copenhagen, Copenhagen, COPENHAGEN, Denmark*

In Darwin's day, tracking the process of biological evolution was limited to studying changes and differences in the physical forms of fossils and living species. For nearly 40 years now, these studies have been supplemented, increasingly powerfully, by DNA analyses. From comparisons of just a few key DNA sequences, DNA technology itself has evolved to now allow evolutionary biologists to compare the entire genomes (all of an organism's DNA) of species. In recent years, researchers have begun to integrate DNA from historic and even ancient specimens into such genomic analyses, following the realization that while analyzing modern samples can tell us about the end point of evolutionary processes, inclusion of samples from the past can literally provide insight into the changes as they happened. We are now seeing the dawn of an era in which population level genomic analyses of ancient and present day populations are possible. I will explore the power of such approaches in this talk using human, dog and maize evolution as examples.

**id #691**

## triose phosphate isomerase: far from perfect, far from done.

**Josh B Gilligan[1], Thomas J Finn[1], Wayne M Patrick[1]**

*1. University of Otago, North Dunedin, Dunedin, OTAGO, New Zealand*

**Background/Aims:**

Triose phosphate isomerase (TPI) is a highly-conserved core metabolic enzyme that catalyses the interconversion of D-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate during glycolysis and gluconeogenesis. This enzyme is described as 'perfect' because the enzyme-catalysed rate of this interconversion is limited only by the rate of diffusion. However, this notion of 'catalytic perfection' was arrived at under *in vitro* conditions, which are not representative of conditions *in vivo*. We have set out to test whether TPI really is perfect, with respect to its activity under *in vivo* conditions and its ability to contribute to host cell fitness.

**Methods:**

We obtained a plasmid library created by comprehensive codon mutagenesis, which contains variants encoding every possible single amino acid mutation of TPI. The enzymatic activities of these TPI variants in an artificial cytoplasm buffer are being determined via high-throughput assays. The effects of the mutations on cell fitness will be analysed using Phenotype Microarray plates.

**Results:**

Data from several hundred TPI variants indicate a wide distribution of activities, from non-functional to above that of the wild type enzyme. Sequencing and characterisation of individual variants is ongoing. We are also determining the effects of these mutations on cell fitness.

**Conclusions:**

Our activity data confirm that TPI is not a 'perfect' enzyme when assayed in artificial cytoplasm. Instead, we have identified mutations that make it even more active. By analysing all possible point mutations, we will ultimately determine the entire activity and fitness landscape of TPI, yielding insights into how natural selection acts upon perfect enzymes.

**id #532**

## Sex and stress: Is cortisol a mediator of sex change in fish?

**Alexander Goikoetxea Pérez de Mendiola[1], Erica V Todd[1], Simon Muncaster[2], Mark Lokman[1], Jodi T Thomas[1], Hui Liu[1], Neil J Gemmell[1]**

*1. University of Otago, Dunedin Central, OTAGO, New Zealand*

*2. Toi Ohomai Institute of Technology, Tauranga, Bay of Plenty, New Zealand*

Sex change occurs as a usual part of the life cycle for many teleost fish. Changing sex is known to enhance the lifetime reproductive success of these fish and the modifications involved (behavioral, gonadal, morphological) are well studied. However, the exact mechanism behind the transduction of the environmental signals into the molecular cascade that underlies this singular transformation remains largely unknown. Cortisol is the main glucocorticoid in fish and the hormone most directly associated with stress. Recent research suggests that this hormone may act as a key factor linking social environmental stimuli and the onset of sex reversal by initiating a shift in steroidogenesis from estrogens to androgens. In this study, we aim to elucidate the role of cortisol in mediating sex change of a protogynous (female-to-male) hermaphrodite, the endemic New Zealand spotty wrasse (*Notolabrus celidotus*). We implanted slow-release cortisol pellets into female spotty wrasses to promote sex reversal under inhibitory conditions. We monitored each female daily for behavioral and morphological signs of sex change. To track the interrenal hormonal changes across the process of sex reversal, we collected blood samples fortnightly. We also obtained brain, pituitary and gonadal tissue to create a histological time series and to conduct transcriptome-wide expression analysis. We anticipate that this study will enhance our understanding of the role of cortisol in the initial stages of sex change, improve our understanding of sex determination and differentiation across vertebrates, and may lead to new tools to control fish sex ratios in aquaculture.

**id #731**

## epigenetics and sex determination

**Jenny Graves[1]**

*1. La Trobe University, Bundoora, VIC, Australia*

Mammals and birds both have well defined sex chromosome systems containing sex determining genes that determine gonad type with apparently no input from the environment. Yet we have long known that there are systems in which sex is determined without the benefit of sex chromosomes. Crocodilians and marine turtles are famous for having no sex chromosomes. Instead, sex of hatchlings is determined by the temperature at which the egg is incubated. Males and females have identical genomes, so the choice of sex must be effected by epigenetic means. The mechanism has been a longstanding mystery.

We now know that there are several species with perfectly respectable sex chromosomes, but sex can be reversed by temperature. The Central bearded dragon has well defined Z and W chromosomes, but at a high temperature ZZ as well as ZW embryos develop as females. The half-smooth tongue sole also has ZZ males and ZW females and *DMRT1* is the sex determining gene (as in birds). The difference is that the *DMRT1* locus is repressed by methylation at moderate temperatures; incubation at higher temperatures releases this inhibition and results in ZW "pseudomales".

The interaction of genes and the environment to precipitate male or female development gives us a chance to explore the epigenetic basis of temperature dependent sex determination. A breakthrough has been the identification of unique transcripts in sex reversed dragons that establish links between stress and sex determining pathways.

**id #752**

## Madagascar's extinct elephant birds: what we know from molecular studies

**Alicia Grealy[1], Matthew Phillips[2], Gifford Miller[1, 3], Michael Bunce[1]**

*1. Trace and Environmental DNA (TrEnD) Laboratory, Department of Environment and Agriculture, Curtin University, Perth, Australia*

*2. Vertebrate Evolution Group, School of Earth, Environmental and Biological Sciences, Queensland University of Technology, Brisbane, Australia*

*3. INSTAAR and Geological Sciences, University of Colorado, Boulder, United States of America*

The elephant birds of Madagascar (Aves: Aepyornithidae) were large, flightless ratites that became extinct around a millennium ago. These birds offer excellent models to study many aspects of evolution, including speciation and extinction, as well as test biogeographic hypotheses and characterise the genetic basis of island phenotypes. In these endeavors, ancient DNA (aDNA) from elephant birds is essential. However, it has been challenging to recover aDNA from skeletal fossils due to their rarity and the warm climate of Madagascar, which is not conducive to aDNA preservation. With approximately 3% endogenous aDNA retrievable from elephant bird eggshell, it is a promising substrate for recovering high-quality aDNA. Using aDNA extracted from fossil eggshell, coupled with target enrichment and next-generation sequencing techniques, we recover complete mitochondrial genomes as well as nuclear loci, and use these to investigate the placement of elephant birds within the avian phylogeny, date their divergence from other birds, revisit elephant bird taxonomy, and provide the first analysis of elephant bird phylogeography. These results offer the first molecular insight into elephant bird biodiversity and speciation, and advocate for a major revision of elephant bird systematics. We foresee that elephant bird whole genome recovery is ultimately achievable, and will provide further insights into the evolution these birds.

**id #567**

## Genomics of a weevil pest and its parasitoid biocontrol agent

**Thomas WR Harrop[1], Ruy Jáuregui[2], Shola Olaniyan[2], Shannon Taylor[1], Sarah Inwood[1], Jeanne Jacobs[2], Stephen Goldson[2], Peter K Dearden[1]**

*1. Department of Biochemistry, The Laboratory for Evolution and Development, University of Otago, Dunedin, New Zealand*

*2. AgResearch Ltd, Christchurch, New Zealand*

The Argentine stem weevil (ASW), *Listronotus bonariensis* (Coleoptera: Curculionidae), is an exotic pasture pest in New Zealand. It was first detected in the 1920s, and by the 1980s was causing hundreds of millions of dollars' damage to pasture per year. In the early 1990s, a parasitoid wasp from the host range of ASW, *Microctonus hyperodae* (Hymenoptera: Braconidae), was introduced as a biocontrol agent. Attack rates were initially around 80%, providing effective control, but have subsequently declined to around 20%, suggesting the evolution of resistance in the host. Along with the agricultural importance of this problem, measuring the genetic variation in both the sexual host and the asexual parasitoid is an exciting opportunity to investigate the evolution of resistance in a biocontrol system. We are working on *de novo*, shotgun genome and transcriptome sequences for both species and population surveys using genotyping-by-sequencing (GBS) and targeted resequencing of the 16S ribosomal RNA gene. These results are guiding our efforts to determine the mechanism of resistance using functional experiments including RNAi, genome editing and RNAseq.

**id #772**

## Characterisation of an *Arabidopsis thaliana* plant cysteine dioxygenase.

**Brooklyn K Hayes[1], Guy NL Jameson [2], Sigurd M Wilbanks[1]**

*1. Department of Biochemistry , University of Otago, Dunedin, Otago, New Zealand*

*2. Bio21 Molecular Science and Biotechnology Institute , University of Melbourne , Melbourne , Victoria , Australia*

Cysteine dioxygenase (CDO) is a non-heme, mononuclear-iron enzyme which catalyses the addition of molecular oxygen to the thiol group of cysteine residues forming cysteine sulfinic acid. In plants, this dioxidation occurs on N-terminal cysteine residues which promotes N-terminal arginylation and ubiquitin mediated degradation of target proteins which mediate the response to oxygen depletion. This pathway facilitates both submergence-tolerance and seed maturation, contributing to enhanced crop yield.

As mammalian CDO is involved in cysteine catabolism, (which prevents the cytotoxic and neurotoxic effects of unregulated free cysteine) determination of its mechanism is clinically relevant. Dual sequence alignment of plant and mammalian CDO suggests that although the same iron-coordinating residues are present in plant CDO, many proposed catalytic amino acids are not. Kinetic- and structural-based characterisation of plant CDO offers a unique perspective on the minimal requirements for CDO activity.

Homology modelling suggests that the central domain of plant CDO is well ordered, adopting a structure similar to mammalian CDO whilst the N- and C- terminal domains are inherently disordered. Bioinformatic analyses show that the N-terminal domain contains a bipartite nuclear localisation signal and *in vitro* experiments indicate that this basic domain also interacts with DNA. Preliminary $^{1}$H NMR of the product of plant CDO with free cysteine show spectral features characteristic of cystine demonstrating that plant CDO is not able to dioxidise free cysteine. In contrast to the lack of activity against free cysteine, colorimetric substrate depletion assays instead show enzymatic activity with short peptide substrates containing an N-terminal cysteine.

**id #735**

## Recombination drives evolution of the pyoverdine locus in *Pseudomonas aeruginosa.*

**Astra Heywood[1], Brian Forde[2], Scott A Beatson[2], Iain L Lamont[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

*2. University of Queensland, Brisbane*

**Background / aims.** *Pseudomonas aeruginosa* is an opportunistic pathogen that secretes molecules called pyoverdines, which chelate iron and deliver it into the bacteria via specific receptors. There are three chemically distinct classes of pyoverdines. The genes required for pyoverdine synthesis and uptake are well characterised and are amongst the most variable within this species with only ~30% identity between types. The aim of this research was to investigate the role of horizontal gene transfer in generating genetic diversity at the pyoverdine locus.

**Methods.** Over 1400 genome sequences of *P. aeruginosa* from publicly available databases were examined for phylogenetic relatedness, pyoverdine type and recombination analysis using RAxML and Gubbins.

**Results.** Each genome was found to encode only one of the three types of pyoverdine. Whole genome phylogenetic analysis showed that strains do not cluster according to pyoverdine type indicating horizontal transfer of genes for pyoverdine synthesis and uptake. Recombination analysis revealed the pyoverdine locus as one of six within the *P. aeruginosa* genome that had a high rate of recombination. The pyoverdine locus itself has five regions that have a high rate of recombination that not only include type specific genes but also genes that are highly conserved between types.

**Conclusion.** Our findings demonstrate the very extensive role of horizontal gene transfer and recombination in the phenotypic evolution of a bacterial pathogen.

**id #728**

## A unified model for the molecular basis and evolution of temperature-dependent sex determination

**Clare Holleley[1], Ira Deveson[2], Arthur Georges[3]**

*1. Australian National Wildlife Collection, CSIRO, Canberra, ACT, Australia*

*2. Genomics and Epigenetics Division, Garvan Institute of Medical Research, Sydney, NSW, Australia*

*3. Institute for Applied Ecology, University of Canberra, Canberra, ACT, Australia*

In many vertebrates, sex of offspring is determined by external environmental cues rather than by sex chromosomes. In reptiles, for instance, temperature-dependent sex determination (TSD) is common. Despite decades of work, the mechanism by which temperature is converted into a sex-determining signal remains mysterious. This is partly because it is difficult to distinguish the primary molecular events of TSD from the confounding downstream signatures of sexual differentiation. Here we use the Australian central bearded dragon, in which chromosomal sex determination is overridden at high temperatures to produce sex-reversed female offspring, as a unique model to identify TSD-specific features of the transcriptome. The link between epigenetic regulation and external temperature appears to involve an unusual mode of gene regulation (differential intron retention) in a family of chromatin-modifying genes that are key players in eukaryotic epigenetic regulation. Significantly, we also observe sex-associated differential retention of the equivalent introns in the same chromatin-modifying gene transcripts expressed in embryonic gonads from other reptiles, indicative of a reptile-wide mechanism controlling TSD. This work is soon to appear in Science Advances.

**id #708**

## Epigenetic memory in vertebrates

**Julian R Peat[1], Oscar Ortega-Recalde[1], Michael P Collins[1], Olga Kardailsky[1], Morgan T Jones[1], Tim Hore[1]**

*1. Department of Anatomy, University of Otago, Dunedin, OTAGO, New Zealand*

Epigenetic modification can provide a mechanism for cells to 'remember' early developmental decisions in the absence of the signals which first initiated them. Methylation of CG dinucleotides is amongst the most iconic of all epigenetic systems because there is a well defined mechanism by which it transmits molecular memory following cell division. We have recently surveyed CG methylation in a wide range of vertebrates (>25 species) and characterised the methylome of a cartilagenous fish, the elephant shark (*Callorhinchus milii*). Despite last sharing a common ancestor with humans ~465 Mya, elephant shark shares many epigenetic characteristics with human, including an association between promoter methylation and gene silencing. These findings position the elephant shark as a valuable model to explore the evolutionary history and function of vertebrate methylation, and stimulate further questions surrounding what drives high levels of epigenetic memory and how it is reprogrammed between generations.

**id #734**

## Evidence for post-transcriptional regulation of dosage compensation in platypus

**William Horspool[1], Carly Conlan[2], Paul Waters[1]**

*1. University of New South Wales, Sydney, NSW, Australia*

*2. Australian National University, Canberra, ACT, Australia*

In Ohno's classic theory for the evolution of dosage compensation, as Y gene function is lost, gene expression on the X chromosome is upregulated to restore ancestral autosomal expression levels in males. This upregulation of X genes carried through to females, who then evolved X chromosome inactivation to transcriptionally silence one X. This hypothesis is challenged by observations in platypus (and other species) where expression from the X's in males is lower than from the autosomes, and from the X's in females. However, all previous large scale studies of dosage compensation have focused only on total mRNA levels, and have ignored the possibility that gene dosage could change post-transcription. In this study we analyse dosage compensation of opossum and platypus X genes at three different stages from the genome to the proteome: 1) total mRNA; 2) mRNAs bound to ribosomes; 3) total protein abundance. As expected, we observed that total mRNA levels of X genes in male platypus were less than 2/3 (0.62) that observed in females. In contrast, mRNA bound to ribosome in males was 3/4 (0.74) of that observed in females. Finally, we observed that protein levels were much closer to balanced between sexes (0.88). These results demonstrate that a lack of global dosage compensation in the transcriptome does not necessarily carry through to the functional unit. This is the first example that dosage compensation of X genes can be corrected post transcriptionally in mammals, and has large implications for inconsistencies observed with dosage compensation in other species.

**id #699**

## Telomere length dimorphism in dasyurid marsupials is based on parental origin of the chromosome

**Emory D Ingles[1], Janine E Deakin[1]**

*1. University of Canberra, Canberra, ACT, Australia*

The marsupial family of dasyurids is characterised by an extraordinary telomeric feature, telomere length dimorphism. For each chromosome pair, one homologue will have relatively long, and the other relatively short, telomeres, forming a non-random distribution of telomere lengths. As it is always the Y chromosome in males that has the longer telomeres, this led to the hypothesis that telomere length in dasyurids is regulated based on the parental origin of the chromosome, with paternal chromosomes having longer telomeres, and maternal chromosomes having shorter telomeres (the parent-of-origin hypothesis). We have tested this hypothesis in females of four dasyurid species. The maternal and paternal X chromosomes in these species can be distinguished by different epigenetic marks associated with paternally imprinted X chromosome inactivation, enabling us to investigate if the parental origin of sex chromosomes is linked with telomere length in females as well as males. Epigenetic marker enrichment on the maternally-derived X was observed using immunofluorescence staining, and telomere length was observed by fluorescent in situ hybridisation. We determined that associations between short telomere length and epigenetic enrichment on the maternal X chromosomes were consistent among cells from an individual, and between individuals. Overall, our results are consistent with the parent-of-origin hypothesis, that paternal chromosomes have long telomeres and maternal chromosomes short telomeres. We are currently in the initial stages of the next step towards testing this hypothesis, which is to determine telomere length in germ cells.

**id #677**

## Predicting the future and reconstructing the past through protein engineering

**Colin Jackson**[1]

*1. Australian National University, Canberra, ACT, Australia*

In this talk I will discuss some recent results from our laboratory in which we have used a variety of protein engineering approaches (loop design, ancestral reconstruction, directed evolution, combinatorial mutagenesis) to answer questions such as: How will TB evolve resistance to next-gen antibiotics? How will pesticide resistance develop? How can enzymes evolve novel functions? And how can enzymatic activity emerge from a non-catalytic binding protein scaffold?

**id #768**

## Genotyping-By-Sequencing for diverse applications including population genetics

**Jeanne Jacobs**[1], **Shannon Clarke**[2], **Marty Faville**[3], **Andrew Griffiths**[3], **Mingshu Cao**[3], **Rachel Tan**[3], **Tracey Van Stijn**[2], **Rayna Anderson**[2], **Rachael Ashby**[2], **Rudiger Brauning**[2], **Alan McCulloch**[2], **Ken Dodds**[2], **John McEwan**[2]

*1. AgResearch, Christchurch, NZ, New Zealand*

*2. Invermay Agricultural Centre, Mosgiel, New Zealand*

*3. Grasslands Research Centre, Palmerston North, New Zealand*

Genotyping-by-Sequencing (GBS) is a method used to develop rapid and cost-effective high-density genetic SNP marker data for diverse applications in biology. The NZ Genomics for Production and Security programme (MBIE C10X1306) has developed the infrastructure and skill base required to apply GBS for different purposes across a wide range of species. Thus far, we have optimised GBS in 50 different species encompassing plants, mammals, shellfish, fish, birds, and insects and have processed well over 100,000 samples. The methodology has been scaled from small sample sizes (< 100) for diversity studies up to many thousands in animal and plant breeding programmes where GBS underpins parentage determination and genomic selection. GBS is also being extended into population and conservation genetics studies. Continuous improvements in wet-lab methods have enabled increased quality and quantity of data generated, with high reproducibility. Furthermore, data analysis has been enhanced through improved bioinformatic pipelines, including a novel statistical method (KGD; Dodds et al., BMC Genomics (2015) 16:1047) designed specifically for utilising GBS data to develop genomic relationship matrices. KGD is particularly suited for the low depth sequencing frequently found with GBS allowing SNP markers with low coverage to be included rather than discarded. In addition KGD does not require imputation making it computationally favourable over other methods. Components of the data analysis pipeline are made available in a public Github repository (https://github.com/Agresearch). Many studies are in collaboration with NZ Crown Research Institutes, universities & research organisations, as well as commercial entities, both nationally and internationally.

**id #678**

## Environmental DNA monitoring detects habitat-specific species assemblages in the marine ecosystem.

**Gert-Jan Jeunen**[1], **Hamish Spencer**[1], **Miles Lamare**[1], **Michael Knapp**[1], **Neil Gemmell**[1]

*1. University of Otago, Dunedin, OTAGO, New Zealand*

**Background:** Declining global biodiversity is driving efforts to find effective approaches to aid ecosystem conservation and management. New tools to rapidly and accurately gather biodiversity data are necessary for informed management. Metabarcoding of environmental DNA (eDNA) allows the simultaneous identification of multiple species from DNA present in environmental samples without biological source material. This technique offers the possibility of monitoring substantial components of biodiversity in a non-invasive, economical and timely manner. An eDNA approach could help marine ecosystems, due to their reduced accessibility, cryptic species, and poorly known taxa. However, water movement between habitats through currents and tidal influences could transport DNA from one area to another, leading to false-positive species detection and inaccurate biodiversity data. **Results:** We examined the accuracy of the eDNA monitoring method in a marine setting by comparing the eDNA signal between two neighboring sites holding different community assemblages (rocky shore vs. sheltered mudflats, <1km distance). 64 species were identified from three amplicon targets. Community structure analysis found a clear difference in the retrieved eDNA signal between our sites and habitat preference of detected species showed little evidence of DNA transport between habitats. **Conclusion:** Our results prove the accuracy of the eDNA monitoring method for a coastal marine setting by showing a lack of evidence for DNA transport through water movement and the possibility of detecting habitat-specific species assemblages. Metabarcoding of eDNA could alleviate the problems of monitoring biodiversity in the marine environment, by accurately and quickly gathering the necessary data for ecosystem conservation and management.

**id #664**

## Wildlife genomics and conservation – lessons from the koala genome

**Rebecca Johnson**[1]

*1. Australian Museum, Sydney, NSW, Australia*

Whether it is policing the illegal rhino horn trade, genome mediated conservation of the koala, or protecting Australia's borders from invasive species, the research undertaken at the Australian Museum, utilising its extensive natural sciences collections, has never been more relevant or translational.

This talk will focus particularly on case studies from Rebecca's work in the illegal wildlife trade and include lessons learned from the from the koala genome. Koalas, *Phascolarctos cinereus*, are iconic Australian marsupials, famous for their large furry ears, prominent black nose and their diet of toxic eucalyptus leaves. The koala is estimated to generate >AUD$1.5 billion per annum in tourism, yet represents a 'conservation conundrum' throughout its range on the Australian east coast where it is widely distributed but under threat from urbanisation, habitat loss, climate change and disease (including chlamydiosis). Rebecca will discuss how this genetic resource is of significant value to conservation and management of this important vulnerable species.

**id #775**

## Regulatory small RNAs in bacteria: Annotation and evolutionary origins

**Bethany R Jose**[1]

*1. University of Canterbury, Christchurch, CANTERBURY, New Zealand*

Bacterial sRNAs are small and highly structured RNA molecules whose complex role in regulating prokaryotic gene and protein expression has only recently been realised. Several sRNA families have been found to regulate virulence factors in pathogenic bacteria, and many are expressed in response to specific environmental pressures, making them a pressing area of study. Despite their important biological function, the annotation and discovery of sRNAs is hindered by a lack of sequence conservation across the tree of life. We are investigating the use of hidden markov models (HMMs) to improve sRNA search methods and better understand the mechanisms behind their origins and rapid evolution.

**id #676**

## Pet dogs, citizen science and complex behavioral genetics

**Elinor Karlsson**[1]

*1. Broad Institute of Harvard & MIT, Cambridge, MA, United States*

To be provided

**id #743**

## Zbtb11 regulates *TP53* and is required for definitive haemopoiesis

**Maria-Cristina Keightley**[1], **Joly Kwek**[1], **Graham J Lieschke**[1]

*1. Australian Regenerative Medicine Institute, Clayton, VIC, Australia*

Haemopoiesis caters to the daily demands of producing and maintaining the various blood cell lineages in the right quantities. In response to injury and infection it must rapidly generate vast numbers of specific lineages. It is important to understand the sophisticated mechanisms regulating this balancing act so that therapeutic targets manipulating blood cell production can be developed for disease treatment. ZBTB11 is a ZBTB (BTB/POZ) transcription factor we have shown is required for early myeloid development and subsequently for maintenance of haemopoietic stem cells in the zebrafish *zbtb11* mutant *mne*. Depletion of other cell lineages at later time points supports a broad failure of definitive haemopoiesis in Zbtb11 deficiency. Master myeloid regulators Pu.1, C/ebpα and Gfi1 regulate both zebrafish and human ZBTB11 luciferase reporters cementing a conserved place for ZBTB11 in the haemopoietic transcription factor hierarchy. *Tp53* is upregulated in *mne* neutrophils and is a direct target of ZBTB11 in luciferase assays in human 293-HEK cells. RNAseq profiling of neutrophils identified apoptosis and cell proliferation as Zbtb11 target gene networks. Evaluation of cell death and proliferation show increased apoptosis in *mne* consistent with the location of proliferative cells in WT. At 48hpf, however, there is no detectable overlap between apoptotic cells and neutrophils. EdU incorporation is strikingly absent throughout *mne*, and is p53-independent, pointing to cell cycle arrest as a potential contributor to Zbtb11-compromised haemopoietic depletion. Together these data demonstrate a critical role for Zbtb11 in zebrafish haemopoiesis and identify Zbtb11-centered pathways that are conserved from zebrafish to mammals.

**id #706**

## functional analysis of a gout-associated noncoding snp variant

**Sarada Ketharnathan**[1], **Megan Leask**[1], **Tony Merriman**[1], **Julia Horsfield**[1]

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Gout is a type of chronic arthritis characterized by high levels of uric acid in the blood, and has an unusually high incidence in Maori and Pacific peoples. Genome-wide association studies identified about 30 genetic loci that increase the risk of high serum urate levels and/or gout.

Here, we functionally characterized a gout-associated single nucleotide polymorphism, rs1967017 located upstream of the *PDZK1* gene, in a region that is highly enriched in gene regulatory features. We found that this variant region showed enhancer activity in the kidney cell line, HEK293 using a luciferase-based reporter system. Enhancer assays in zebrafish further revealed that the enhancer activity is highly specific to the pro-nephric ducts, which is also the site of expression of the *pdzk1* gene in zebrafish. Interestingly, rs1967017 disrupts the binding site of the transcription factor HNF4A, which is crucial for kidney and liver development.

We propose that rs1967017 may lead to disrupted serum urate levels by aberrant regulation of *PDZK1* gene expression, assisted by altered binding of the HNF4A transcription factor. This might impair the function of PDZK1 as a scaffolder that holds various urate transporters together.

**id #749**

## Out of Australia - Are New Zealand's extinct giant birds of prey descendants of recent Australian migrants?

**Michael Knapp[1], Jessica Thomas[2], Stefan Prost[3], Olga Kardailsky[1], James Haile[4], Mike Bunce[5], Tom Gilbert[4], Paul Scofield[6]**

*1. Department of Anatomy, University of Otago, Dunedin, New Zealand*

*2. School of Biological Sciences, Bangor University, Bangor, United Kingdom*

*3. Department of Integrative Biology, University of California, Berkeley, USA*

*4. Natural History Museum of Denmark, Copenhagen, Denmark*

*5. Department of Environment and Agriculture, Curtin University, Perth, Australia*

*6. Canterbury Museum, Christchurch, New Zealand*

Prior to human arrival in the 13th century, two large birds of prey formed the top of the food chain in New Zealand. In the absence of non-volante mammals, Haast's Eagle, the largest Eagle in the world, and Eyles' Harrier one of the largest Harrier in the world, had filled ecological niches that are elsewhere occupied by - for example - large cats or canines. Genetic evidence has identified Haast's Eagle as a close relative to the small Australian Little Eagle, but the phylogenetic relationships of Eyles' Harrier are unknown. Here we use mitochondrial genome data to show that, like Haast's Eagle, Eyles' Harrier is a close relative of a small Australian open land species and that both New Zealand island giants diverged from their small Australian relatives as recent as the Pleistocene. The study sheds light on the biogeographic and evolutionary history of New Zealand's prehistoric apex predators.

**id #524**

## Growing a Pair: Directing pluripotent stem cells towards human testis cell lineages

**Ingrid M Knarston[1, 2], Katie L Ayers[1, 2], Irene Ghobrial[1], Alexander N Combes[1, 3], Melissa H Little[1, 2], Andrew H Sinclair[1, 2]**

*1. Murdoch Children's Research Institute, Parkville, Victoria, Australia*

*2. Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia*

*3. Department of Anatomy and Neuroscience, The University of Melbourne, Parkville, Victoria, Australia*

**Background:** Human gonads initially develop in a bipotential form that subsequently differentiates into either testes or ovaries. Disruptions to gonad development often result in Disorders of Sex Development (DSD) in humans. Genomic analysis of these patients has identified variants in both known and novel DSD genes. However functional analysis of these variants is severely hampered by the lack of a human fetal gonad cell line. Recent studies have differentiated human pluripotent stem cells (hPSCs) into renal lineages. Aggregates of these cells, termed kidney organoids, grown *in vitro*recapitulate features of functional embryonic kidneys.

**Aim:** Given the shared developmental origin of gonad and kidney, we aim to optimise protocols to allow differentiation of hPSCs into male gonad cells, to create “testes in a dish”.

**Methods:** Starting with male hPSCs, ten different growth factor conditions were tested for their ability to induce gonadal fate after seven days treatment. We also undertook a comprehensive analysis of RNA-Seq data to determine markers of the bipotential gonad and fetal testis. Differentiated cells were screened using qRT-PCR and immunofluorescence.

**Results:** Differentiation studies showed high induction of bipotential gonad markers when hPSCs are treated for four days with CHIR99021 followed by three days with FGF9/BMP4. Furthermore, up-regulation of the testis marker *SOX9* indicated these cells are differentiating towards a testis fate.

**Conclusions:** These data provide evidence for the induction of gonadal lineages from hPSCs. We are now aggregating these differentiated cells to create testis organoids.

**Funding Source:** Murdoch Children’s Research Institute

**id #698**

## Rock wallabies as a model for chromosome speciation: fine-scale mapping of chromosome rearrangements.

**Maya Kruger-Andrzejewska[1], Janine Deakin[1], Sally Potter[2], Mark Eldridge[3]**

*1. University of Canberra, Bruce, ACT, Australia*

*2. Australian National University , Canberra*

*3. Australian Museum Research Institute, Sydney*

Despite speciation being a fundamental biological process crucial to creating biodiversity, we still lack a complete understanding of the mechanisms involved. Chromosome rearrangements are known to actively contribute to the divergence of some species, however, the mechanisms driving these genetic incompatibilities that lead to speciation have been much debated. Three mechanisms proposed to contribute to chromosomal speciation are being explored in this study. The first is known as underdominance, in which the hybrid offspring of two chromosomally diverse individuals are infertile as a result of meiotic missegregation. A second mechanism, recombination suppression, occurs when rearranged regions of chromosomes are suppressed from recombining at meiosis. The third mechanism explores the role of epigenetic incompatibilities in chromosomal speciation.

We are using a combination of cytogenetic, genomic and epigenetic techniques to determine the mechanisms and synergistic effects of genic and chromosomal variation in causing reproductive isolation in the chromosomally diverse rock-wallabies (*Petrogale*). We target four of the six parapatric northeast Queensland rock-wallabies which have recently diverged and have simple to complex chromosomal rearrangements. The first step in this study is to compare the gene order on chromosomes using fluorescence in situ hybridisation in order to fully characterise the chromosome rearrangements that have occurred across these species. The fine scale gene mapping has detected rearrangements not previously identified using lower resolution cytogenetic techniques. These mapping data will provide the backbone for the genomics and epigenetics aspects of this study.

**id #704**

## Intergenerational effects of atrazine exposure during juvenile development in zebrafish

**Simon SDL Lamb[1], Sheri SLJ Johnson[2]**

*1. Zoology, University of Otago, North Dunedin, Dunedin, New Zealand*

*2. Zoology, University of Otago, North Dunedin, Dunedin, New Zealand*

Anthropogenic environmental stressors are rapidly becoming ubiquitous across environmental systems. Effects in the initial exposed generation have been shown to directly affect fitness (either positively or negatively) in the following generation, as well as a myriad of sub-lethal effects that

may in turn affect reproduction and survival, e.g. behaviour. Recent research highlights possible epigenetic (non-genetic) mechanisms as a key step in the transmission of environmental information, via germ cells, from one generation to the next. Zebrafish (*Danio rerio*) is a model organism frequently used in developmental biology, toxicology, genetic and behavioural studies, and thus makes an ideal candidate to investigate intergenerational and transgenerational effects in a controlled environment. More importantly the collective methylation pattern (methylome; an epigenetic modifier) is shown to be inherited via sperm. Here I investigate how differing levels of atrazine (a common herbicide and endocrine disruptor) exposure at common environmental levels during juvenile development influences the behavioural phenotype (personality traits; aggression, exploration, activity and anxiety/stress responses) of subsequent generations. We also aim to investigate whether alterations in behaviour are underpinned by changes in gene expression.

**id #736**

## Evolution of antibiotic resistance in *Pseudomonas aeruginosa*

**Iain L Lamont[1], Sam L Taylor-Wardell[1], Wayne M Patrick[1], Lois W Martin[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

**Background/ Aims.** *Pseudomonas aeruginosa* infects patients with a wide range of predisposing conditions. The bacterium intrinsically has low antibiotic resistance, but constant exposure to antibiotics results in resistant strains that are difficult or impossible to treat. Resistance arises through mutations that alter antibiotic target proteins or increase efflux of antibiotics from the bacterial cells. However understanding of the genetics underlying resistance is far from complete. The aim of this research was to carry out genome-wide analysis of resistance-causing mutations in order to more comprehensively determine the range of antibiotic resistance genes.

**Methods.** Mutants that were highly resistant to either meropenem or tobramycin, two widely used anti-*Pseudomonas* antibiotics with different protein targets, were selected *in vitro*. Whole genome sequencing was carried out to identify the associated mutations.

**Results.** Fifteen mutants with high resistance to meropenem and thirteen with high resistance to tobramycin were selected. Whole genome sequencing showed that each mutant contains multiple mutations. Many of these are in known antibiotic-resistance genes, validating our approach, but many are in genes not previously recognised as conferring antibiotic resistance. Seven of the meropenem-resistant mutants had large (>200 kb) deletions. The nature of the mutated genes suggests previously under-appreciated mechanisms of antibiotic resistance in *P. aeruginosa*. Bioinformatic analysis indicated that resistance alleles in the laboratory mutants are also commonly present in clinical isolates of *P. aeruginosa*.

**Conclusions.** Whole genome sequencing of *in vitro*-derived mutants provides a powerful tool for identifying genes that contribute to antibiotic resistance of *P. aeruginosa*.

**id #746**

## New structural and functional insights into the type II fungal ATP-binding cassette transporter *Candida albicans* Cdr1

**Erwin Lamping[1], Hee Ji Lee[1], Richard D Cannon[1]**

*1. Sir John Walsh Research Institute, Faculty of Dentistry,, University of Otago, Dunedin, New Zealand*

**Background:** ATP-binding cassette (ABC) transporters are one of the largest protein super-families found in all kingdoms of life. They are active transporters that can be divided into type I, type II, and energy-coupling factor (ECF) importers (only found in prokaryotes), and type I and type II exporters. *Candida albicans* Cdr1 is a member of the pleiotropic drug resistance (PDR) ABC exporter sub-family unique to plants and fungi. Overexpression of Cdr1 causes life-threatening drug resistance in clinical isolates of the opportunistic human fungal pathogen, *C. albicans*. PDR transporters typically have two large extracellular loops, EL3 and EL6, containing PDR transporter-defining motifs, PDRA and PDRB, and EL6-motif and EL6-helix.

**Methods:** The role of the four PDR-specific motifs in Cdr1 structure and function was investigated by alanine scanning mutagenesis and functionally overexpressing and characterizing individual mutants in the genetically modified heterologous host *Saccharomyces cerevisiae* AD$\Delta\Delta$.

**Results:** We discovered seven amino acids in PDRA, PDRB and the EL6 motif critical for pump function and identified three important extracellular disulfide bonds that are conserved in all fungal PDR transporters. Cdr1 has 23 cysteines, and progress has been made towards the creation of an entirely cysteineless, but functional, Cdr1 - an essential first step for cysteine-cross-linking studies to confirm predicted structural interactions.

**Conclusion:** The PDR motifs together with the two large extracellular domains of fungal PDR transporters are stabilized by three unique disulfide bonds, and the interaction between these motifs and the extracellular domains contribute to a transport mechanism that is unique to PDR transporters

**id #657**

## unravelling the genetics of macadamia: integration of linkage and genome maps.

**Kirsty S Langdon[1], Catherine J Nock[1], Graham J King[1], Abdul Baten[1], Bruce Topp[2], Craig Hardner[2]**

*1. Southern Cross Plant Science, Institute for Innovative Agriculture, Southern Cross University, Lismore, NSW, Australia*

*2. Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia*

Background and Aims:

Macadamia is endemic to the lowland subtropical rainforest regions of north east NSW and south east Queensland, Australia. The macadamia industry is based on cultivars from two species *M. integrifolia* and *M. tetraphylla*. In 2016, a draft genome for *Macadamia integrifolia* was published. This draft provides access to most macadamia genes but is highly fragmented. In order to anchor chromosomal scale sequence a high density linkage map is required. This project aims to use genome wide single nucleotide polymorphism (SNP) markers to construct a SNP saturated genetic linkage map for *M. integrifolia*.

Methods:

Mapping populations with over 400 individuals were developed including open pollinated, bi-parental and self pollinated progeny. Total genomic DNA was extracted from individuals in these mapping populations and SNP markers were identified based on a combined process of complexity reduction and next generation sequencing.

Results and conclusions:

Over 3000 SNP markers have been identified and are currently being used to construct maternal, paternal and composite linkage maps. Preliminary results indicate that sufficient data will be generated to construct a high-density composite linkage map. This will improve genome assembly as well

as facilitating gene discovery and marker assisted selection in macadamia breeding. The latest results on the development of genetic linkage maps and progress towards anchoring the genome will be presented.

Funding Source:

Horticulture Innovation Australia, Australian Macadamia Society and Southern Cross University.

**id #670**

## Using population genetics and genomics as next-generation approaches for the control of invasive insects: social wasps as a case study

**Phil Lester[1], Neil Gemmell[2], Dan Tompkins[3], Aidin Jalilzadeh[3], Peter Dearden[4]**

*1. School of Biological Sciences, Victoria University of Wellington, Wellington*

*2. Department of Anatomy, University of Otago, Dunedin*

*3. Landcare Research Limited, Dunedin*

*4. Department of Biochemistry, University of Otago, Dunedin*

A major goal of New Zealand's National Science Challenge is to develop socially acceptable, cost-effective and highly targeted nest generation technologies for pest control. In this talk we will discuss three genetics based approaches being developed for the control of social insects as model pests. The first approach uses the Trojan Female Technique, which utilises naturally occurring mitochondrial mutations associated with low fertility. We have identified wasp haplotypes in New Zealand that are associated with low fitness and mathematically modelled their use in pest control. The second approach uses gene silencing or RNAi, referred to in pest control as "the next generation of insecticides". Our preliminary gene silencing trials have indicated it is possible to alter gene expression in social insects. Reducing the expression of immune genes can result in higher levels of virus loads in invasive insects, such as Argentine ants. The third suggested approach examines the use of gene drives for the eradication of wasps from New Zealand. Each control approach has advantages, disadvantages and needs social licence to operate before they can be implemented.

**id #541**

## Commemorating 60 years of bidirectional selection: Applying genomics to the Virginia body weight chicken lines

**Mette Lillie, Christa F Honaker[1], Paul B Siegel[1], Örjan Carlborg**

*1. Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA*

This year marks the 60$^{th}$ anniversary of the founding of the Virginia body weight chicken lines. With each year representing one generation of bidirectional selection on 8-week body weight, this White Plymouth Rock population represents a valuable resource for studying long-term selection for a complex trait. Response to selection has resulted in over 16-fold difference in the body weight between the high and low line, but also in drastic physiological, metabolic, and behavioural changes. We apply pooled genome sequencing to the Virginia lines to characterise the genomic consequences of this long-term selection. We characterised over 50 regions with extreme differentiation between the two lines, revealing the wide genomic impacts of this selection regime. Overlap between differentiated regions and growth QTL previously mapped in this population lend support to these candidate selective sweeps. We observed a general trend across the candidate sweep regions where one line was fixed, creating long homozygous blocks, whereas the other line would still maintain high heterozygosity, with many haplotypes continue to segregate. Therein lies an indication of the haplotypic complexity that would have been present in the founder population. By investigating the significant *growth1* QTL on chromosome 1 of the pooled genomes, we are able to show this haplotypic complexity in more details and identify a strong candidate variant present from standing genetic variation. Overall, we have gained better insights into the genomics of this experimental model system, where standing variation at many loci have contributed to the strong response to bidirectional selection.

**id #680**

## Independent loss of introns during evolution across multiple fungal clades

**Chun Shen Lim[1], Christopher Brown[1]**

*1. Department of Biochemistry, University of Otago, Dunedin, New Zealand*

Most eukaryotic genes are interrupted by introns. Intron removal requires an expensive, complex splicing mechanism. Since discovery, introns have been shown to have multiple roles in transcription and translation. Here, we used over 600 fungal genomes to investigate the evolution of introns in fungi. Current evolutionary models propose that the genes in the last eukaryotic common ancestor have a density of introns of ~4 intron/kb. Intron density varies widely in fungal kingdom, ranging from 0.1 intron/kb in the *Saccharomyces cerevisiae* (less than 5% of genes have introns) to over 4 intron/kb in other ascomycetes. Some distantly related basidiomycetes also have low intron density, e.g., below 0.5 intron/kb in *Ustilago maydis* (only 28% genes have introns). This raises the question — why do fungi retain introns when most can be dispensed with? To address this question, over 1000 fungal orthologous genes were identified and patterns of intron distribution examined. We found that the introns that are independently retained during evolution are enriched in some classes of genes, e.g., ribosomal protein genes. Whereas introns are depleted in others, e.g., base-excision repair genes. Analysis of publicly available ribosome profiling datasets for intron-poor *S. cerevisiae*, *Candida albicans, Schizosaccharomyces pombe*, and *Neurospora crassa* suggest that the remaining introns are associated with genes with higher translation efficiency. These findings suggest that one reason for the retention of introns during evolution relates to a role in translation.

**id #551**

## New insights into sexual plasticity in fish using RNA-Seq

**Hui Liu[1], Melissa S Lamm[2], Kim Rutherford[1], Erica V Todd[1], Michael A Black[3], John R Godwin[2], Neil J Gemmell[1]**

*1. Department of Anatomy, University of Otago, Dunedin, NZ*

*2. Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA*

*3. Department of Biochemistry, University of Otago, Dunedin, NZ*

Most plants and animals reproduce sexually with individuals developing as either male or female and remaining the same sex throughout life. However, in some vertebrates, notably teleost fishes, individuals do not lose the potential of sexual plasticity during their life cycle. One of the most

dramatic examples is functional sex change, which is widespread in many marine fishes. For example, the Caribbean bluehead wrasse (*Thalassoma bifasciatum*) displays socially-controlled female-to-male (protogynous) sex change that entails radical alterations at multiple levels, which include a complete ovary-to-testis transformation occurring in 1 to 2 weeks. The molecular basis underpinning such sexual plasticity remains poorly understood. Here we present the first global analysis of gonadal gene expression across protogynous sex change using RNA-sequencing approaches. Whole-transcriptome expression analysis in bluehead wrasses revealed a clear trend whereby many female-pathway genes were steadily down-regulated until mid sex-change stages when a male-specific expression network was progressively up-regulated. The gonadal aromatase gene that governs estrogen production was silenced prior to other feminizing genes, indicating that interruption of estrogen signaling is a key event initiating gonadal sex change. We also identified genes and pathways never before implicated in sex determination or differentiation in any vertebrate system, and interesting expression patterns of some key sex-related genes that indicate novel roles of these genes in regulating sex change. These results provide knowledge of how a usually committed developmental process remains plastic in sex-changing fishes, which is of fundamental importance for understanding sexual plasticity and the evolution of vertebrate sex determination and differentiation systems.

**id #686**

## To the Caribbean and beyond: complete mitogenomes of ancient guinea pigs as a proxy for human interaction post-AD500

**Edana Lord[1], Lisa Matisoo-Smith[1], Susan deFrance[2], Michelle LeFebvre[3]**

*1. Department of Anatomy, University of Otago, Dunedin, Otago, New Zealand*

*2. Department of Anthropology, University of Florida, Gainsville, Florida, United States*

*3. Florida Museum of Natural History, University of Florida, Gainsville, Florida, United States*

The late ceramic age (AD500-1500) in the Caribbean was associated with increased interaction between the islands and mainland South America. The domestic guinea pig (*Cavia porcellus*) was introduced to the Caribbean post-AD500 through human transportation. This study aimed to use guinea pigs as a commensal model for identifying likely human migration routes and interaction spheres within the wider Caribbean region, using complete mitogenomes of ancient guinea pigs. Possible origins of early historic European and North American guinea pigs were also determined.

Complete mitogenomes of 23 ancient and two modern guinea pigs were obtained. The identified haplogroups indicate that two introductions of guinea pigs to the Caribbean occurred, and that ancient Caribbean guinea pigs were most closely related to those from Peru. The first introduction occurred through previously established trade networks from Peru through coastal Colombia to Puerto Rico post-AD500. A second introduction occurred post-AD1000 to the Southern Lesser Antilles, likely as a result of coastal migrations via the northern coasts of Colombia and Venezuela into the Caribbean. A potential origin for European domestic guinea pigs was found to be in the Andean region encompassing Peru and Bolivia and a historic period North American guinea pig was found to have come from the Caribbean. This study is the first to use next-generation sequencing to obtain complete mitogenomes of a commensal animal to investigate prehistoric interaction in the pan-Caribbean region, and results are in agreement with current archaeological evidence for human mobility in the Caribbean.

**id #543**

## Evolving Eusociality: Using *Drosophila* to understand how queen pheromone inhibits reproduction in worker honeybees

**Mackenzie R Lovegrove[1], Elizabeth J Duncan[2], Peter K Dearden[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

*2. School of Biology, University of Leeds, Leeds, United Kingdom*

Eusociality is the social structure in which one individual reproduces, and the others rear her offspring. Queen honeybees produce QMP, which represses reproduction in their workers. This repression is through the highly conserved Notch cell signalling pathway. Interestingly, the non-social, 350 million year diverged *Drosophila melanogaster* also has their reproduction impaired by exposure to honeybee QMP, similar to the repression observed in worker bees.

We aim to understand how eusociality has evolved in honeybees, in particular how queen mandibular pheromone (QMP) induces reproductive constraint in the worker caste. We are using the easily manipulable and genetically tractable *Drosophila melanogaster* to investigate this process.

Confirming previously published results; we show that QMP exposure causes a significant reduction in the number of mature oocytes in *Drosophila* ovaries. We have demonstrated that this response is plastic and reversible by removing *Drosophila* from the QMP source and allowing ovarian development to proceed, leading to a significant recovery of phenotype. RNA-seq is currently being carried out on ovaries from QMP exposed *Drosophila* across various time points, as well as those with recovered phenotypes. This allows us to identify genes that alter their expression during this removal of QMP.

That *Drosophila* responds to QMP despite being so diverged, implies that there may be ancestrally conserved mechanisms by which insects respond reproductively to their environment that have been co-opted into this role in honeybees.

**id #787**

## Using large-scale genomic databases to interpret genetic variation

**Daniel MacArthur[1]**

*1. Massachusetts General Hospital & Broad Institute, Boston, MASSACHUSETTS, United States*

Despite the tremendous successes of genomic approaches to rare disease diagnosis, the majority of rare disease patients remain undiagnosed, and the majority of the genes underlying these diseases remain undiscovered. One of the most powerful resources for understanding the functional impact of human genetic variation is the distribution of naturally occurring genetic variation across the population. In this talk I'll describe the development of massive-scale resources of human genetic data, spanning exome and genome data from over 135,000 individuals, and the applications of these resources to improving diagnosis of rare disease, understanding the patterns of constraint against gene-disrupting variation, the penetrance of disease-causing variation, and the likely feasibility of specific genes as therapeutic drug targets.

## id #547

## genome-wide association study of gout in 111,098 people of european ancestry

Tanya J Major[1], Murray J Cadzow[1], Marilyn E Merriman[1], Amanda J Phipps-Green[1], Ruth KG Topless[1], Abhishek Abhishek[2], Mariano Andreas[3], Linda A Bradbury[4], Russell RC Buchanan[5], Katie Cremin[6], Cloe Erika de Guzman[4], Janak R de Zoysa[7], Michael Doherty[2], Catherine L Hill[8], Tom WJ Huizinga[9], Timothy L Jansen[10], Matthijs Janssen[11], Leo AB Joosten[12], Fina AS Kurreeman[9], Susan Lester[8], Frederic Liote[13], Donia McCartney[14], Hirotaka Matsuo[15], Geraldine M McCarthy[16], Sally PA McCormick[1], Rinki Murphy[7], Karel Pavelka[17], Fernando Perez-Ruiz[18], Juan Garcia Puig[19], Tim RDJ Radstake[20], Philip L Riches[21], Maureen Rischmueller[8], Ed Roddy[22], Malcolm D Smith[23], Eli A Stahl[24], Blanka Stiburkova[17], Richard S Stubbs[25], Anne-Kathrin Tausche[26], Rosa Torres Jimenez[19], Rob J Walker[1], Ken Yamamoto[27], Matthew A Brown[4], Hyon K Choi[28], Nicola Dalbeth[7], Alex So[29],

Lisa K Stamp[1], Tony R Merriman[1]

*1. University of Otago, Dunedin, New Zealand*
*2. University of Nottingham, Nottingham, England*
*3. Universidad Miguel Hernandez, Alicante, Spain*
*4. Queensland University of Technology, Brisbane, Australia*
*5. University of Melbourne, Melbourne, Australia*
*6. University of Queensland, Brisbane, Australia*
*7. University of Auckland, Auckland, New Zealand*
*8. Queen Elizabeth Hospital, Adelaide, Australia*
*9. Leiden University Medical Centre, Leiden, Netherlands*
*10. VieCuri Medical Centre, Venlo, Netherlands*
*11. Rijnstate Hospital, Arnhem, Netherlands*
*12. Radboud University Medical Centre, Nijmegen, Netherlands*
*13. Hospital Lariboisiere, Paris, France*
*14. Environmental and Scientific Research, Wellington, New Zealand*
*15. National Defence Medical College, Saitama, Japan*
*16. University College Dublin, Dublin, Ireland*
*17. Institute of Rheumatology, Prague, Czech Republic*
*18. Hospital de Cruces, Vizcaya, Spain*
*19. Hospital Uinversitario La Paz, Madrid, Spain*
*20. University Medical Centre Utrecht, Utrecht, Netherlands*
*21. University of Edinburgh, Edinburgh, Scotland*
*22. Keele University, Newcastle, England*
*23. Flinders Medical Centre and Repatriation Hospital, Adelaide, Australia*
*24. Mount Sinai School of Medicine, New Your, United States of America*
*25. P3 Research Limited, Wellington, New Zealand*
*26. University of Dresden, Dresden, Germany*
*27. Kurume University, Kurume, Japan*
*28. Harvard Medical School, Boston, United States of America*
*29. University of Lausanne, Lausanne, Switzerland*

**Background/Aims:** Gout progresses through three disease stages: hyperuricaemia, monosodium urate crystal deposition, and innate immune response to crystals. Genome-wide association studies (GWAS) have provided insight into the molecular control of hyperuricaemia; however, less is known about progression from hyperuricaemia to gout. Our aim was to conduct the largest GWAS of gout (to date) in European people.

**Methods:** This study used three data-sets: EuroGout (2,242 clinically-ascertained cases; 1,302 controls), the Health Professionals Follow-Up (HPFS) and Nurses' Health Studies (NHS) (1,038 self-ascertained cases; 1,095 controls), and UK Biobank (2,432 cases ascertained by self-report, hospital records, and/or urate-lowering therapy use; 102,989 controls). Whole-genome genotyping was performed using the Illumina CoreExome-v24 array (EuroGout), Illumina OmniExpress-v12 array (HPFS/NHS), and Affymetrix Axiom array (UK Biobank). Overlapping markers between arrays (279,939) were identified and associated with gout (adjusted for sex and age) within each data-set separately using PLINK-v1.9. An inverse-variance weighted meta-analysis was then performed per marker using meta-v4.4 within R-v3.2.3.

**Results:** Seven loci had genome-wide significant evidence for an association with gout ($P<5x10^{-8}$) – *ABCG2, GCKR, PDZK1, SLC2A9, SLC17A1-A4, SLC22A12,* and *TRIM46*.

**Conclusions:** These seven loci have all been associated with serum urate levels in previous genome-wide studies. Our data emphasises the relative importance of genetic control of serum urate, compared to the genetic control of monosodium urate crystal formation or the innate immune response, in determining gout. Further analyses are required to assess whether these loci play a role in gout irrespective of their influence on serum urate levels.

**Funding:** HRCNZ

## id #617

## Cell-type specific profiling of chromatin states within the brain

**Owen Marshall**[1]

*1. Menzies Institute for Medical Research, Hobart, TAS, Australia*

A key question in developmental biology is how cellular differentiation is controlled during development. Particular interest has focused upon changes in chromatin state, with transitions between Trithorax-group (TrxG) and Polycomb-group (PcG) states vital for the differentiation of ES cells to multipotent stem cells. Recently a number of other chromatin states have been shown to exist in cell culture, including a repressive "Black"

chromatin state devoid of common chromatin marks. However, little is known as to the role of chromatin states during the development of complex organs such as the brain.

In order to understand the role chromatin states play in neural development, we used the Targeted DamID system to profile chromatin states within the developing fruit fly brain. We obtained genome-wide binding profiles of five key chromatin proteins in three separate cell types – neural stem cells (NSCs), immature neurons and mature neurons – and we determined chromatin states through a Hidden Markov Model approach.

We demonstrate that the majority of genes that are activated during neuronal differentiation are repressed by the Black chromatin state in NSCs. Furthermore, almost all key NSC genes are switched off via a transition to HP1-mediated repression. Interestingly, PcG-mediated repression does not play a significant role in regulating either of these transitions; instead, PcG chromatin specifically regulates lineage-specific transcription factors that control the spatial and temporal patterning of the brain. Combined, our data suggest that forms of chromatin other than canonical PcG/TrxG transitions take over key roles during neural development.

**id #776**

## Structural investigation of the Hsc70 heat shock cognate protein for applications in radiotherapy

**Jamin L.B Martin[2, 1], Sigurd M Wilbanks[2]**

*1. Radiation Oncology, Southern District Health Board, Dunedin, New Zealand*

*2. Department of Biochemistry, University of Otago, Dunedin, New Zealand*

Hsp70s are a ubiquitous family of molecular chaperones and heatshock (or stress response) proteins. Expression of Hsp70 family members has been shown to be induced in many tumour types. This overexpression is likely driven by the stresses of the tumour microenvironment. Hsp70 expression of can be stimulated by the stresses in the tumour cell environment, including pH and loss of proteostasis. In addition, survival in the tumour environment exerts selective pressure on hypoxic cells for increased expression of Hsp70s, including that owing to mutation and genetic rearrangement. This overexpression of Hsp70s contributes to tumour cells being able to thrive in their microenvironment, bypassing limits on their growth and induction of apoptosis. Its increased presence in hypoxic cells confers resilience to damage of radiation therapy, perhaps by contributing to the low biological effectiveness of photons (x-rays). Therefore, pharmacological inhibitors of Hsp70s could potentially increase the effectiveness of conventional radiotherapy, as well as decreasing the risk of resurgent hypoxic cells post treatment. This work is aimed at an x-ray crystal structure of the ATP-bound form of Hsc70, the most abundant, constitutively expressed human Hsp70. It uses variants containing a crosslink in order to stabilize the C-terminal substrate binding domain, increasing the likelihood and quality of protein crystals. Elucidation of the ATP-bound structure of Hsc70 and description of its active site will facilitate inhibitor design. Two crosslinkable variants have been designed and expressed in *Escherichia coli* with sufficient yield and solubility. Crystallization trials will follow purification and oxidative crosslinking.

**id #694**

## Whole genome insight into Kea's alpine lifestyle

**Denise Martini[1], Michael Knapp[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Kea and Kaka are both native threatened parrot species in New Zealand. The two species are thought to have evolved about 5 million years ago, when the uplifting of the Southern Alps created a new alpine niche that allowed for their ecological differentiation. While the Kaka remained a forest specialist, the Kea is the only alpine parrot in the world and as such is particularly exposed to the effects that the current climate change scenario might have on its preferred habitat. Given the relatedness of the two species and the high degree of conservation in avian genomes in general, it is possible to investigate the Kea's adaptations to an alpine lifestyle through a genome comparison with Kaka.

Using NGS techniques for this study we sequenced and constructed a Kaka whole genome assembly. We used it in conjunction with the already available resources for the Kea[1] to identify the genomic differences between the two species. This whole genome approach enables us to recognise neutral and adaptive variation and to find potential candidate genes that might explain how this species copes with such different habitat and resources. The functional variation identified will offer an insight on the Kea's resilience to the warming climate and may help to inform management decisions for the future conservation of this and other species linked to the alpine environment.

[1] Zhang G, Li C, Li Q, Li B, Larkin DM, *et al.* (2014). Comparative genomics reveals insights into avian genome evolution and adaptation. *Science* **346**: 1311–20.

**id #769**

## Ancient DNA contributions to understanding the human settlement of the Pacific

**Lisa Matisoo-Smith[1]**

*1. University of Otago, Dunedin, New Zealand*

Since the 1980s a general scenario regarding the human settlement of the Pacific has been constructed based on linguistic, archaeological and some basic genetic data. This picture focuses on two major colonization events – an early Pleistocene settlement of Near Oceania (Australia, New Guinea and the Solomon Islands) followed by the later Lapita colonization of Remote Oceania (the islands east of the Solomon Islands). These Lapita colonists are generally seen as the immediate ancestors of the Polynesians and most Micronesians. Mitochondrial DNA and whole genome data, both ancient and modern, obtained initially from commensal animals (those transported by humans during colonisation) and more recently from human populations suggest that things may be slightly more complicated than this traditional two wave theory often presented. In this talk I argue that it may be time to reconsider our ideas about population origins and history particularly of Remote Oceania. I will discuss how the data generated from humans, ancient samples of dogs, rats, chickens and various plants introduced to the Pacific are contributing to our understanding of Pacific settlement history.

**id #770**

## Mechanisms of Active DNA Demethylation in somatic cells

**Issam Mayyas[1], Rob Weeks[1], Mark Hampton[2], Ian Morison[1]**

*1. Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand*

*2. Department of Pathology, Christchurch School of Medicine, University of Otago , Christchurch, New Zealand*

While, every cell in an organism is genetically identical there are marked phenotypic differences between tissues and organs that are controlled by epigenetic modifications. The most stable epigenetic modification is methylation of cytosine. Many cancers show significant global loss of methylation. Our research investigates the mechanism of changes in DNA methylation as they occur during human life using a *barcoded hairpin-bisulphite sequencing technique*. We have observed rapid demethylation in cultured Jurkat cells (T cell leukaemia) implicating novel mechanisms of active demethylation that have not yet been recognised by researchers in the field. It is likely that the demethylation pathways that we are studying operate during the onset of cancer and the existence of molecules (ascorbate or transition metals) that alter ten-eleven translocation (TET) activity may have implications for modification of this process. While there is a substantial amount to be done before making therapeutic or dietary recommendations, our results might provide a rationale for long-term intervention to alter an individual's epigenetic risk.

**id #713**

## Gender bias and late onset idiopathic disease.

**Jeremy McCallum-Loudeac[1], Greg Anderson[1], Megan Wilson[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Adolescent Idiopathic Scoliosis (AIS) is a 3-Dimensional rotational curvature of the spine. Affecting between 1-4% of the population, onset typically coincides with puberty in otherwise health individuals. A key characteristic of AIS is an overwhelming sex-bias, 90% of progressive cases are female with no known explanation. Genome wide association studies have recently identified the transcription factor gene, *Lbx1*, as a candidate gene for AIS. *Lbx1* is the only consistently replicated finding across multiple ethnic groups. Lbx1 has a role in spinal cord development, specifying dorsal horn interneuron populations during embryo development. However, how Lbx1 is linked to AIS susceptibility and progression in females is unknown.

We propose a sex-specific interaction between genetic susceptibility and female-specific hormonal changes occurring through puberty. RNA-sequencing was initially conducted to identify any significantly differentially expressed genes in spinal cords from juvenile and adult mice. A total of 382 differentially expressed genes were identified. Ten genes of interest were selected (that were also predicted target genes of Lbx1), and RT-qPCR was used to determine their expression in male and female mice before and after puberty. Subsequent studies into sex-specific interactions occurring across puberty were examined in gonadectomy experiments, with and without hormonal replacement. Together these experiments suggest expression of Lbx1 and downstream targets are altered in response to gonadal hormones.

Future steps will involve determining the function of Lbx1 in the juvenile mouse spinal cord and the generation of a mouse model of AIS (not currently available) using CRISPR-Cas9 gene editing.

**id #528**

## Innovative approaches to disease gene discovery in motor neuron disease

**Emily P McCann[1], Jennifer A Fifita[1], Kelly L Williams[1], Sandrine Chan[1], Ingrid S Tarr[1], Dominic B Rowe[1], Garth A Nicholson[1, 2], Ian P Blair[1]**

*1. Centre for MND Research, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia*

*2. ANZAC Research Institute, University of Sydney, Concord Hospital, Sydney, NSW, Australia*

Motor neuron disease, MND, is an ultimately fatal neurodegenerative disease. Approximately 10% is hereditary (familial). MND is genetically heterogeneous, with over 20 causal and 14 associated MND genes identified to date. Mutations in these genes are the only proven cause of disease, however one third of MND families carry an unidentified mutation. These families are often small and exhibit incomplete penetrance, inhibiting traditional disease gene discovery. Using whole exome sequencing and custom bioinformatics, we employed two innovative approaches to identify novel genetic contributors to familial MND. Analysis of four MND families identified 52, 55, 66 and 112 shared variants respectively. Custom bioinformatics filtering reduced these numbers to 21, 24, 22 and 74. Having exhausted genetic analysis, we assessed the potential pathogenicity of each variant using *in silico* tools, including protein predictions and conservation, genic tolerance, and presence in other MND cohorts, to rank and categorise each as having a high, medium or low likelihood to cause MND. This resulted in just a handful of high priority variants for downstream *in vitro* analysis and confirmation of pathology. Additionally, 34 candidate genes implicated in disease by proteomic, transgenic mouse models or other genetic studies were screened through 61 probands. Five novel variants potentially causing MND were identified and processed through the above pipeline. Further, 14 known variants were found to be either over- or under-represented in MND patients. Elucidating the remaining genetic contribution to MND is crucial for enhancing our understanding of disease and inspiring downstream studies, particularly therapeutic development.

**id #777**

## Inbreeding on the rise: Molecular-based pedigree reveals an early warning sign of gene diversity loss in an island population of Tasmanian devils (*Sarcophilus harrisii*)

**Elspeth A McLennan[1], Rebecca M Gooley[1], Phil Wise[2], Katherine Belov[1], Carolyn J Hogg[1], Catherine E Grueber[1]**

*1. The University of Sydney, Camperdown, Sydney, NSW, Australia*

*2. Save the Tasmanian Devil Program, Department of Primary Industries, Parks, Water and Environment, Hobart, TAS, Australia*

Tasmanian devils have experienced an 85% population decline since the emergence of an infectious cancer, devil facial tumour disease (DFTD). In response, a captive insurance population was established in 2006 with a subpopulation later introduced onto Maria Island, Tasmania. The aim of our study was to 1) examine the genetic parameters of the Maria Island population as a stand-alone site and within its broader metapopulation context, 2) give evidence on the efficacy of assisted colonisations, and 3) inform future translocation events. We reconstructed the pedigree of 86 island-born devils using 31 polymorphic microsatellite loci. We used a combination of molecular and pedigree analysis to monitor change in population genetic parameters since colonisation. Molecular analysis alone revealed no significant change in genetic diversity over the four years of occupation on Maria Island. In contrast, DNA-reconstructed pedigree analysis revealed a statistically significant increase in inbreeding over time due to skewed founder representation. Pedigree modelling predicted that gene diversity would only be maintained above the threshold of 95% for a further 2 years, dropping to 77.1% after 40 years. Modelling of alternative supplementation strategies revealed that introducing eight new founders every three years will enable the population to retain 95% gene diversity until 2056. Our study highlights the value of combining pedigree analyses with molecular data, from both a single-site and metapopulation viewpoint, for analysing changes in genetic parameters within populations of conservation concern. Further, we emphasise the importance of post-release genetic monitoring in an established population, given how quickly inbreeding can accumulate and gene diversity be lost.

## id #765

### Germline Variant in Tumour Supressor *p16$^{INK4A}$* and Long Non-Coding RNA *ANRIL* in Breast Cancer

**Paulomi Mehta[1], Tania Slatter[1]**

*1. University of Otago, Dunedin Central, OTAGO, New Zealand*

**Overview:** Breast cancer is the second most prevalent cancer in New Zealand with a largely unclear genetic etiology. Recent progress has demonstrated that expression of tumour suppressor proteins is regulated by long non-coding RNA. At chromosome position 9p21 the tumour suppressor p16$^{INK4A}$ and the long non-coding RNA *antisense non-coding RNA in the INK4 locus* (*ANRIL*) are both encoded. Inherited variants in *p16$^{INK4A}$* including the minor allele of the rs11515 (C/G500) polymorphism at the 3'UTR region are more frequent in breast cancer patients. The rs11515 allele was associated with increased *ANRIL* and reduced *p16$^{INK4A}$* expression. This study aimed to develop a method to type the rs11515 alleles in formalin fixed tissue for use when only archival material is made available. The study also aimed to confirm the previous association between *ANRIL* and *p16$^{INK4A}$*and identify the cell type responsible for increased *ANRIL* in breast cancer tissues. Lastly the study aimed to investigate *ANRIL* expression in a wide range of tissues to obtain a better understanding of where *ANRIL* is expressed.

***Hypothesis*:** The rs11515 CG genotype is associated with higher *ANRIL* in malignant breast cancer cells. Expression of *ANRIL* will vary between tissues and a better understanding of *ANRIL* expression in multiple tissues will identify future tissue types to investigate the effect of rs11515 genotypes

**Conclusion:** Increased *ANRIL* and *p16$^{INK4A}$* were found in rs11515 heterozygotes whereas the presence of ER/PR status influenced the co-expression in both genotypes. The increased *ANRIL* with the CG genotype was consistent with that found earlier; however, the positive correlation between *ANRIL* and *p16$^{INK4A}$* differed from the inverse correlation found previously. *In Situ Hybridisation* showed *ANRIL* in malignant cells. The multiple tissue based analysis suggested a tissue specific co-expression profiles for *ANRIL* and *p16$^{INK4A}$*

## id #683

### De novo assembly and reconstruction of complete circular chloroplast genomes using Geneious

**Moreland Gibbs[1], Matt Kearse[1], Hilary Miller[1]**

*1. Biomatters Ltd, Auckland, NZ, New Zealand*

Chloroplast genomes contain large (≈25,000 bp) almost perfect inverted repeats (IR). During de novo assembly individual repeats cannot be resolved unless the paired-read insert size is larger than the repeat unit. This means a complete circular plastome cannot be resolved during assembly if using only short-read data. However, the use of paired-read data, combined with identification of the repeat and truncated repeat boundaries, can allow reconstruction of the complete circular plastome.

Geneious contains all of the tools required to do rapid and accurate de novo assembly of chloroplast genomes from short-read NGS data. The NGS data may be derived DNA extracted from purified chloroplasts, or "skimmed" from whole-genome sequence of total DNA derived from chloroplast-rich leaf material. In this poster, we take a short-read NGS data set, available for download from the NCBI Sequence Read Archive (SRA), and describe how to use Geneious to reconstruct a complete, circular, annotated chloroplast genome.

## id #701

### evolution of the odorant receptor multigene family in insects: a tale from coloured fish to flying dragons

**Melissa D Jordan[1], Michael Thoma[1], Amali Thrimawithana[1], Christine Missbach[2], Thomas Buckley[3], Bill S Hansson[2], Ewald Grosse-Wilde[2], Richard D Newcomb[1]**

*1. Plant and Food Research, Auckland, New Zealand*

*2. Institute for Chemical Ecology, Max Planck, Jena, Germany*

*3. Landcare Research, Auckland, New Zealand*

Olfaction is an essential sense in animals especially insects. A large family of odorant receptors (ORs) has evolved in insects, to become involved in behaviours such as finding food, mates and avoiding predators. Insect ORs are ligand-gated ion channels formed as a complex between a ligand-binding OR and an obligate co-receptor, Orco expressed together in each sensory neuron. Genomes of higher insects sequenced to date typically contain one Orco and up to several hundred ligand-binding ORs. We are undertaking genomic and antennal transcriptomic surveys from species at the base of the Hexapoda to understand the origins and patterns of expansion of this multigene family. The presence of multiple Orcos was detected in the antennae of the silverfish, *Lepisma saccharina*. In comparison what look like ligand-binding ORs, together with a single Orco were detected in the antennae of the giant bush dragonfly, *Uropetala carovei*. The nature of the OR complex and what these receptors detect remains to be determined. Phylogenetic analysis with these and other OR sequences is building a picture of the evolution of insect ORs initially through duplication of Orco followed by the evolution and expansion of specialised ligand-binding ORs. This evolutionary scenario remains consistent with the idea that the evolution of the OR multigene family was associated with the evolution of flight in insects and the requirements to rapidly navigate based on volatile cues within a three dimensional gaseous medium.

## id #672

### Decoding obesity & type-2 diabetes co-morbidity

**Tayaza Fadason[1], Cameron Ekblad[1], John R Ingram[2], William Schierding[1], Justin O'Sullivan[1]**

*1. Liggins Institute, The University of Auckland, Auckland, New Zealand*

*2. The New Zealand Institute of Plant and Food Research, Auckland, New Zealand*

The mechanisms that underlie the association between obesity and type-2 diabetes have yet to be fully understood. Here we interpreted the combined impacts of diabetes and obesity associated single nucleotide polymorphisms (SNPs) by integrating data on the genes with which they physically interact and the functional (*i.e.* expression quantitative trait loci [eQTL]) outcomes associated with these interactions. We identified enrichment for spatially regulated genes involved in lipid metabolism in adipose, skeletal muscle, and pancreas (p-value = 1.57 x $10^{-2}$). The spatial eQTL SNP-gene interactions occur in a tissue and disease specific manner. For example, obesity associated eQTL SNP-gene interactions occurred most frequently in the thyroid (23.16%) and tibial nerve (17.90%) while those associated with type-2 diabetes occurred most frequently in the thyroid (22.16%) and subcutaneous adipose (19.32%). Our results are consistent with differential regulation of genes by spatially connected regions that

are marked by disease associated SNPs in tissues involved in regulating energy homoestasis and adiposity. Investigating these putative spatial SNP-gene interactions may shed more light on the development of obesity and type 2-diabetes.

**id #545**

## Is migration propensity linked to genetic "switches" in a great "speciator" silvereyes *Zosterops lateralis*?

**Graeme Oatley[1], Sonya M Clegg[2], Bruce BC Robertson[1]**

*1. University, Dunedin, OTAGO, New Zealand*

*2. Department of Zoology, Edward Grey Institute of Field Ornithology, University of Oxford , Oxford, UK*

Background

The "paradox of the great speciators" has puzzled evolutionary biologists, requiring excellent dispersal ability to explain wide distributions, but reduced dispersal abilities to explain high numbers of divergent, isolated forms. Rapidly changing dispersal abilities are assumed, but identifying a mechanism has proved elusive. We take a novel approach by examining genetic changes at four "migration" genes in a great speciatior, *Zosterops lateralis*.

Methods

Genotypic data at four candidate loci from 320 *Zosterops lateralis* individuals from 21 populations of varying migratory capabilities in Australia and New Zealand (and associated islands), New Caledonia and Vanuatu. Seven neutral genetic microsatellite loci were used to assess neutral genetic variation, and to provide inferences of gene flow among populations. Using linear mixed models, we assessed if allele length variation was significantly associated with migratory ability.

Results

Gene flow estimates inferred that high levels of migration occurred among Australasian populations, particularly Tasmania and New Zealand. Allelic polymorphisms were observed in ADCYAP and CREB1, with CREB1 showing a pattern of longer mean allele length in Australasia/New Zealand populations, but shorter in New Caledonia and Vanuatu populations. Linear mixed models did not show any significant association of mean CREB1 or ADCYAP allele length with inferred gene flow.

Conclusions

The four migration-linked candidate genes do not appear to be associated with migratory ability in the silvereye. This work presents a first look into the genetic mechanisms behind the loss of migratory ability in silvereyes with the next step involving the analysis of genome-by-sequencing SNP data.

Funding

Marsden Fund no: UOO1410

**id #707**

## Cyclotides: deployment of the small circular peptides for butterfly pea (*Clitoria ternatea*) plant defence

**Georgianna K Oguis[1], Edward K Gilding[1], Aaron Poth[1], Mark A Jackson[1], David Craik[1]**

*1. Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD, Australia*

Cyclotides are knotted, head-to-tail cyclised peptides comprising of around 30 amino acids. They are currently being exploited as ultrastable scaffolds for the production of peptide-based pharmaceuticals due to their exceptional stability. Cyclotides also merit utility in agriculture as they exhibit insecticidal activity, a role in which they are hypothesised to have evolved for. To date, cyclotides are reported in five angiosperm families. Of particular interest in this study, are the cyclotides found in butterfly pea (*Clitoria ternatea*), which is currently the only species in the legume plant family that is known to produce cyclotides. Why the butterfly pea cyclotides are produced in such great abundance, and how these are produced and metabolised, are not well understood. To shed light on these questions, the peptide profiles of over a hundred seed-grown butterfly pea accessions were compared; and the promoter region of the most highly-expressed cyclotide (CterM) in the vegetative tissues was characterised. Results showed that the different accessions have variable cyclotide expressions—some of which do not produce CterM. Further scrutiny revealed that these incurred numerous missense mutations, and variability in the regulatory elements, likely contributing to the lack of CterM expression. The promoter region showed numerous binding sites for WRKY, NAC;NAM and EIN3 transcription factors, recapitulating on the hypothesis that cyclotides are indeed metabolised for plant defence. The results also revealed that there are two unique CterM promoters, hinting to the possibility of having two CterM versions—which may either be located in the same loci or different genomic regions.

**id #554**

## Phenotypically normal worker honey bees (*Apis mellifera capensis*) with three alleles at multiple microsatellite loci

**Benjamin Oldroyd[1]**

*1. University of Sydney, Sydney, NSW, Australia*

*Apis mellifera capensis* (hereafter Capensis) is a subspecies of honey bee, whose distribution is confined to the Cape provinces of South Africa. We induced virgin Capensis queens to reproduce thelytokously (production of female offspring by parthenogenesis) by repeated narcosis with $CO_2$, and then artificially inseminated them with drones from unrelated colonies. Many (24.3%) of the eggs laid post-insemination carried both maternal alleles and the alleles of one or more fathers. We then inseminated four virgin *A. m. capensis* queens with the semen of a single male of another subspecies. Many (16.6%) adult, female, offspring of these crosses carried two maternal alleles and one paternal allele, and the distribution of these alleles was homogeneous across tissues. SNP genotyping of the mothers, the fathers, and the three-allele female progeny showed that the progeny were diploid mosaics that most likely arose when two maternal pronuclei fused with a different sperm nucleus in the egg. We believe that this is the first report of two-zygote genetic mosaics in any insect.

**id #684**

## Germline memory: Understanding epigenetic reprogramming in vertebrates

**Oscar Ortega-Recalde[1], Neil Gemmell[1], Tim Hore[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Primordial germ cells (PGCs) are germinal stem cells, the precursors of gametes – eggs and sperm. In mammals, PGCs are reprogrammed from somatic cells early in development and are subjected to genome-wide erasure of epigenetic modifications, in particular, DNA methylation. This process ensures that non-genetic traits acquired in the lifetime of an organism are almost never inherited to the offspring. In non-mammalian organisms this process has not been studied quantitatively and some studies indirectly suggest absence of epigenetic reprogramming in the germline. Lack of epigenetic reprogramming in PGCs may support a currently unappreciated process of transgenerational inheritance. Using the model organism zebrafish (*Danio rerio*) expressing the transgene *Tg(vasa:vasa-EGFP)*, a specific molecular marker of PGCs, we have isolated these germline cells using fluorescence-activated cell sorting (FACS). Measurement of DNA methylation status is being undertaken using a high-throughput bisulfite sequencing technique called post-bisulfite adaptor tagging (PBAT). Initial bootstrap sampling experiments have shown that relatively few sequencing reads are required in order to accurately estimate genome-wide methylation levels (10,000 reads, ±4.8%), greatly reducing sequencing costs. Our study aims to explore the epigenetic reprogramming dynamics in non-mammalian vertebrates and a potential mechanistic evidence for transgenerational inheritance.

**id #519**

## Identifying methylome changes in response to heavy, long-term cannabis use, in a large longitudinal cohort

**Amy Osborne[1], John Pearson[1], Tim Hore[2], John Horwood[1], Donia Macartney-Coxson[3], Neil Gemmell[2], Martin Kennedy[1]**

*1. University of Otago Christchurch, Christchurch City, New Zealand*

*2. Anatomy, University of Otago, Christchurch, New Zealand*

*3. Biomarkers Team, Health Programme, Institute of Environmental Science and Research Limited (ESR), Porirua, New Zealand*

Marijuana has been highly publicised of late, with controversy and debate surrounding legalisation and its application for medical purposes. These debates have emphasised the medicinal and therapeutic benefits of cannabis, but there is also strong evidence for negative psychosocial consequences of prolonged cannabis use. Much of the evidence on health effects derives from the work of the Christchurch Health and Development Study (CHDS) which has followed the lives of 1265 people since they were born in 1977. Many drugs impact on the pattern of epigenetic marks that control genome function, and while the effects of cannabis at a genomic level are not well understood, we do know that cigarette smokers, have distinct patterns of cytosine methylation compared with non-smokers and these "methylome" changes likely impact on the regulation of genes that underlie the effects of cigarettes. It stands to reason that cannabis may similarly affect the methylome, so we recently carried out a genome wide analysis of methylation in blood of 96 CHDS subjects (48 cannabis smokers vs. 48 controls). Preliminary analyses identified many intriguing differentially methylated regions (DMRs) in heavy cannabis users, the majority are which are novel, and include genomic sites near the opioid receptor (OPRM1) and the glutamate transporter (SLC17A7) genes. Interestingly, these cannabis-associated DMRs do not appear to be differentially methylated in tobacco smokers. We hypothesise that many are specifically affected by cannabis constituents, and that these genomic regions are, or contain, important regulatory sites for genes and biochemical pathways relevant to the effects of cannabis.

**id #786**

## Discovery of new natural products from uncultivated microbes

**Jeremy G Owen[1], Matthew A Storey[1], Luke J Stevenson[1], Mark J Calcott[1], David F Ackerley[1], Robert A Keyzers[1], Liwei Jui[1]**

*1. Victoria University of Wellington, Welllington, WELLINGTON, New Zealand*

Hidden all around us are complex microbial communities in which a myriad of bacterial species must compete with their foes and communicate with their brethren in order to survive. Interactions in these communities are mediated by secondary metabolites, a collection of small molecules whose structures and biological activities are vastly diverse. Beyond their natural roles, microbial secondary metabolites have been incredibly valuable as a source of antibiotics, anticancer agents, immunosuppressants and many other compounds used in both medicine and basic research. Traditionally, microbial secondary metabolites have been examined via the isolation and laboratory culture of microbes, however it is estimated that less than one percent of the microbes present in a given environment are currently able to be cultured in the laboratory. It is now possible to access the biosynthetic potential of the remaining 99 % of bacterial species using a cultivation independent approach to discovery. This entails direct extraction of microbial genomic DNA from environmental samples and archiving this DNA as libraries. Fragments encoding small molecule biosynthesis can then be identified and transferred to a cultivable host that can read the new instructions and build the compounds they specify. In this talk I will discuss experimental and computational methods that allow rapid identification of genome fragments encoding biologically active small molecules. I will also present recent results from screening of New Zealand soil, sea sponge and lichen microbiomes.

**id #763**

## *SLC2A9* and hyperuricemia: Identification of population-specific genetic variants in New Zealand Maori and Pacific (Polynesian) people.

**Padmini Parthasarathy[1], Tony Merriman[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Hyperuricemia (HU), elevated levels of serum urate, is a prerequisite for gouty arthritis. The *SLC2A9* gene that encodes a urate transporter tops the list of hyperuricemic genes [1]. It is a key genetic determinant of serum uric acid (SUA) levels and explains about 3% of SUA variance [2].

Analysis of resequence data to identify and characterize variants within the *SLC2A9* locus conferring susceptibility to hyperuricemia specifically in the New Zealand Māori and Pacific Islander (Polynesian) population.

The *SLC2A9* locus was resequenced in approximately 800 individuals comprising hyperuricemic cases and normouricemic controls**.** Based on self-reported ancestry, the cohort was split into two subsets (Polynesian, n=440 and European, n=368). All Polynesians were from NZ while Europeans were from NZ and the United States. Association analysis was carried out to identify non-synonymous risk variants within the *SLC2A9* locus that confer risk for HU. Multiply adjusted logistic regression analysis was carried out using R.

A total of 3964 variants were identified within the *SLC2A9* locus. Over a hundred variants were found to be significant in the Polynesian population (OR=0.10[0.01;0.88]-5.43[1.93;15.33], $P_{OR}$=0.00028-0.049, $MAF_{controls}$=0.014-0.535, $MAF_{cases}$=0.002-0.546). Twenty five of these variants were found to be Polynesian-specific, eleven known and fourteen novel. These Polynesian-specific variants will be further analysed, annotated, and genotyped in a larger cohort as a continuation of this study.

These findings will aid in the identification of penetrant variants that could be applied in precision medicine and public health genomics.

1. Mandal, A.K., and Mount, D.B. (2015). The molecular physiology of uric acid homeostasis. Annu. Rev. Physiol. 77, 323–345.

2. Köttgen, A., Albrecht, E., Teumer, A., Vitart, V., Krumsiek, J., Hundertmark, C., Pistis, G., Ruggiero, D., O'Seaghdha, C.M., Haller, T., et al. (2013). Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nat. Genet. *45*, 145–154.

**id #722**

## Interactions between PvdA and PvdF, two enzymes involved in pyoverdine biosynthesis in *Pseudomonas aeruginosa*

**Priyadarshini Devi Philem[1], Sigurd Wilbanks[1], Iain L Lamont[1]**

*1. University of Otago, Dunedin, New Zealand*

**Background:**

During infections the bacteria, *Pseudomonas aeruginosa* secretes an iron scavenging compound, pyoverdine. Pyoverdine biosynthetic enzymes are proposed to form a complex, the "siderosome". Pyoverdine consists of a peptide group containing unusual amino acid, formylhydroxyornithine, a chromophore and an acyl side chain. Formyl-hydroxyornithine is synthesized by PvdA and PvdF. PvdA (a monooxygenase), converts ornithine to hydroxy-ornithine, which is highly unstable. PvdF is a formyltransferase which converts hydroxyornithine to formylhydroxyornithine. Formylhydroxyornithine is then incorporated into pyoverdine by a non-ribosomal peptide synthetase. The instability of hydroxyornithine indicates substrate channeling between PvdA and PvdF.

**Aim:**

The aim of this research is to investigate the interaction of PvdA and PvdF.

**Methods:**

A bacterial two-hybrid system and a co-purification pull down method were used to study the interaction of PvdA and PvdF.

**Results:**

The bacterial two-hybrid system was unable to detect the interaction. However co-purification of PvdF with PvdA showed that PvdF interacts with PvdA.

**Conclusions:**

PvdA and PvdF may have a weaker or transient interaction. The observed interaction between PvdA and PvdF is consistent with their existence as part of the siderosome.

**id #546**

## Pleiotropic effect of ABCG2 in gout

**Amanda J Phipps-Green[1], Rebekah Wrigley[1], James Boocock[1], Philip Riches[2], Anne-Kathrin Tausche[3], Timothy R Radstake[4], Matthijs Janssen[5], Leo Joosten[6], Timothy Jansen[7], Alexander So[8], Jennie Harré Hindmarsh[9], Lisa K Stamp[10], Nicola Dalbeth[11], Tony R Merriman[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

*2. University of Edinburgh, Edinburgh, United Kingdom*

*3. University Clinic "Carl-Gustav-Carus", Dresden, Germany*

*4. University Medical Centre Utrecht, Utrecht, The Netherlands*

*5. Rijnstate Hospital , Arnhem, The Netherlands*

*6. Radboud University Medical Center, Nijmegen, The Netherlands*

*7. VieCuri Medical Centre, Venlo, The Netherlands*

*8. University of Lausanne, CHUV, Lausanne, Switzerland*

*9. Ngati Porou Hauora Charitable Trust , Te Puia Springs, New Zealand*

*10. University of Otago, Christchurch, CANTERBURY, New Zealand*

*11. University of Auckland, Auckland, New Zealand*

*Background/Purpose:* The ABCG2 Q141K (*rs2231142*) variant is an established cause of hyperuricaemia in Europeans. Although the effect size of *rs2231142* on serum urate levels is ~60% that of *SLC2A9,* its effect on gout is consistently greater than that of *SLC2A9*[1,2]. We tested the hypothesis that *ABCG2* plays a role in gout additional to causing hyperuricemia by testing for association of *rs2231142* with gout using asymptomatic hyperuricemic controls.

*Methods:* There were 1,672 European gout cases and 15,367 controls and 1,197 New Zealand Polynesian gout cases and 1,371 controls, with Polynesian divided into Eastern (EP) and Western Polynesian (WP). Association testing was performed using logistic regression with adjustment for confounding variables.

*Results:* In European, the 141K allele was strongly associated with asymptomatic hyperuricemia compared to normouricemic controls (OR=1.55, $P$=4.3x10$^{-18}$), and with gout compared to asymptomatic hyperuricemia controls (OR=1.83, $P$=2.6x10$^{-14}$). In Polynesian, 141K was not associated with asymptomatic hyperuricemia compared to normouricemic controls ($P$>0.35), whereas there was a strong risk for gout compared to asymptomatic hyperuricemia (WP: OR=2.35, $P$=3.9x10$^{-5}$; EP: OR=2.15, $P$=0.010). In comparison, *SLC2A9 rs11942223* showed no positive association with gout compared with asymptomatic hyperuricemia in European (OR=0.82, $P$=0.022), WP (OR=0.81, $P$=0.69) or EP (OR=1.39, $P$=0.41).

*Conclusion:* These data are consistent with a role for *ABCG2* 141K in gout pathogenesis when hyperuricemia is established, potentially through formation of monosodium urate crystals and/or regulation of the inflammatory response. In Polynesian people, 141K does not play a role in determining hyperuricemia.

1. Kottgen et al. *Nat Genet* 2013;45:145-54
2. Phipps-Green et al. *Ann Rheum Dis* 2016;75:124-30.

**id #717**

## Emergence of RNA editing in an evolution experiment

**Anthony Poole**[1], **Alicia Lai**[2]

*1. School of Biological Sciences, University of Auckland, Auckland, New Zealand*

*2. School of Biological Sciences, University of Canterbury, Christchurch, New Zealand*

Covello and Gray proposed a model for the evolution of RNA editing wherein genetic drift results in fixation of genetic changes that must be transcriptionally corrected for production of a functional protein product. In this model, the enzymatic activity that alters the transcript is already present, and it is the chance fixation of an editable sequence that leads editing to be necessary for continued protein production. We previously observed that RNA polymerase slippage is required for cotranscriptional correction of frameshifted genes in *Buchnera*, an endosymbiont of aphids. Slippage is an intrinsic property of RNA polymerase, in *Buchnera* and in its relative, *E. coli*. However, *E. coli* lacks frameshifted open reading frames, whereas these are widespread among *Buchnera*, which is prone to Muller's Ratchet. We reasoned that, under conditions where *E. coli* is subjected to bottlenecks, frameshift mutations may become fixed through drift. Following repeated single-cell bottleneck events, we observed emergence of dozens of frameshifts that require slippage for production of protein. We show that slippage has a real fitness cost, and that frameshifting impacts protein production. Our work is consistent with Covello & Gray's evolutionary model. We see no evidence of adaptive cooption of slippage in our bottlenecked lines, consistent with the expectation that the majority of slippage events would yield non-functional or deleterious alternative products. Our results demonstrate the nonadaptive origins of editing, that we conclude selection normally keeps in check, and reveal the initial evolutionary path taken by genes that have evolved additional functions through editing or slippage.

**id #525**

## Hierarchical metapopulation structure in the southern Australia coastal bottlenose dolphin (*Tursiops* cf. *australis*)

**Eleanor AL Pratt**[1, 2], **Luciano B Beheregaray**[2], **Kerstin Bilgmann**[3], **Nikki Zanardo**[1, 2], **Fernando Diaz-Aguirre**[1, 2], **Luciana M Moller**[1, 2]

*1. Cetacean Ecology, Behaviour and Evolution Laboratory, School of Biological Sciences, Flinders University, Adelaide, South Australia, Australia*

*2. Molecular Ecology Laboratory, School of Biological Sciences, Flinders University, Adelaide, South Australia, Australia*

*3. Biological Sciences, Maquarie University, Sydney, New South Wales, Australia*

Little is known about the population ecology of the recently described bottlenose dolphin species *Tursiops australis*. The species is thought to be comprised of small and genetically distinct populations (including sub-populations under increasing anthropogenic threats) and is likely endemic to coastal southern Australia. Mitochondrial DNA (mtDNA) control region sequences and microsatellite loci were used to assess genetic variation and hierarchical population structure of coastal *T.* cf. *australis* across a range of spatial scales and environmental discontinuities between southern Western Australia (WA) and central South Australia (SA). Overall, genetic diversity was similar to that typically found for bottlenose dolphins, although very low mtDNA diversity was found in Gulf St. Vincent (GSV) dolphins. We found historical genetic subdivision and likely differences in colonisation between GSV and Spencer Gulf, outer- and inner-gulf locations, and SA/WA and previously identified Victorian/Tasmanian populations. A hierarchical metapopulation structure was revealed along southern Australia, with at least six genetic populations occurring between Esperance, WA and southern Tasmania. In addition, fine-scale genetic subdivision was observed within each SA/WA population. In general, contemporary migration was limited throughout southern Australia, but an important gene flow pathway was identified eastward along the Great Australian Bight. Management strategies that promote gene flow among populations should be implemented to assist with the maintenance of the inferred metapopulation structure. Further research into the population ecology of this species is needed to facilitate well-informed management decisions.

**id #544**

## A bimolecular luminescence complementation assay to identify a bee-friendly insecticide

**Charlotte Pushparajan**[1], **Andrew G Cridge**[1], **Peter K Dearden**[1]

*1. University of Otago, Dunedin, OTAGO, New Zealand*

A critical event in insect development is specification of embryonic termini by the gap gene *tailless* in a process called terminal patterning.In the majority of insects,including several pest species,nuclear expression of *tailless* is triggered through a MAP kinase signalling cascade initiated by activation of the receptor tyrosine kinase *torso* by its ligand *trunk*.The honeybee is a beneficial insect whose genome lacks orthologues for *torso* and *trunk* and *tailless* expression is not activated by their interaction.Our aim is to identify a selective inhibitor of *torso-trunk* signalling which can be used as an insecticide to disrupt development in pests but spare bees.We have developed a bimolecular luminescence complementation reporter system in Sf9 cells,which allows us to quantify the strength of the interaction and its modulation in response to antagonists.Plasmid constructs encoding *Torso* and its downstream effector *corkscrew* fused to the respective amino- and carboxyl-terminal fragments of *Renilla* luciferase were transiently co-expressed in Sf9 cells,together with or without *Trunk*.Reconstitution of luciferase activity in the presence of substrate was significantly higher when *trunk* was present at 48h post-transfection,indicating ligand-mediated dimerization of *torso* and binding of phosphorylated *corkscrew* to activated *torso*.On the other hand,luciferase activity decreased when cells co-expressing *trunk*, *torso* and *corkscrew* were treated with tyrosine kinase inhibitors.A stable cell line is being generated for antibiotic-inducible expression of the interaction partners.Sustained gene expression at consistent levels in a clonal cell-based assay will improve reproducibility of hits obtained in high-throughput screening of compound libraries in our search for a bee-friendly insecticide.

**id #642**

## Drosophila and mouse genetic models provide clues into the inexplicable tumour suppressor behaviour of FUBP1 in oligodendroglioma

**Linna Guo**[2, 1], **Olga Zaysteva**[2, 1], **Naomi C Mitchell**[2], **Ross D Hannan**[2, 3], **David L Levens**[4], **Leonie Quinn**[2, 1]

*1. University of Melbourne, Parkville, VIC, Australia*

*2. John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia*

*3. Peter MacCallum Cancer Centre, Melbourne, Australia*

*4. National Cancer Institute, NIH, Bethesda, USA*

Human FUBP1 was discovered over 25 years ago, as a single stranded DNA binding protein, and transcriptional activator of the *MYC* oncogene. In accordance, FUBP1 is upregulated in many cancers, including breast, liver, bladder, kidney, lung, prostate. Moreover, our recent *Drosophila* studies revealed the FUBP1 ortholog (dFUBP1/Psi) interacts with the transcriptional Mediator (MED) complex, to integrate developmental signals, activate *MYC* and promote cell and tissue growth in the wing epithelium. Paradoxically, our recent unpublished data demonstrate expansion of the

glial lineage in loss-of-function *dFUBP1* mutants, suggesting anti-proliferative capacity in the context of the brain. The developmental context-dependency of FUBP1 function is also clear from analysis of the *Fubp1* knockout mice; while ***hypo***proliferation is observed in the embryonic blood lineage, ***hyper***cellularity occurs in the brain.

*FUBP1* loss-of-function ranks in the top 10% of predicted driver mutations in oligodendroglioma, the second most common primary brain cancer in adults. The anti-proliferative function of FUBP1 in the mouse and fly brain could provide a rationale for the otherwise inexplicable prediction that FUBP1 behaves as a tumour suppressor in glial lineage tumours. The prolonged survival time (around 15 years) of patients with these low grade, but invasive, brain tumours is associated with significant morbidity. Moreover, they typically recur following current treatments with unpredictable outcomes; some remain low grade while others become more aggressive. Thus, to improve patient outcomes we are currently using our genetic models to determine molecular mechanisms of FUBP1-dependent glial lineage overproliferation, toward development of new prognostic markers and potential drug targets.

**id #531**

## DNA methylation and aging in zebrafish

**Alexandria Ragsdale[1], Tim Hore[1], Sheri Johnson[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

The processes of aging are incredibly complex and still somewhat unclear; however, epigenetic features such as DNA methylation, are beginning to emerge as key markers of the aging process and may constitute a "biological clock". Biological clocks have been the interest of research for some time, and have included telomere length, microsatellite mutations, and tumor suppression gene expression. Nevertheless, DNA methylation clocks appear to be surprisingly accurate, predicting biological age in a wide variety of human cell and tissue types (Horvarth, 2013). Use of methylation clocks could become a standard diagnostic tool for early detection of age-related disease; however, current progress in this area is hampered by the lack of an appropriate model system. In addition to human health, a DNA methylation-based biological clock would be valuable in other model and non-model species, especially in species where age might otherwise be unknown and/or difficult to predict. We aim to produce a DNA-methylation clock for zebrafish (*Danio rerio*), using next-generation bisulfite sequencing, for males aged between 4 and 28 months. Recently, researchers have demonstrated an overall decrease in DNA methylation of somatic cells using a candidate gene approach in zebrafish, but a pattern of hypermethylation in the male germ line with increasing age, which is also in line with our preliminary findings for young *vs.* old males. Determining a DNA methylation-based biological clock in zebrafish will have wide-ranging implications, as zebrafish are a frequently used model organism in aging studies.

**id #751**

## Ancient DNA reveals extinction and replacement of New Zealand's unique flight-reduced semi-terrestrial swans

**Nic Rawlence[1], Afroditi Kardamaki[1], Luke J Easton[1], Alan J D Tennyson[2], R. Paul Scofield[3], Jonathan M Waters[1]**

*1. Otago Palaeogenetics Laboratory, Department of Zoology, University of Otago, Dunedin, New Zealand*

*2. Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand*

*3. Canterbury Museum, Christchurch, New Zealand*

Prehistoric human impacts on megafaunal populations have dramatically reshaped ecosystems worldwide. However, the effects of human exploitation on smaller species, such as anatids (ducks, geese and swans) is less clear. In this talk we apply ancient DNA and osteological approaches to reassess the history of Australasia's iconic black swans (*Cygnus atratus*) including the palaeo-behaviour of prehistoric populations. Our study shows that at the time of human colonization, New Zealand housed a genetically, morphologically and ecologically distinct black swan lineage (*C. sumnerensis*), distinct from modern (Australian) *C. atratus*. Morphological analyses indicate *C. sumnerensis* exhibited classic signs of the 'island rule' effect, being larger, semi-terrestrial and flight-reduced compared to *C. atratus*. Our research further reviewed sudden extinction and replacement events within this anatid species complex, coinciding with recent human colonization of New Zealand. This research highlights the role of anthropogenic processes in rapidly reshaping island ecosystems and raises new questions for avian conservation, ecosystem re-wilding and de-extinction.

**id #766**

## Human papilloma virus in the placenta: the role of p53

**Sankalita Ray[1, 2], Amanda Fisher[1, 2], Sara Bowie[1, 2], Celia Devenish[3], Tania Slatter[1, 2], Noelyn Hung[1, 2]**

*1. University of Otago, Dunedin Central, OTAGO, New Zealand*

*2. Department of Pathology , Dunedin School of Medicine, University of Otago, Dunedin*

*3. Department of Women's and Children's Health, Dunedin School of Medicine, University of Otago, Dunedin*

Pre-eclampsia (PE) is a hypertensive related syndrome in pregnant women, one of main reasons of foeto-maternal morbidity and mortality. Although a lot of research has been done, the pathophysiology of this condition is still unknown. Transplacental viral transmissions are known to cause pregnancy complications. Recent work done in the "Pathogenesis Laboratory" based at the University of Otago, found 100% of PE associated placentas had the high risk (HR) human papilloma virus (HPV) present. However, if and how HPV directly contributes to pregnancy complications remains unknown. Some evidences have shown up-regulated expression of the tumour protein 53 (p53) in PE placentas. This project investigated if HPV was associated with increased p53. Seventy-one placentas were selected based on the presence or absence of HPV from the existing OPuS study and were divided into specific cohorts according to the presence of HR or low risk (LR) HPV along with the presence or absence of pregnancy complications. Three independent methods were used to detect HPV and p53 expression in placentas including immunohistochemistry for HPV L1 and p53 protein, *in situ* DNA hybridization to identify high-risk HPV DNA, and *in situ* RNA hybridization (RNAScope) to identify HR HPV E6 and E7 gene expression. The one-way ANOVA test corrected for multiple comparisons was used to show significantly increased p53 positive cells in the HPV associated cohorts including PE, HR HPV matched, HR HPV non-complicated (all $P < 0.0001$) and LR HPV cohorts ($P < 0.0004$) in comparison with HPV negative cohorts. HPV E6 and E7 expression was detected by RNAscope in all three PE placentas investigated. Increased p53 was correlated with the presence of HR and LR HPV in the placenta. Further investigations are planned to determine if the expression pattern of p53 and HPV are cell type specific and also determine the effect of HPV p53 related pathways.

**id #745**

## Detecting genetic divergence in a relict New Zealand seabird

**Aisling Rayne[1], Chris P Gaskin[2], Mark Miller[3], Stephanie M H Ismar[4, 5], Karen A Baird[6], Tammy E Steeves[1]**

*1. School of Biological Sciences, University of Canterbury, Christchurch, New Zealand*

*2. Northern New Zealand Seabird Trust, Warkworth, New Zealand*

*3. Centre for Tropical Environmental and Sustainability Science, James Cook University, Cairns, Queensland, Australia*

*4. GEOMAR Helmholtz Center for Ocean Research, Experimental Ecology, Düsternbrooker Weg 20, Kiel, Germany*

*5. School of Biological Sciences, University of Auckland, Auckland, New Zealand*

*6. Forest & Bird, Auckland, New Zealand*

The inclusion of a conservation genomic approach promises to be indispensable for detecting genetic divergence, particularly for relatively poorly studied species with divergent phenotypes. We are using genotyping-by-sequencing (GBS) data to determine whether 'summer' and 'winter' breeding populations of New Zealand's threatened Kermadec petrel (*Pterodroma neglecta neglecta*) represent genetically divergent lineages. The Kermadec Island group, ~1,000 km northeast of New Zealand, currently hosts >10,000 breeding pairs of Kermadec petrel in which two divergent breeding behaviours are represented. Most are 'winter' breeders (laying February-April), however a small number 'summer' breeders (~250 breeding pairs, laying October-November) have been identified on the Meyer Islands, 4 km from Raoul Island (the largest of the Kermadec Island group). These 'summer' breeders represent the last survivors of a great population once hosted by Raoul Island that was effectively extirpated during the mid-20$^{th}$ century through predation. Raoul Island became predator-free in 2004 and later expeditions have confirmed that 'winter breeders' have recolonised the island, while 'summer' breeders remain absent. Preliminary genetic evidence based on a small number of known 'winter' (n=22) and 'summer' (n=6) breeders indicates no shared mitochondrial cytochrome oxidase 1 haplotypes. Should genomic data, which will represent both putatively adaptive and non-adaptive variation, indicate that 'winter' and 'summer' breeders are genetically distinct, conservation action for 'summer' breeders will be warranted - particularly if this is further supported by non-genomic data. To this end, our findings will inform a larger interdisciplinary collaboration investigating the genetic, ecological and behavioural distinctiveness of Kermadec petrels across the Indo-Pacific.

**id #724**

## Understanding evolution of ciprofloxacin resistance in *Pseudomonas aeruginosa*

**Attika Rehman[1], Wayne M Patrick[1], Iain L Lamont[1]**

*1. Biochemistry, University of Otago, Dunedin, New Zealand*

**Background/Aims:** The lungs of the patients with cystic fibrosis become chronically infected with the bacterium *Pseudomonas aeruginosa*. Infection can be treated with antibiotics such as ciprofloxacin but ciprofloxacin resistance in *P. aeruginosa* is increasing, making this drug less effective. The mechanisms of ciprofloxacin resistance in *P. aeruginosa* are partially understood. The purpose of this study was to find mutations in the *P. aeruginosa* genome associated with ciprofloxacin resistance in order to understand how the bacteria resist ciprofloxacin and how treatment influences the evolution of ciprofloxacin resistance.

**Methods:** Nine individual ciprofloxacin resistant strains driven from *P. aeruginosa* strain PAO1 were evolved in the laboratory using an antibiotic gradient agar plate method. Minimal inhibitory concentration (MIC) testing and Whole genome sequencing (WGS) was carried out.

**Results:** MIC showed that the evolved strains had ~4000 higher ciprofloxacin resistance than the parental PAO1 strain. WGS showed that mutations in *gyrA* (gyraseA), PA3491(probable ferredoxin), *nfxB* (negative regulator of efflux), *parC/parE* (topoisomerase IV subunits) and *pil* (pilin) genes are strong contributors of ciprofloxacin resistance. Mutations in *parC* were associated with high level of resistance. WGS of strains with intermediate resistance showed that mutations are acquired in a specific order. Comparison of mutations in the lab-evolved *P. aeruginosa* with the sequences of strains from cystic fibrosis patients showed that alleles conferring resistance in laboratory evolved samples are also present in strains from patients. **Conclusion:** Overall, our findings provide new insights into how *P. aeruginosa* evolves resistance to a key anti-Pseudomonal antibiotic.

**id #548**

## Convergence of Deformed Wing virus recombinant strains in honey bee populations with *Varroa*-resistance

**Emily Remnant[1], Stephen Martin[2], Laura Brettel[2], Tjeerd Blacquière[3], Madeleine Beekman[1]**

*1. University of Sydney, Sydney, NSW, Australia*

*2. University of Salford, Manchester, UK*

*3. Wageningen University & Research, Wageningen, The Netherlands*

The viral landscape of honey bees *(Apis mellifera)* has changed dramatically since the emergence of the ectoparasitic mite, *Varroa destructor*. The global spread of *Varroa* caused decreased honey bee health and increased colony losses throughout the beekeeping world. It is now clear that the pathology caused by mites is largely due to the spread of virulent viruses that *Varroa* harbours and transmits to bees upon feeding. In particular, the spread of one RNA virus, Deformed Wing virus (DWV) has been described as a global pandemic: DWV titres increase dramatically upon infestation with mites. However, multiple DWV strains exist, and specific strains are apparently associated with increased virulence. Furthermore, recombination between DWV strains frequently occurs, generating novel viral haplotypes.

In most cases, chemical miticides are necessary to prevent colony loss. However, multiple honey bee populations have naturally evolved or been selected for *Varroa*-resistance. While these populations can withstand *Varroa* infestation, it is unclear whether the bees also differ in their response to viruses. Therefore, we examined the viral landscape of *Varroa*-resistant honey bee populations from Europe, Africa and the Pacific. We find that DWV titres can be extraordinarily high, similar to levels found in collapsing, *Varroa*-sensitive colonies. However, we see multiple instances of similar DWV recombinant strains that have occurred in different populations of *Varroa*-resistant bees. These results indicate that the relationship between honeybees, *Varroa* mites and DWV is constantly evolving, and subtle differences in viral genomes could alter the balance between colony survival and mortality.

**id #716**

## Phylogenetic affinities of white-chinned petrels: questions for conservation management

**Kalinka Rexer-Huber[1], John McEwan[2], Tracey Van Stijn[2], Andrew Veale[1], Jon Waters[1], Bruce Robertson[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

*2. Invermay Agricultural Centre, AgResearch, Mosgiel, Otago, New Zealand*

Background. White-chinned petrels are caught as fisheries bycatch more than almost any other seabird on the planet, but taxonomic uncertainty hinders risk assessment and species management efforts. The focus of uncertainty is in boundaries of taxa in the New Zealand region. We tested whether any NZ colonies (Antipodes, Auckland, and Campbell Islands) are distinct from each other and/or from colonies around other sectors of the Southern Ocean.

Methods. We obtained DNA sequence data for the mitochondrial cytochrome *b* gene (~1143 base pairs, bp), the nuclear intron β-fibrinogen 7$^{th}$ intron (~933 bp), and the mitochondrial control region domain I (~519 bp). Sequences were investigated using maximum-likelihood, Bayesian and distance analyses.

Results. These data did not support a separate taxon for the Antipodes breeding population. Instead, white-chinned petrels from all NZ islands clustered together and a second, separate taxon included all other island colonies. However, whole-genome data (~80,000 SNPs) revealed a mismatch: SNPs confirmed the NZ regional taxon seen with other markers, and showed a previously unsuspected split between colonies in the South Atlantic (South Georgia and Falkland Islands) and southern Indian Ocean (Marion, Crozet and Kerguelen Islands).

Conclusions. These groupings indicate three ocean-basin level genetic management units for white-chinned petrels. The challenge: to manage the vast fisheries bycatch problem according to biologically relevant boundaries that span political boundaries.

Funding. NZ Department of Conservation's Conservation Services Programme (to B.R.), Otago Museum Linnaeus Taxonomy Fellowship and Department of Zoology allocation (to K.R.H.), and Ministry for Business Innovation and Employment project funding (to J.M.).

**id #702**

## WITHDRAWN propr: an r package for measuring associations in any -omics data

**Thomas Quinn[1], Mark Richardson[1], David Lovell[2], Tamsyn Crowley[1]**

*1. Deakin University, Waurn Ponds, VIC, Australia*

*2. Queensland University of Technology, Brisbane, Queensland, Australia*

Correlation is a commonly used method for measuring the association between genomic elements. However, correlation yields spurious (i.e., falsely positive) results when applied to relative data. In contrast to absolute data, relative data contain measurements that only carry meaning when compared to another measurement (e.g., when the two values [50, 100] equate to [500, 1000]). Common examples of relative data include anything measured in percent or parts per million (ppm). However, relative data also can include biological data sets produced by high-throughput RNA-sequencing, chromatin immunoprecipitation (ChIP), ChIP-sequencing, or Methyl-Capture sequencing. Here, we present propr: an R package implementation of proportionality analysis that provides a valid alternative to correlation that is suitable for any and all data sets. Unlike correlation, proportionality yields the same result for relative data as its absolute counter-part, all without generating spurious results. Using a real data set, we show how propr can fit within a larger -omics pipeline to enrich the findings of conventional differential expression analysis. Unlike other analytical pipelines, this one makes no assumption about the underlying distribution of the data and does not require any normalization.

**id #719**

## WITHDRAWN Gene expression in conspecific-supressed cane toad tadpoles

**Mark F Richardson[1], Jack G Reid[1], Michael Crossland[2], Richard Shine[2], Lee A Rollins[1]**

*1. Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Geelong, VIC, Australia*

*2. School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia*

*Background/Aims*

Many anurans exhibit complex interactions with conspecifics during early life-history stages, including plastic responses to chemical cues during development. In the cane toad (*Rhinella marina*) a waterborne chemical cue released by tadpoles suppresses the growth and survival of developing embryos. This species-specific suppressor pheromone might provide an avenue for targeted control across the toad's invasive range in Australia. Yet, we know little about how gene expression changes underlie conspecific suppression.

*Methods*

Using RNA-Seq we quantified gene expression from tadpoles exposed to three older conspecifics during embryonic development (suppressed) and compared the profiles to tadpoles that developed without exposure (control), for four full-sibling clutches – two from both the invasion front and the long colonised range-core.

*Results*

We find significant suppression of growth, but not developmental stage, at the invasion front but not the range-core. However, clutches from both regions exhibit differential expression between treatments. Thirteen genes down-regulated in suppressed tadpoles (from both regions) are mainly involved in regulating innate immune responses. In contrast, uniquely down-regulated genes at the invasion front also regulate immune responses, while those uniquely up-regulated have roles in energy production, maintenance of epithelia and other physiological processes – including development.

*Conclusions*

Our results provide new insights into the physiological processes involved in suppression of conspecifics, suggesting a trade-off between maintaining immune responses and development. The candidate genes identified here could be vital to future efforts of invader control in this species.

*Funding Sources:* CIE, Deakin University (MR); ARC DE150101393 (LA); FL120100074 (RS).

**id #710**

## Using mitochondrial DNA sequencing and nuclear Genotyping-by-Sequencing to identify bycaught seabirds

**Peter A Ritchie[1], Jana R Wold[1], Kate McKenzie[1], Geoff K Chambers[1], Chris J R Robertson[2]**

*1. Victoria University of Wellington, Wellington, NI, New Zealand*

*2. Wild Press, Wellington, New Zealand*

The correct identification of the seabird species caught in fishing gear is of particular conservation concern. Northern Buller's Albatross (*Thalassarche bulleri platei*) and Southern Buller's Albatross (*Thalassarche bulleri bulleri*) are often bycaught by fishing vessels in New Zealand, but it has been difficult to determine the provenance of caught birds and identify species. We used a combination of mitochondrial DNA (mtDNA) and

nuclear genome sequencing to assess the genetic differences between the Buller's Albatross subspecies and whether there is suitable genetic marker for determining provenance. DNA was isolated from samples collected from 73 birds, which comprised of two Northern colonies (n = 26) and two Southern colonies (n = 47). DNA sequences from the mtDNA control region showed a high level of genetic differentiation between the Northern and Southern groups. All bycaught individuals could be assigned to their subspecies group. An analysis of molecular variance did not find any significant population structuring among the breeding colonies of each subspecies. A nuclear DNA set of single-nucleotide polymorphism (SNP) markers was obtained using a Genotyping-by-Sequencing (GBS) approach. We found 26 319 putative loci and 54 061 SNPs. A filtered-set of SNPs showed two distinct genetic clusters that corresponded to Northern and Southern Buller's albatrosses, but there were limited fine-scale differences among colonies. The mtDNA and GBS data sets appear to produce similar findings.

**id #738**

## Mitochondrial variation and heteroplasmy in Australian and Hawai'ian cane toads

**Lee A Rollins[1], Mark F Richardson[1], Daniel M Selechnik[2], Andrea J West[1], Timothy G Amos[3], Richard J Edwards[3], Richard Shine[2]**

*1. School of Life and Environmental Sciences, Centre for Integrative Ecology, Deakin University, Geelong, VIC, Australia*

*2. School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia*

*3. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia*

*Background/Aims*

Invasive species can adapt to new environments despite low levels of standing genetic diversity due to small founding numbers or sequential introductions. The iconic Australian cane toad was sourced from an introduced population in Hawai'i and conflicting evidence exists regarding the level of genetic diversity across these invasions.

*Methods*

We extracted mitochondrial sequence data from the genome of one individual sequenced using the PacBio RSII and Illumina X10 platforms. From these data, we assembled and annotated the mitochondrial genome. RNAseq data from 18 individuals collected from Hawai'i and 68 individuals from Australia were aligned to the reference sequence. We quantified polymorphism across samples and heteroplasmy (multiple mitochondrial haplotypes within individuals).

*Results*

A complete, annotated mitochondrial reference genome was constructed consisting of 18154 base pairs (bp), the largest reported bufonid mitochondrial genome. We aligned RNAseq data to the entire reference sequence, with the exception of a 347bp region containing several 104bp repeats. We identified 16 polymorphisms (17 haplotypes); one haplotype was common to 65 individuals sampled in both introductions. Heteroplasmy was detected at most polymorphic sites and also at multiple sites where the predominant haplotype was common to all individuals.

*Conclusions*

Mitochondrial diversity is low in Australian and Hawai'ian cane toads. Our findings add to the growing body of evidence that heteroplasmy may be ubiquitous across taxa. Selection within heteroplasmic individuals (recently demonstrated in expanding populations) may provide an important source of variation in genetically depauperate populations.

*Funding*

Australian Research Council DE150101393 (LAR) and FL120100074 (RS)

**id #737**

## The genetic and mechanistic basis of worker sterility in the honey bee

**Isobel Ronai[1], Benjamin P. Oldroyd[1], Deborah A. Barton[1], Vanina Vergoz[1]**

*1. The University of Sydney, Sydney, NSW, Australia*

Worker sterility is a defining feature of social insects. To understand how worker sterility has evolved, it is important to identify both its genetic and mechanistic basis. We utilise an 'evo-devo' framework to propose that worker sterility can be conceptualised as 'reproductive control points' (specific mechanisms that reduce the reproductive capacity of workers). We provide empirical evidence for a control point in honey bee (*Apis mellifera*) workers: the environmental cue of the queen's pheromone triggers the abortion of adult honey bee workers' oocytes at mid-oogenesis. We show that when workers are exposed to the queen's pheromone, their germ cells degenerate midway through development. Further, the degeneration of the germ cells has the morphological hallmarks of programmed cell death. We also find that the candidate gene, *Anarchy*, is the most promising gene identified so far for the mid-oogenesis control point in adult honey bee workers. *Anarchy* is associated with the termination of oogenesis and *Anarchy*'s expression is responsive to the presence of the queen in the colony. In addition, *Anarchy*'s transcripts localise to degenerating oocytes at the mid-oogenesis stage of development and when we knocked down the expression of *Anarchy* we found that this gene is associated with the programmed cell death pathway. We also establish that exposure to queen pheromone quantitatively increases programmed cell death activity (caspase activity) in the ovaries of workers. In summary, the mechanism underlying all the reproductive control points, and therefore worker sterility, is likely to be programmed cell death.

**id #779**

## A FRET reporter of conformation in Hsc70

**Malcolm T Rutledge[1], Sigurd M Wilbanks[1]**

*1. Department of Biochemistry, University of Otago, Dunedin, New Zealand*

Hsp70s are the most well conserved protein family known, and yet little of their mechanism within the cell is understood. Hsc70 is a constitutively expressed member of the Hsp70 family found in the cytosol of all eukaryotic cells. Hsc70 has many functions within the cell, including assisting with the folding of nascent peptides, targeting proteins for degradation, and remodelling protein complexes. Hsp70s consist of two highly conserved domains, the nucleotide binding domain (NBD) and the substrate binding domain (SBD), joined by a flexible linker region. The juxtaposition of the two domains is dependent on the nucleotide bound by the NBD. Many previous studies of Hsp70s have relied on the bacterial form, DnaK. In this study, Hsc70 constructs were produced based on previous DnaK versions. Using these constructs, the nucleotide dependant conformational shifts

of Hsp70s were probed by Förster resonance energy transfer (FRET) using fluorescent dyes attached to each domain of the protein. This allowed direct comparisons to be made between Hsc70 and DnaK. One of the aims of this study was to find an Hsc70 construct that would produce a strong FRET signal, which could them be used in future single molecule and *in vivo* studies. The variant E318C/T427C/C574S/C603S gave the strongest signal of the four variants tested. It also showed that while broadly similar, Hsc70 and DnaK conformations are not the same, with ADP-bound DnaK spending less time with the linker region exposed to solvent than does the ADP-bound Hsc70 does.

**id #565**

## Myth or relict: Does ancient DNA detect the enigmatic Upland seal?

**Alexander T Salis[2, 1], Luke J Easton[1], Bruce C Robertson[1], Neil Gemmell[3], Ian W Smith[4], Marshall I Weisler, Jonathan Waters[1], Nic J Rawlence[1]**

*1. Department of Zoology, University of Otago, Dunedin, New Zealand*

*2. University of Adelaide, North Adelaide, SOUTH AUSTRALIA, Australia*

*3. Department of Anatomy, University of Otago, Dunedin, New Zealand*

*4. Department of Anthropology and Archaeology, University of Otago, Dunedin, New Zealand*

The biological status of the so-called 'Upland seal' has remained contentious ever since historical records described a distinct seal from the uplands of New Zealand's (NZ) remote sub-Antarctic islands. Subsequent genetic surveys of the NZ fur seal (Arctocephalus forsteri) detected two highly-divergent mtDNA clades, hypothesized to represent a post-sealing hybrid swarm between 'mainland' (Australia– NZ; A. forsteri) and sub-Antarctic (putative 'Upland'; A. snaresensis) lineages. We present the first ancient-DNA analyses of prehistoric mainland NZ and sub-Antarctic New Zealand fur seals, revealing that both genetic lineages were already widely distributed across the region at the time of human arrival. These findings indicate that anthropogenic factors did not contribute to the admixture of these divergent lineages, and cast doubt on the validity of the Upland seal. Human-mediated impacts on Arctocephalus genetic diversity are instead highlighted by a dramatic temporal haplotype frequency-shift due to genetic drift in heavily bottlenecked populations following the cessation of industrial-scale harvesting. Furthermore, significant population structuring was identified between Australia, New Zealand and the sub-Antarctic, with New Zealand further split into northern and southern groups with a zone of genetic disjunction, possibly resultant of a founder effect following recolonization. These extinction–recolonisation dynamics add to a growing picture of human-mediated change in NZ's coastal and marine ecosystems.

**id #667**

## Using conservation genomics to predict adaptive potential in hihi

**Anna Santure[1]**

*1. University of Auckland, Auckland, AUCKLAND, New Zealand*

Multi-generational studies of organisms in the wild give a unique opportunity to investigate the genetic basis of traits, and of fitness itself. For species of conservation concern, knowledge of the genetic architecture of traits linked to fitness will enable more accurate predictions of adaptive potential, and can also improve our general understanding of the forces maintaining genetic variation despite the effects of drift and inbreeding. Hihi (stitchbird, *Notiomystis cincta*) are a threatened endemic New Zealand passerine that have been undergoing intensive conservation management since the 1980s. They have been studied extensively over that time and are a model species for successful ongoing reintroduction biology. Pedigree, reproductive and morphological data is available for the Tiritiri Matangi island sanctuary population dating back to 2005, and we have recently designed a 55K SNP chip for hihi and genotyped 1,475 individuals. We used this data to determine the genomic architecture of three morphological traits, all of which are linked to fitness, using genome wide association scans (GWAS). We demonstrate that all traits are influenced by many loci distributed throughout the genome, suggesting that future adaptation in this threatened species may be constrained by interactions with other linked traits.

**id #718**

## Identification and characterisation of two putative ecdysteroid kinases in *Drosophila melanogaster*

**Jack Scanlan[1], Rebecca Smith[1], Charles Robin[1]**

*1. University of Melbourne, North Melbourne, VICTORIA, Australia*

Ecdysteroids regulate arthropod development, reproduction and behaviour. In insects, ecdysteroid activity is modulated by reversible conjugation reactions, the most common of which is phosphorylation. However, the functions of specific phosphate conjugates, and the identities of the kinases that synthesise them, are almost entirely unknown. 26-hydroxylation is an intermediate step in the irreversible 26-carboxylation of ecdysteroids, an essential deactivation reaction during development. In the *Drosophila melanogaster* S2 cell line, 26-carboxylation is blocked by the formation of 26-phosphate conjugates, suggesting ecdysteroid catabolism may be regulated by the activity of ecdysteroid kinases throughout insect development. We hypothesise that *CG13813*, a member of the poorly characterised EcKinase gene family, encodes the ecdysteroid 26-kinase active in S2 cells, due to a collated body of published data, including its regulation by ecdysteroids and ecdysteroid-response pathways, and its unique co-expression with the ecdysteroid 26-hydroxylase *Cyp18a1*. Our phylogenetic analyses of EcKinases demonstrate that *CG13813* and its paralog *CG1561* are orthologs of the only characterised ecdysteroid kinase, found in *Bombyx mori*. Functional analyses of *CG13813* and *CG1561* show they are essential for development at the embryonic/larval and pupal stages, respectively. Intriguingly, knockdown of *CG1561* produces a "drowning in food" phenotype post-eclosion and head eversion failure, and its ectopic misexpression during development blocks pupariation, consistent with a capacity to also regulate ecdysteroid signalling. Our ongoing work will explore the functions of ecdysteroid-phosphate conjugates by characterising the genes responsible for their formation.

**id #720**

## GWAS on Post-term Birth: Analysis of Successive Finnish Birth Cohorts Identifies TKT and SSBP2 Loci

**William Schierding[1], Jisha Antony[2], Ville Karhunen[3, 4], Marja Vääräsmäki[3, 4], Steve Franks[3, 4], Paul Elliott[3, 4], Eero Kajantie[3, 4], Sylvain Sebert[3, 4], Alex Blakemore[3, 4], Julia A Horsfield[2], Marjo-Riitta Järvelin[3, 4], Justin M O'Sullivan[1], Wayne S Cutfield[1]**

*1. University of Auckland, Auckland, New Zealand*

*2. Department of Pathology, Dunedin School of Medicine, The University of Otago, Dunedin, New Zealand*

*3. Department of Epidemiology and Biostatistics, MRC–PHE Centre for Environment & Health, School of Public Health, Imperial College London, London, UK*

*4. Center for Life Course Health Research, Faculty of Medicine, University of Oulu, Oulu, Finland*

Gestation is a critical time-point in human development. Deviations from the 37-41 weeks that form the healthy gestational age at birth correlate with both acute and long-term health effects for the child. Both short and long gestational ages at birth (*i.e.* pre- and post-term, respectively) affect a significant portion of the population (rates vary between populations, but each affect over 5% of the population). Additionally, both are heritable and are influenced by the pre- and perinatal environment (*i.e.* infection status, nutrition, antepartum bleeding, twins). Despite the heritable component, the specific genetic influences underlying differences in gestational age are poorly understood. This study identifies genetic variants within the introns of *TKT*, *ARGHAP42*, and *ADAMTS13* genes and intergenic upstream (5') of the *B3GALT5* and *SSBP2* genes that are associated with prolonged gestation in 9,141 Northern Finnish (white European) individuals from two birth cohorts (*i.e.* 1966 and 1986). Spatial and mRNA expression analyses identify the regulatory affects and corresponding consequences of post-term birth. The variants in the *B3GALT5*, *ADAMTS13*, and *TKT* loci are linked to alterations in gene expression levels (*i.e.* cis-eQTLs) with the nearest genes. The SSBP2 and ARGHAP42 loci were not found to be associated with expression differences of the nearest genes. These five loci also have putative effects on the regulation of processes involved in human development such as growth and metabolism, and, more specifically, hematopoiesis. Overall, our findings provide the first evidence of a specific genetic influence associated with prolonged gestation, including putative explanations of the underlying biological cause.

**id #591**

## One big family? Population genetic structure of the endangered black-fronted tern (*Chlidionias albostriatus*)

**Ann-Kathrin V. Schlesselmann[1], Bruce C. Robertson[1]**

*1. Department of Zoology, University of Otago, Dunedin, New Zealand*

*Background/Aims*

Black-fronted terns (*Chlidonias albostriatus*) are nationally and internationally classified as Endangered and only breed in the braided rivers of the South Island, New Zealand. They are in strong decline due to a multitude of threats, predominantly predation as well as ongoing habitat degradation and loss. There is an urgent need to manage black-fronted tern populations and reverse the current population trends. Critical information about the species' biology, particularly the connection of breeding colonies within and between catchments, is however currently lacking. The aim of this research is to fill this gap by assessing the current connectivity between black-fronted tern breeding colonies and characterise the level of genetic diversity within and between them.

*Methods*

We collected DNA of 422 black-fronted terns covering 31 breeding colonies spanning their entire breeding range. Genetically distinct populations were inferred using spatially and non-spatially explicit Bayesian clustering algorithms as well as applying a Discriminant Analysis of Principal Components using 17 microsatellite markers. To complement this, we undertook a phylogeographic analysis using mitochondrial sequence data.

*Results*

We did not find any evidence for an isolation-by-distance pattern on a national scale and clustering and multivariate analyses indicate that genetic diversity within and connectivity between breeding populations is high, despite the strong population declines.

*Conclusions*

We recommend that black-fronted terns are managed as a single evolutionary significant unit (ESU) aiming at retaining the high genetic diversity and connectivity between breeding populations throughout the country.

**id #695**

## Invader immune profiles? Differential gene expression patterns in cane toad populations match predictions about invasive species immune function

**Daniel Selechnik[1], Mark F Richardson[2], Richard Shine[1], Lee A Rollins[3]**

*1. University of Sydney, Sydney, NSW, Australia*

*2. Centre for Bioinformatics, Deakin University, Waurn Ponds, VIC, Australia*

*3. Centre for Integrative Ecology, Deakin University, Waurn Ponds, VIC, Australia*

*Background*

Invasive species lose many co-evolved pathogens and parasites after translocation, but are also faced with novel selection pressures in an introduced range. Thus, the predicted immune profile of an invader involves investment in components of the immune system that are energetically inexpensive, rather than in those that produce excessive inflammation. Because some invaders remain near the site of introduction while others may disperse to novel environments, comparisons between range core and invasion front populations of an invasive species may provide similar results to those between native and newly invasive populations. Since their introduction to Queensland in 1935, cane toads have exhibited remarkable phenotypic flexibility, including in immune function, as their range has expanded across northern Australia. However, the genetic underpinnings of population-level differences in toad immune function have yet to be explored. We sought to investigate whether gene expression differences across toad populations matched predictions about invader immune profiles.

*Methods*

Spleen RNAseq data from toads across their Australian range was used to perform differential expression analysis.

*Results*

Pro-inflammatory genes had higher expression at the ends of the range, while anti-inflammatory genes had higher expression in the middle. Middle populations down-regulated cytotoxic natural killer cells, and up-regulated genes involved in immunoglobulin diversification. These results were consistent with expression patterns across the range determined by soft clustering.

*Conclusions*

Our results suggest that there is indeed an invader immune profile, which may provide insight into how vertebrates cope immunologically with changing environments.

*Funding*

Australian Research Council [FL120100074, DE150101393]

**id #549**

## Association of genetic variants in AAT encoded SERPINA1 gene with gout in Europeans and Polynesian

**Amara Shaukat[1], Amanda Phipps-Green[1], Nicola Dalbeth[2], Lisa Stamp[3], Tony Merriman[1]**

*1. Biochemistry, University of Otago, Dunedin, Otago, New Zealand*

*2. School of Medicine, University of Auckland, Auckland, New Zealand*

*3. Department of Medicine, University of Otago, Christchurch, Otago, New Zealand*

Gout is an auto-inflammatory arthritis caused by deposition of crystallized monosodium urate (MSU) in and around joints in the presence of hyperuricemia. However, there are other factors that control progression from hyperuricemia to gout. Alpha-1 antitrypsin (AAT; alpha1-proteinase inhibitor encoded by SERPINA1), is an abundant hepatic acute phase protein in humans. In response to inflammation AAT levels increase dramatically in blood. Reduced inflammation in an animal model of gout has been observed when injected with alpha-1-anti-trypsin-IgG1-Fc. Moreover, previous GWAS studies in Japanese and Swiss cohorts reported a significant correlation between SERPINA1 polymorphisms and AAT levels. The aim of our study was to test for association of SERPINA1 polymorphisms (*rs12884390*,*rs28929474*,*rs11621961*,*rs4905197*) with gout in European and Polynesian groups. A total of 6268 clinically-ascertained gout cases and 14459 controls of various populations of European ancestry (NZ Caucasian, ARIC, FHS, CARDIA, CHS, UK Biobank) and New Zealand Māori and Pacific (Polynesian) ancestry were utilised. Taqman® genotyping was carried out, followed by multivariate-adjusted (age, sex and self-reported grand-parental ancestry in Polynesian) association analysis in R 3.2.2 with gout as the outcome. The T-allele of SERPINA1;*rs12884390* exhibited significant association with gout risk in European (OR=0.912,$P_{OR}$=4.01×10$^{-04}$ ***). However, a non-significant association of the *rs12884390* T-allele was found with gout in NZ Polynesian (OR= 0.907, $P_{OR}$=0.183). The minor T-allele of the *rs11621961* also conferred a nominal association with gout in Polynesian (OR= 0.872, $P_{OR}$=0.056). This suggests a potential role for the SERPINA1 gene in the inflammatory process that leads to the development of gout.

**id #750**

## Ancient DNA from museum specimens: techniques for minimizing damage

**Lara Shepherd[1]**

*1. Museum of New Zealand Te Papa Tongarewa, Wellington, WELLINGTON, New Zealand*

Museum collections are an important source of DNA, particularly for rare or extinct species, or where historical perspective is required. However, museum specimens are a limited resource and sampling them for ancient DNA studies may restrict their future use for both geneticists and other researchers. To this end sampling methods that minimize damage to specimens are desirable in order to maintain the long-term integrity of museum collections. In this talk I review sampling techniques, which aim to minimize or avoid damage to specimens. I also present a non-destructive sampling technique for herbarium specimens and test its application and success in a range of plant species.

**id #723**

## Determining the regulation of *Lbx1* gene expression during mouse spinal cord development.

**Kathleen J Sircombe[1], Megan J Wilson[1]**

*1. University of Otago, Dunedin, DUNEDIN, New Zealand*

Background

Adolescent Idiopathic Scoliosis (AIS) is a disease of the spine, affecting between 1-3% of the population. This disease manifests as a lateral curvature of the spine that can lead to a range of conditions such as back pain and respiratory issues. Recent advances in genomics have allowed researchers to identify variations in the genomes of affected individuals associated with AIS. Several single nucleotide polymorphism (SNP) identified are linked to the transcription factor *Ladybird Homeobox 1* (*LBX1*) gene, which is important for development of limb muscles and the dorsal spinal cord. We are focussing on rs11190870, a SNP which has been verified to be significant in multiple cohorts.

Aims

The aim of my research is to begin to understand the molecular basis that underlies the pathology of AIS associated SNPs. This will allow us to establish a genetic model for *LBX1*-associated AIS.

Methods

The three main methods of my investigation are ChIP-qPCR, RT-qPCR and *in situ* hybridisation. These techniques allow me to investigate interactions between *Lbx1* and potential regulators of *Lbx1* mRNA, and whether these potential regulators are binding the region surrounding SNP rs1190870 in the dorsal spinal cord of mice.

Conclusions

We anticipated Pbx1/Pbx3 to regulate *Lbx1* mRNA, this has been found to not be the case. I am currently exploring other potential regulators of *Lbx1*. Initial findings show that EZH2 appears to be binding at SNP rs1190870.

**id #517**

## evidence for a large expansion and neofunctionalisation of neuroglobin-like genes in sea anemones

**Hayden L Smith[1], Ana Pavasovic[2], Joachim Surm[2], Matthew Phillips[1], Peter Prentis[1]**

*1. School of Earth, Environmental and Biological Sciences, Queensland University of Technology, Brisbane, QLD, Australia*

*2. School of Biomedical Sciences, Queensland University of Technology, Brisbane, Qld, Australia*

The globin gene superfamily has been well characterised in vertebrates, however, there has been limited research in early diverging lineages, such as phylum Cnidaria. This study aimed to identify globin genes in multiple cnidarian lineages, and to characterise the evolution, structure and expression of these genes. Bioinformatic approaches were used to identify globin genes, and to determine the protein structures and expression patterns for these genes. Analyses have shown that all cnidarians possess neuroglobin-like (Ngb-like) genes. Furthermore, actiniarians have undergone a large-scale expansion of Ngb-like genes with a hexacoordinate conformation, as well as, a neofunctionalisation event resulting in a single lineage of pentacoordinate Ngb-like gene. Some Ngb-like genes displayed tissue and development specific expression with very few orthologous genes similarly expressed across species. Overall our analyses suggest that a Ngb-like gene was probably the ancestral globin gene present in the last common ancestor of eumetazoans. The identification of a large-scale expansion and neofunctionalisation of Ngb-like genes in actiniarians provides an excellent starting point to further our understanding of the evolution and function of the globin gene superfamily.

**id #740**

## Testing the Kinship Theory of Genomic Imprinting in African honey bees

**Nicholas MA Smith[1], Benjamin P Oldroyd[1]**

*1. The University of Sydney, Sydney, NEW SOUTH WALES, Australia*

Colonies of haplodiploid Hymenopteran insects serve as model systems for examining how cooperative behaviour can evolve. More recently, insect colonies have emerged as important systems for understanding within-genome conflict.In eusocial species such as honey bees, queens are polyandrous and mate early in life with 10-20 males. The queen stores the spermatozoa of each drone in an organ known as the spermatheca, and utilises this sperm to fertilize queen- or worker-destined eggs. Honey bee colonies are therefore comprised of subfamilies of workers each of which share the same father. This generates the potential for conflict between males to increase the reproductive success of their female offspring. A father able to influence the expression of genes in offspring, so his daughters are more likely to develop as a queen or a reproductive worker, has a greater probability of reproductive success than another male that fails to do so. Recently, genes that influence worker reproduction have been found to have paternally-biased expression. That is the allele derived from the father is expressed whereas the maternal allele is switched off. This suggests reproductive conflict between the paternal and maternal genomes of females. The mechanism that influences this paternal bias remains unknown, but DNA methylation has been proposed as the most likely.To identify the genes modified by fathers and the mechanisms underlying differentially expressed genes, we generated replicate reciprocal crosses between two African honey bee subspecies. We then sequenced both parents (whole-genome) and offspring (transcriptome and methylome). The current findings will be discussed

**id #527**

## the transcription factor petal loss suppresses growth between sepals in arabidopsis

**David R Smyth[1], Aydin Kilinc[1], Edwin R Lampugnani[1], Martin O'Brien[1], Tezz Quon[1]**

*1. Monash University, Clayton, VIC, Australia*

Flowers develop from hemispheres of undifferentiated cells called floral meristems. Organs arise on the meristem flanks in defined numbers and identities. We have been characterising the role of a transcription factor gene called PETAL LOSS in regulating the development of sepals, the outer leaf-like organs, using the model species *Arabidopsis thaliana*. PETAL LOSS is a member of the plant-specific trihelix family. Loss of function mutants show additional growth between sepals, and outgrowth of the sepal edges. *PETAL LOSS* is specifically expressed in these regions, and its role seems to be to help maintain space between the four sepals, and to limit their lateral growth. Physical interactions occur between PETAL LOSS and AKIN10, the low-energy sensing kinase SnRK1 (orthologous to Snf1 of yeast and AMPK1 of animals). PETAL LOSS also interacts genetically and physically with ROXY1, a glutaredoxin. These proteins can sense hypoxic regions and some activate transcription factors by reducing dithiols. Our working hypothesis is that PETAL LOSS is the target of such sensors of energy deficit and/or hypoxia in specific regions of the developing flower, and that it then induces the expression of growth-suppressing genes. Loss of PETAL LOSS function also leads to the loss of some petals, but this is likely an indirect disruption of auxin signalling of petal initiation. The intricate architecture of the flower may depend on the interplay of many regulatory genes like PETAL LOSS that, together with hormones like auxin, define its blueprint and the diverse development of its floral organs.

**id #784**

## SWItching it up: Purification of fungal SWI/SNF complexes reveals compositional differences from their yeast counterparts.

**Brianna Steed[1], Brendon Monahan[2], Alex Andrianopoulos[1]**

*1. School of BioSciences, The University of Melbourne, Parkville, VICTORIA, Australia*

*2. Walter and Eliza Hall Institute of Medical Research, Parkville, VICTORIA, Australia*

*Talaromyces marneffei* is a pathogenic fungus, endemic to South-East Asia, capable of causing lethal systemic infection in immunocompromised humans. In response to temperature changes, *T. marneffei* alternates between hyphal and pathogenic yeast growth forms: a process known as dimorphic switching. As a potential avenue to design new anti-fungal therapies, we are interested in the molecular mechanism of dimorphic switching and how it is regulated at the chromatin level.

SWI/SNF chromatin-remodelling complexes are evolutionarily conserved, multi-subunit protein complexes, acting as DNA translocases to alter nucleosome position. These complexes regulate transcription by remodelling nucleosomes in the promoter regions of genes, facilitating access to transcriptional machinery. Tandem-Affinity Purification (TAP) coupled with Mass spectrometry (MS) identifies the subunit compositions of the *T. marneffei* SWI/SNF complexes; SWI/SNF and RSC. These purifications reveal compositional differences between the *T. marneffei* SWI/SNF complexes and those purified from yeast, including the identification of four novel proteins conserved across the filamentous fungi.

Purification of SWI/SNF and RSC from the model filamentous fungus *Aspergillus nidulans* suggests these compositional differences are conserved in other filamentous fungi, and confirms the presence of three of these novel proteins in the homologous *A. nidulans* complexes. These findings highlight similarities and differences between the compositions of fungal SWI/SNF complexes and those previously published. Going forward, we have identified clear targets for interrogation of the functions of SWI/SNF complexes in filamentous and pathogenic fungi.

**id #794**

## Maximising genetic diversity in captive breeding for translocation programmes: a conservation genomics approach

**Tammy Steeves[1]**

*1. University of Canterbury, Christchurch, CANTERBURY, New Zealand*

For recovery programmes of 400+ species captive bred for release to the wild across the globe, including more than 20 such programmes in Aotearoa New Zealand, one of the most common questions asked is: How can we choose the 'best' individuals for captive pairing to ensure species have sufficient genetic diversity to adapt to environmental change? The majority of these programmes struggle to use existing methods due to shallow, incomplete or error-prone pedigrees. Using the critically endangered kakī/black stilt as a proof-of-concept, we aim to provide conservation managers of poorly-pedigreed captive populations with a forward-thinking, cost-effective and rapid conservation genomics approach that eliminates the need for robust pedigrees. In this talk, I will present preliminary research for kakī and a second threatened endemic wading bird, tuturuatu/shore

plover, that suggests the most efficient approach for making effective captive pairing decisions is to combine high-throughput next-generation sequencing with an innovative reference-guided approach to generate genomic-based measures of relatedness.

## id #730

### scratched, then sniffed? exploring the role of chemotaxis in host invasion

**Laura RK Stringer**[1], **Monica L Gerth**[1]

*1. Department of Biochemistry, University of Otago , Dunedin*

**Background/Aims:**

Motile bacteria are attracted by certain chemicals and repelled by others, a behaviour that enables them to navigate towards favourable niches for growth and survival. This process – chemotaxis – is used by many plant pathogens, to navigate over the plant surface in order to locate potential entry sites.

*Pseudomonas syringae* pv. *actinidae* (*Psa*) is a destructive pathogen of kiwifruit, that is currently causing severe economic losses in New Zealand and worldwide. *Psa* is motile, and invades host plant tissues through natural openings (*e.g.* stomata, lenticels), or via lesions or wounds. It also has an unusally complex chemosensory system, with 43 putative chemoreceptors encoded in its genome.

The goal of this research is to identify the function of individual chemoreceptors, and to explore their role in the colonisation of kiwifruit leaves by *Psa*.

**Methods:**

Chemoreceptor deletion strains are currently being generated using allelic exchange by homologous recombination. Wild-type *Psa* and deletion strains will be fluorescently labelled, and tested using a variety of approaches, including leaf colonisation and capillary assays.

**Results:**

Progress towards functional characterisation of the fluorescently labelled wild-type and deletion strains will be presented.

**Conclusions:**

By characterising the chemoreceptors of *Psa*, this research will provide important insights into how this bacterium recognizes its host.

## id #742

### Linkage disequilibrium and linked identity by decent for loose linkage

**John Sved**[1], **Igor Chybicki**[2]

*1. Evolution and Ecology Research Centre, UNSW, Sydney*

*2. Department of Genetics, Kazimierz Wielki University, Bydgoszcz, Poland*

# LINKAGE DISEQUILIBRIUM AND LINKED IDENTITY BY DESCENT FOR LOOSE LINKAGE

JOHN SVED, Evolution and Ecology Research Centre, UNSW, Australia
IGOR CHYBICKI, Department of Genetics, Kazimierz Wielki University, Poland

**Abstract.**

Linked identity by descent (LIBD) gives a close approximation to the expected level of linkage disequilibrium (LD) in a simple Wright-Fisher model population (Sved, 1971):

$$E[r^2] = \frac{(1-c)^2}{1+(2N-1)(2c-c^2)} \quad (1)$$

where $r$ is the correlation of gene frequencies, $c$ is the recombination frequency and $N$ is the population size.

Weir and Hill (1980) suggested a different formula:

$$E[r^2] = \frac{(1-c)^2+c^2}{2N(2c-c^2)} \quad (2)$$

which seems inappropriate for very small values of $c$, but can be shown by simulation to work much better than (1) when $c$ becomes large. In particular, for the case $c = 1/2$, for unlinked loci, the two formulae differ by a factor of 2, and (2) is clearly correct.

The essential difference between the two formulae is the $c^2$ term in the numerator of (2). In this study, we show that this term arises from the difference between a haploid and a diploid model, and that the LIBD method can be modified to take this difference into account.



**id #566**

## sex-differential microrna expression in the developing mouse brain

**Susie Szakats[1], Megan J Wilson[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Sex bias in the prevalence of various neurodevelopmental disorders suggests that differences in sex development may leave one sex more susceptible to disease. Subtle differences between the developing male and female brain can be driven by modulating gene expression levels. MicroRNAs (miRNAs), a class of small, non-coding RNAs, function to finely tune gene expression levels but little is known about their role in sex-dimorphic brain development.

We hypothesised that sex-differential miRNA expression occurs during mouse brain development. Small RNA sequencing was performed with RNA isolated from E15.5 mouse brains. Differences in expression were validated using real time quantitative RT-qPCR adapted for miRNA quantification using the PolyA tailing method.

We found 12 miRNAs to be significantly differentially expressed between the sexes (n=3 per sex, Padj < 0.05). RT-qPCR confirmed differential expression of a portion of these miRNAs, including *miR-10*, encoded within the *Hox* gene locus. LNA *in situ* hybridisation targeting *miR-10* showed high expression in the hindbrain and anterior spinal cord of embryos at E15.5. As miRNAs are expressed in a temporally specific manner, novel miRNAs may be specific to the developing mouse brain. Further deep sequencing aims to discover novel miRNAs throughout the genome.

Future work aims to explore the causes and consequence of sex-dimorphic miRNA expression. How embryo sex determines miRNA expression will be investigated by manipulating hormone levels. To confirm predicted target mRNAs I will use luciferase reporter assays. Ultimately, we aim to compare wildtype brain with a knock-out line for a miRNA of interest.

**id #689**

## Modelling rare disease in *Xenopus laevis* using CRISPR/Cas9

**Amy Taylor[1], Caroline Beck[1], Louise Bicknell[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Background and Aims

Meier-Gorlin syndrome is a rare autosomal recessive disorder characterized by a triad of features: short stature, microtia and absent patellae. Patients can also display a range of skeletal abnormalities. Five genes causative genes involved the formation of the pre-replication complex have been identified namely: ORC1, ORC4, ORC6, CDT1 and CDC6 as well as CDC45, a protein involved in the pre-initiation complex.

Skeletal development is conserved between frogs and humans. We aimed to investigate the skeletal features of this disease using cutting edge CRISPR/Cas9 technology to disrupted *orc1* in *X. laevis*.

Methods

gRNA were designed to target X.orc1, these were microinjected into one cell stage embryos along with Cas9 mRNA. We also designed Antisense morpholino oligios against *orc1* which inhibit translation of *orc1* mRNA into protein these were microinjected into two cell embryos.

Results

*X.orc1* edited tadpoles display skeletal abnormalities reminiscent of Meier-Gorlin syndrome including delayed bone age and movement deformities at the knee joint. *X.orc1* edited tadpoles are developmentally delayed and smaller in size when compared with control siblings at late tadpole stages. Surprisingly they do not display obvious growth phenotype early in development.

Antisense morpholino oligios were used in to attempt to recapitulate the early growth phenotype seen in Meier-Gorlin patients. Morpholino oligio treated embryos show demonstrable reduction in growth when compared to control siblings.

conclusions

These results show proof of concept that CRISPR/Cas9 edited *Xenopus* frogs have potential to act as models for rare human diseases.

**id #665**

## Bridging the conservation genetics gap by identifying barriers to uptake for conservation practitioners

**Helen R Taylor[1], Nicolas Dussex[2, 1], Yolanda van Heezik[3]**

*1. Department of Anatomy, University of Otago, Dunedin, Otago, New Zealand*

*2. Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Stockholm, Sweden*

*3. Department of Zoology, University of Otago, Dunedin, Otago, New Zealand*

Despite its recognized importance for species persistence, integrating genetics into conservation management has proved problematic, creating a "conservation genetics gap". This gap could widen with the advent of advanced genomic techniques, but these techniques are undoubtedly important for the future of threatened species management. Genetics is frequently a segregated topic at all major interdisciplinary conservation conferences, leaving interaction between conservation geneticists and practitioners all but discouraged. Bridging the conservation genetics gap requires a clear understanding of the barriers to uptake of genetics by conservation practitioners, but few (if any) papers on this topic involve direct consultation with practitioners themselves. We surveyed 148 conservation practitioners in New Zealand's Department of Conservation. Although practitioners were largely receptive to using genetics for conservation management, access to expertise and funding remain barriers to uptake. Practitioners would like to collaborate with geneticists at universities or other institutes, but do not necessarily know who to talk to or fully understand how genetics might benefit them. We contend these barriers or similar likely exist at an international level, suggest ways they might be overcome and emphasize the need for clearer communication between geneticists and practitioners.

**id #789**

## Identifying functional roles for novel cis-regulatory elements predicted to regulate the *Runx1* gene

**J Marsman[1], Amarni Thomas[1], M Osato[2], J O'Sullivan[3], J A Horsfield[1]**

*1. Department of Pathology, Dunedin School of Medicine, University of Otago, Dunedin, New Zealand*

*2. 2International Research Center for Medical Sciences, Kumamoto University, Kumamoto, Japan*

*3. Liggins Institute, The University of Auckland, Auckland, New Zealand*

*RUNX1* is essential for definitive haematopoiesis and requires precise regulation for normal development. *RUNX1* disruption by mutation or translocation are frequently associated with Acute Myeloid Leukaemia (AML). Despite the well-established role of RUNX1 in haematopoiesis and leukaemogenesis, there is limited knowledge of the *cis*-regulatory mechanisms that drive cell type-specific *RUNX1* expression.

Previously, the +24 *Runx1* mouse conserved non-coding element (mCNE) was identified as an enhancer in haematopoietic cells in zebrafish and mice. The Osato research group identified several additional mCNEs in regions surrounding *Runx1*, which have not yet been tested for functionality.

Circular Chromosome Conformation Capture (4C) identified long range interactions between the *Runx1* promoters and putative *cis*-regulatory elements. This 4C was successfully applied to mouse haematopoietic progenitor (HPC-7) cells and the results provided a clear picture of

chromosome conformation information with potential to regulate *Runx1* expression. Nine putative enhancers were identified to interact with *Runx1*-P1 in HPC-7 cells. Two of the nine enhancers had been predicted by the Osato research group.

Zebrafish enhancer assays show that eight of the nine enhancers drive expression in haematopoietic tissue. Since the putative enhancers interact long-range with *Runx1*, they are likely to have a functional role in regulating *Runx1* expression. This research has contributed to the understanding of *RUNX1* regulation by *cis*-regulatory elements, and it will be of future interest to determine if mutation of these elements contributes to AML development.

**id #533**

## Investigating early genetic regulators of sex change in labrid fish: a multispecies candidate gene approach

**Jodi T Thomas[1], Erica V Todd[1], Hui Liu[1], Florence Gléonnec[1], Melissa S Lamm[2], Kim Rutherford[1], Kiyoshi Soyano[3], John R Godwin[2], P Mark Lokman[4], Neil J Gemmell[1]**

*1. Anatomy Department, University of Otago, Dunedin, New Zealand*

*2. Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA*

*3. Institute for East China Sea Research, Nagasaki University, Nagasaki, Japan*

*4. Zoology Department, University of Otago, Dunedin, New Zealand*

Teleost fishes are the only vertebrate lineage to show sequential hermaphroditism, where individuals change sex as a natural part of their life cycle. Teleost sex change has been well studied at many levels; from understanding sex change in an ecological and evolutionary context, to characterising the behavioural, anatomical, and hormonal changes that occur. However, the molecular mechanisms underlying this remarkable example of sexual plasticity remain elusive. From previous whole transcriptome analyses in a protogynous (female to male) sex-changer, the bluehead wrasse (*Thalassoma bifasciatum*), we have identified candidate genes for instigating sex change. Here, we discuss quantitative real-time PCR (qPCR) expression data for two candidate genes in the gonad (gonadal aromatase, anti-müllerian hormone) and two candidate genes in the brain (brain aromatase, isotocin) across sex change in three related protogynous labrids (wrasses): bluehead wrasse, New Zealand spotty wrasse (*Notolabrus celidotus*), and kyusen wrasse (*Parajulis poecilepterus*). Our qPCR and transcriptome data together support a role for these genes as early regulators of protogynous sex change in labrids. The labridae are a monophyletic family in which protogyny is the ancestral state. Common expression patterns of these candidate genes across wrasse species suggests that the proximate molecular mechanisms underlying sex change may be evolutionarily conserved in this group. These results contribute to ongoing work developing the New Zealand spotty as a local model organism for sex change research, and increases our understanding of molecular processes involved in phenotypic plasticity, sex determination, and development.

**id #682**

## Functional analysis of the SOXB1 bound Nestin enhancer using CRISPR

**Ella Thomson[1], Ruby Moffat[1], Dale McAninch[1], Fatwa Adikusuma[1], Chee H'ng[1], Sandra Piltz[1], Paul Thomas[1]**

*1. School of Biological Sciences, University of Adelaide, Adelaide, South Australia, Australia*

The SOXB1 group of transcription factors (SOX1, SOX2 & SOX3) are expressed within the neural progenitor/stem cells of the CNS during early embryogenesis, and are essential for proper development of the embryo. We have previously reported ChIP-Seq data showing the binding of SOX3 within mouse neural progenitor cells at predicted enhancers within the genome, and discovered a region within the intermediate filament gene, *Nes,* that contains 6 putative SOXB1 binding sites. This region is within a known enhancer commonly used to direct expression within neural progenitor cells in transgenic animals and cell lines. Whilst this region has been used extensively in vitro, it has not previously been removed in vivo; making it difficult to quantify the contribution of the enhancer to overall nestin expression. Using CRISPR we have generated independent mouse models that lack the binding sites, and by using qPCR, in situ hybridisation and immunohistochemisty to assess both RNA and protein expression we show decreased levels of nestin within embryos from 9.5 to 15.5dpc, indicating the enhancer is active during these developmental stages. Curiously, ectopic expression of nestin is also seen within the vasculature of mice lacking the enhancer, a tissue that does not express SOXB1's, suggesting the enhancer may have additional SOXB1 independent repressor functions. This work shows the application of CRISPR in the functional assessment of putative enhancers, whilst also providing greater insight into how the SOXB1 transcription factors control target gene expression, furthering our understanding of SOX proteins in embryonic development.

**id #539**

## genetic effects on social isolation anxiety in zebrafish (*Danio rerio*)

**Hilary Thomson[1], Sheri Johnson[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Differences in personality (within-individual consistency in behaviours across multiple contexts) are a widespread phenomenon across the animal kingdom. Variation in some personality traits has been previously linked to polymorphisms in the Serotonin transporter gene (SERT), associated with anxiety and risk avoidance. Zebrafish (*Danio rerio*) are shoaling fish, so prolonged isolation from conspecifics can cause them stress. Chronic stress may cause changes in individuals' expression of behavioural syndromes; for example, previous research has found higher levels of anxiety in zebrafish after three weeks of individual housing. Accordingly, we explored the effects of social isolation on anxiety in zebrafish. We have undertaken a long-term behavioural repeatability experiment and shown anxiety levels of individual zebrafish, measured as the proportion of time spent in the bottom half of a novel tank over ten minutes, to be significantly repeatable over five weeks. Thus, we used this measure to phenotype zebrafish as anxious or non-anxious. Fish were then either kept individually or in groups for three weeks before reassessing anxiety levels, to determine whether individual housing changed anxiety levels, and whether any changes seen were dependent on initial levels of anxiety. We then examined whether individual variation in anxiety is linked to a genetic polymorphism, by sequencing regions of the SERT genes of the anxious and non-anxious fish. This research will help to refine our understanding of the effects of social isolation on anxiety, as well as identifying a potential genetic basis for these differences in zebrafish and other shoaling fishes.

**id #732**

## Determining the molecular effects of 5-fluorocytosine on the pathogenicity of *Pseudomonas aeruginosa*

**Jessica M Thomson[1], Iain L Lamont[1]**

*1. University of Otago, Dunedin, OTAGO, New Zealand*

Background/Aims

*Pseudomonas aeruginosa* is an opportunistic pathogen responsible for a variety of infections. Like many pathogenic bacteria, *P. aeruginosa* presents a challenge to conventional antibiotic therapeutics because of the evolution of resistance. One approach to antibiotic discovery is to screen drugs approved for other purposes for antibiotic activity. Identified compounds can potentially be repurposed and fast-tracked into clinical use. This approach has identified the antimycotic drug 5-fluorocytosine as reducing pathogenicity of *P. aeruginosa* in a murine infection model. The aim of the presented research is to determine how 5-fluorocytosine reduces pathogenicity of *P. aeruginosa*.

Methods

RNAseq and proteomics were used to identify genes and proteins whose expression is affected by 5-fluorocytosine.

Results

After 4 h of treatment, over 300 genes involved in a variety of different functions and pathways were found to be differentially expressed using RNAseq. A large portion of the genes were pathogenicity-related. In particular, 5-fluorocytosine strongly increased the expression of genes required for the production of pyocyanin. Pyocyanin is implicated in facilitating redox balance in *P. aeruginosa*. A proteomics approach also identified proteins involved in pyocyanin synthesis as upregulated in 5-fluorocytosine treated bacteria.

Conclusions

RNAseq showed that 5-fluorocytosine treatment had a broad effect on *P. aeruginosa* gene expression, likely explaining why this compound reduces pathogenicity. This information on differentially expressed genes is currently being used to help determine the mechanism of action of 5-fluorocytosine on *P. aeruginosa*.

**id #771**

## Purification and chromophore composition of an unusual phycoerythrin from New Zealand red alga, *Polysiphonia strictissima*

**Pauline I Uyseco[1], Malcolm T Rutledge[1], Sigurd M Wilbanks[1]**

*1. University of Otago, North Dunedin, DUNEDIN, New Zealand*

Phycoerythrin is a water soluble photosynthetic protein present in cryptomonads, cyanobacteria, and red algae. The relative amounts of its constituent chromophores, phycourobilin and phycoerythrobilin, determines phycoerythrin's spectral properties and commercial value. Phycourobilin absorbs light at 495 nm, and phycoerythrobilin at 565 nm. With an absorption at 495 nm, and emission wavelength of 575 nm, phycoerythrin is a complementary dye to other dyes, such as fluorescein. With its absorption coefficient and great Stokes shift, phycourobilin-rich phycoerythrin offers enhanced sensitivity for biomedical assays. A phycourobilin-rich phycoerythrin was purified from the New Zealand red algae, *Polysiphonia strictissima*, obtained from the Otago Harbour, and characterised. Purification used differential ammonium sulfate precipitation and anion exchange chromatography. The subunits were then separated by reverse phase chromatography and the ratios of chromophores associated with each subunit were then determined by visual absorption spectroscopy. Peptides containing one chromophore will be generated and characterised by *de novo* sequencing using mass spectrometry to establish at which residue chromophores are covalently attached. *P. strictissima* harvested in the summer carries two phycoerythrobilin chromophores on its Ongoing analysis suggests that during autumn there is a rise in the phycourobilin content of phycoerythrin from *P. strictissima*. Complete analysis of the seasonal variation of this phycoerythrin will determine its potential commercial value, how the seasons affect this, and potentially determining the ideal time for harvest.

**id #516**

## Untangling the evolutionary history of the European Bison (*Bison bonasus*)

**Ayla L. van Loenen[1], Bastien Llamas[1], Julien Soubrier[1], Emilia Hofman-Kamińska[2], Rafał Kowalczyk[2], Alan Cooper[1]**

*1. Australian Center for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, SA, Australia*

*2. Mammal Research Institute, Polish Academy of Sciences, Białowieża, Poland*

The evolutionary history of the European bison (*Bison bonasus*) has often been debated and remains cryptic despite recent ancient DNA and genomic studies. Nonetheless, bison are one of the few species to have survived the mass megafaunal extinction during the Pleistocene/Holocene transition (12-9ky BP), and they can inform on the response of megafaunal populations to periods of rapid environmental change. Ancient DNA (aDNA) provides a unique opportunity to directly observe genetic evolution by investigating the changes in genetic structure of species and populations in real time. A previous study containing predominantly mitochondrial control region sequences as well as 13 complete mitochondrial genomes of bison samples from a restricted geographical range in Eastern Europe has revealed dynamic series of events through time correlated with environmental changes (Soubrier et al., 2016). Here we describe the evolutionary patterns observed in high-resolution mitochondrial sequencing data from over 60 ancient European bison samples across Eurasia, specifically delineating patterns of succession of various bison ecomorphs across a broad geographical and temporal range.

**id #781**

## Genotyping-By-Sequencing for diverse applications

**Tracey van Stijn[1], Shannon Clarke[1], Marty Flaville[2], Andrew Griffith[2], Mingshu Cao[2], Rachel Tan[2], Jeanne Jacobs[3], Rayna Anderson[1], Rachael Ashby[1], Rudiger Brauning[1], Alan McCulloch[1], Ken Dodds[1], John McEwan[1]**

*1. AgResearch, Mosgiel, OTAGO, New Zealand*

*2. Grasslands Research Centre, Palmerston North, New Zealand*

*3. AgResearch Ltd, Lincoln Science Centre, Christchurch, New Zealand*

Genotyping-by-Sequencing (GBS) is a method used to develop rapid and cost-effective high-density genetic SNP marker data for diverse applications in biology. The NZ Genomics for Production and Security programme (MBIE C10X1306) has developed the infrastructure and skill base required to apply GBS for different purposes across a wide range of species. Thus far, we have optimised GBS in 50 different species encompassing plants, mammals, shellfish, fish, birds, and insects and have processed well over 100,000 samples. The methodology has been scaled from small sample sizes (< 100) for diversity studies up to many thousands in animal and plant breeding programmes where GBS underpins

parentage determination and genomic selection. Continuous improvements in wet-lab methods have enabled increased quality and quantity of data generated, with high reproducibility. Furthermore, data analysis has been enhanced through improved bioinformatic pipelines, including a novel statistical method (KGD; Dodds et al., BMC Genomics (2015) 16:1047) designed specifically for utilising GBS data to develop genomic relationship matrices. KGD is particularly suited for the low depth sequencing frequently found with GBS allowing SNP markers with low coverage to be included rather than discarded. In addition KGD does not require imputation making it computationally favourable over other methods. Components of the data analysis pipeline are made available in a public Github repository (https://github.com/Agresearch). Many studies are in collaboration with NZ Crown Research Institutes, universities & research organisations, as well as commercial entities, both nationally and internationally.

**id #550**

## Use it or lose it? The genomic mechanisms underlying wing-loss in New Zealand's alpine stoneflies.

**Andrew Veale[1], Brodie Foster[1], Peter Dearden[1], Jonathan Waters[1]**

*1. University of Otago, Dunedin, New Zealand*

Wing polymorphism is a prominent feature of numerous insect groups, but the evolutionary genomic basis for this diversity remains poorly understood. Stoneflies present an excellent model system for understanding the evolution of insect flight, due to their early divergence within Pterygota (winged insects), and because they exhibit a diversity of wing morphologies and flight abilities. The widespread New Zealand stonefly *Zelandoperla fenestrata* species complex contains populations ranging from fully winged (macropterous) to completely wingless (apterous), with the latter phenotype often present at high altitude. Given the presence of non-dispersive, flightless forms on multiple mountain ranges, separated by lowland winged populations, wing reduction has probably evolved independently multiple times. To study the genomic bases for the apparent convergent evolution of alpine wing reduction in *Z. fenestrata*, we sequenced and assembled a draft genome assembly and transcriptome assemblies using Illumina sequencing. We then constructed GBS libraries from sympatric winged and wingless individuals, sampled along altitudinal transects conducted across several streams. Using outlier detection tests between paired winged and wingless samples, we have identified several highly divergent SNPs, likely to represent islands of divergence linked to the genes responsible for wing-loss in this species. Understanding how these genes control wing development may elucidate critical regulatory pathways in wing developmental biology across Pterygota.

**id #709**

## Evolution of *Pseudomonas aeruginosa* during long term infection in a patient with cystic fibrosis

**Samuel J.T Wardell[1], Lois W Martin[1], Wayne M Patrick[1], Iain L Lamont[1]**

*1. Department of Biochemistry, University of Otago, Dunedin, Otago, New Zealand*

**Background.** The opportunistic pathogen *Pseudomonas aeruginosa* is a major factor in the morbidity and mortality of cystic fibrosis (CF) sufferers. It causes chronic infections in the lungs of these patients that can last decades. *P. aeruginosa* is widespread in the environment, providing a reservoir for infection of individuals with CF. Currently, there is an incomplete understanding of how *P. aeruginosa* evolves to survive the hostile environment of the lung. To this end, we have collected isolates of *P. aeruginosa* from the lungs of a CF patient in 1991, and isolates from the same patient 21 years later. Aiming to further understand the processes involved in developing chronic infections.

**Methods.** The isolates were characterised using a combination of whole genome sequencing, RNA-seq, and phenotypic approaches.

**Results.** Whole genome sequence analysis indicates the modern isolates are derived from those obtained in 1991. Several hundred non-synonymous mutations separate the modern sequences from the ancestral sequences. Many mutations identified are in genes with unknown function. Also present are large deletions, bacteriophage acquisition, and significant differences in gene expression. Phenotypic differences between ancestral and modern isolates are consistent with the mutations and regulatory changes that have occurred in the 21-year infection. These include, changes in growth dynamics, motility, biofilm production, and antibiotic resistance, with most modern isolates being multi-drug resistant.

**Conclusions.** This research provides detailed insight into adaptation of *P. aeruginosa* to the CF lung environment over 21-years. A complete understanding of the evolution of these bacteria during infection may suggest novel strategies for treating infections.

**id #748**

## Torso-like interacts with the insulin signalling pathway to regulate growth and developmental timing

**M A Henstridge[1], L Aulsebrooke[1], C K Mirth[1], Coral Warr[1]**

*1. School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia*

Membrane Attack Complex / Perforin-like (MACPF) proteins are best known for roles in mammalian immunity, where they function to disrupt cell membranes by forming oligomeric pores. However, several MACPF proteins instead perform roles in development. The *Drosophila* MACPF protein, Torso-like (Tsl), is well known for its role in activating the Torso (Tor) receptor tyrosine kinase to pattern the embryo termini, and our studies suggest it does so by enabling extracellular accumulation of the Tor ligand Trunk[1]. Tor and Tsl also play roles later in development in growth and developmental timing. Tor functions in the prothoracic gland (PG) as the receptor for prothoracicotropic hormone (PTTH), and *tor* mutants show developmental delay and increased body size[2,3]. While Tsl is also expressed in the PG, and *tsl* mutants show developmental delay, *tor:tsl* double mutants show a much more severe delay than either single mutation alone[4]. This suggests that Tsl can act independently of Tor in growth. In support of this, *tsl* mutants have a smaller body size, not larger as seen with loss of function of PTTH/Tor signalling. In fact the phenotype of *tsl* mutants more closely resembles that of mutations in the insulin signalling pathway. To investigate this we performed metabolic studies and found that *tsl* mutants have increased hemolymph glucose and triglyceride content but unchanged hemolymph trehalose levels, consistent with the known metabolic phenotype of insulin pathway mutants. In addition, *tsl* mutants display reduced nutritional plasticity in a similar manner to *chico* mutants. We have also performed genetic interaction experiments between *tsl* and components of the insulin signalling pathway. Over-expression of PI3K in the prothoracic gland using *phm-Gal4* results in a shorter time to pupariation, and this phenotype is fully suppressed in a *tsl* mutant background. Conversely, the reduced pupariation time caused by $InR^{CA}$ overexpression is not suppressed by loss of Tsl, suggesting that Tsl acts upstream of InR. We therefore tested for interaction with the dILPs. Flies lacking three dILPs (2, 3 and 5) show a greatly extended time to pupariation, and this is not rescued by removal of Tsl. Taken together, our data suggests that Tsl plays a role in activation of the insulin receptor by the dILPs.

1. Johnson, Henstridge *et al.* (2015) *Nat. Commun.* **6**, 8759; 2. Rewitz *et al.* (2009) *Science* **5958**, 1403-1405 ; 3. Grillo *et al.* (2012) *Sci. Rep.* **2**, 762; 4. Johnson *et al.* (2013) *PNAS* **110**, 14688-14692

## id #669

### Ancient DNA: testing for climatic and anthropogenic drivers of ecosystem change in the Southern Hemisphere

**Jonathan M Waters[1], Ceridwen I Fraser[2], Justin J Maxwell[3], Nicolas J Rawlence[1]**

*1. Department of Zoology, University of Otago, Dunedin, New Zealand*

*2. Fenner School of Environment and Society, Australian National University, Canberra, Australia*

*3. Department of Anthropology and Archaeology, University of Otago, Dunedin, New Zealand*

Genetic 'turnover' — the extinction and replacement of biological diversity — represents a fascinating but poorly-understood issue in Southern Hemisphere prehistory. Here we present ancient DNA and radiocarbon analyses of archaeological remains to assess the chronologies and causes of prehistoric megafaunal extinction and replacement in southern New Zealand. The collated data include ancient DNA sequences from over 200 ancient sea-lion (*Phocarctos*) and penguin (*Megadyptes*) specimens, in addition to 150 modern genetic samples. Temporal genetic analyses show that sudden, synchronous megafaunal turnover events occurred in New Zealand at around 1500 AD, coinciding with the Little Ice Age onset and an associated drastic human demographic decline in the south of the country. We conclude that a combination of climatic and human demographic shifts facilitated northward expansion of subantarctic sea lion and penguin lineages, replacing extirpated mainland New Zealand megafauna. Broadly, the interaction between human pressure and late Holocene climatic change is implicated as a cause of multiple biological turnover events in the Southern Hemisphere.

## id #727

### The shape of silencing: differential DNA methylation between the sexes of genes subject to X chromosome inactivation in marsupials

**Shafagh Waters[1], Alexandra M Livernois[2], Hardip Patel[3], Denis O'Meally[4], Paul D Waters[1]**

*1. University Of New South Wales, Sydney, NSW, Australia*

*2. University of Canberra, Canberra, ACT, Australia*

*3. The Australian National University, Canberra, ACT, Australia*

*4. University of the Sunshine Coast, Sippy Downs, QLD, Australia*

X chromosome inactivation is an epigenetic phenomenon in therian (eutherian and marsupial) mammals that results in the transcriptional silencing of one X chromosome in the somatic cells of females. In eutherian mammals, hypermethylation at gene promoters is a late, stabilizing step that maintains transcriptional silence on the inactive X. Previous single loci in different marsupial models demonstrated that there was no differential DNA methylation of promoters between the sexes, leading to the long standing conclusion that it plays no role in marsupial X-inactivation.

Using reduced representation bisulfite sequencing, we analysed CpG methylation to generate male and female methylation profiles in a marsupial representative (grey short-tailed opossum). In contrast to mouse, opossum displayed no differential DNA methylation between the sexes at promoters of genes subject to X-inactivation. However, regions adjacent to promoters of these genes were hypomethylated in females, "flattening" the DNA methylation profile. This is the first observation of non-random hypomethylation on the X in a female marsupial representative, which we propose acts as a silencing signal during X inactivation.

## id #688

### differential gene expression in brain tissue of cane toads across the australian invasive range

**Andrea J West[1], Mark Richardson[1], Richard Shine[2], Lee A Rollins[1]**

*1. School of Life and Environmental Sciences, Centre for Integrative Ecology, Deakin University, Waurn Ponds, VIC, Australia*

*2. School of Life and Environmental Sciences, University of Sydney, Sydney, NSW, Australia*

Background/Aims

Many invasive species exhibit rapid evolution of behavioural traits that facilitate dispersal, such as locomotor activity, risk-taking and exploration. In invasive Australian cane toads, variation in morphology and behaviours linked to dispersal coincide with spread across their expanding range; for example, invasion front cane toads are more exploratory than are those from the range-core. However, little is known about the role of genetic mechanisms that may underpin these shifts in behaviour during the cane toad invasion. Because behavioural traits that assist dispersal facilitate range expansion, we predict that genes underlying those traits may be differentially expressed across the cane toad's invasive range.

Methods

We used RNAseq data to analyse gene expression in whole brain tissue from wild-caught toads sampled across a transect from the original introduction site to the invasion front.

Results

We identified 17 differentially expressed genes and cluster analysis revealed that genes associated with assisting spatial memory, central nervous system repair, DNA repair and reduction of oxidative stress were up-regulated towards the invasion front. Conversely, genes associated with learning ability, increased toxin resistance, and dealing with stress are up-regulated at the original introduction site.

Conclusion

This study demonstrates that both physiological and behavioural changes are associated with range expansion and may highlight important candidate genes underpinning invasion ability in this species. This knowledge is vital to future gene technologies, which may assist in the management of both invasive and conserved populations.

Funding Source

Funding provided by the Australian Research Council (DE150101393).

## id #526

### Establishing an invertebrate chordate model to study whole body regeneration

**Simon Blanchoud[1], Lisa Zondag[1], Kim Rutherford[1], Neil Gemmell[1], Megan Wilson[1]**

*1. Anatomy, University of Otago, Dunedin, OTAGO, New Zealand*

Tunicates are filter feeding marine invertebrates that compose the closest phylogenetic group to the vertebrates. This chordate subphylum contains a particularly diverse range of reproductive methods, regenerative abilities and life-history strategies. Consequently, tunicates provide an extraordinary perspective into the emergence and diversity of chordate traits. Currently published tunicate genomes include three Phlebobranchiae (sessile solitary benthic), one Thaliacean (planktonic pelagic solitary), one Larvacean (planktonic pelagic solitary) and one Stolidobranchian (sessile colonial benthic). To gain further insights into evolution of the tunicate phylum, we have sequenced the genome of the colonial ascidian *Botrylloides leachii*.

The predicted *B. leachii* genome size of ~194 Mb is much smaller than the *Botryllus schlosseri* genome, estimated to be ~750 Mb, but is similar to *Ciona intestinalis* (160 Mb). This difference is largely due to an increase in repetitive DNA content between *B. leachii* (17%) and *B. schlosseri* (65%). Analysis of homeobox genes typically found in gene clusters, identified many examples of multiple cluster breaks and gene dispersion, suggesting several lineage-specific genome rearrangements occurring during tunicate evolution. These findings further support that this subphylum undergoes uniquely rapid genetic evolution.

In addition, we have found lineage-specific gene gain and loss within the Wnt, Notch and retinoic acid pathways. These diverse changes to key conserved biological pathways are major examples of the genetic strategies yielding the diverse biology observed in the tunicate phylum. Altogether, the *B. leachii* genome provides is a new resource for the understanding of chordate evolution.

sdfaf

## id #692

### Using the mouse model to understand the interplay between genetic susceptibility and idiopathic scoliosis progression during puberty.

**Megan Wilson[1], Jeremy McCallum-Loudaec[1], Kathleen Sircombe[1], Carol A Wise[2]**

*1. Anatomy, University of Otago, Dunedin, OTAGO, New Zealand*

*2. Texas Scottish Rite Hospital for Children, Dallas, Texas, USA*

Scoliosis, the most common type of spine deformity in children, occurs in around 1-3% of the population worldwide and 90% of those patients that develop a severe curvature are female. The most common form of scoliosis is adolescence idiopathic scoliosis (AIS), which occurs in otherwise healthy children with no obvious structural problems with the spine. The biological origin of AIS is poorly understood. We will use mouse models to understand AIS pathogenesis at the molecular and cellular level.

Many AIS-associated single nucleotide polymorphism (SNP) regions identified in previous GWAS studies are located within non-coding sequences near the *Lbx1* gene. Lbx1 is an evolutionally conserved transcription factor essential for normal embryonic development, with roles in muscle development and the specification of dorsal spinal cord somatosensory interneurons. We investigated the function of Lbx1 by determining the direct targets of Lbx1 in the developing mouse spine, using chromatin-immunoprecipitation followed by sequencing (ChIP-seq). Over 2000 potential direct targets for Lbx1 were identified and are currently being validated using a CRISPR-CAS9 *Lbx1* deletion mouse line. Gene ontology analysis revealed significant enrichment for genes involved in neurogenesis, axon guidance and cell migration consistent with a role for Lbx1 in determining neuronal cell fate in the spinal cord. Lbx1 target genes include several other genes previously linked to AIS, suggesting molecular pathways that may lead to AIS susceptibility.

## id #725

### Lhx9 is required for urogenital ridge formation and ovarian development.

**Stephanie Workman[1], Yisheng Yang[1], Megan Wilson[1]**

*1. The University of Otago, Dunedin, OTAGO, New Zealand*

*Background/Aims*

The distinction between sexes is one of the most obvious examples of morphological dimorphism in the animal kingdom. While there has been extensive research into the differentiation of the ovaries and testes, there is much to learn about the urogenital ridge, a bipotential tissue that makes this all possible. The LIM-homeobox gene, *Lhx9* is a transcription factor critical for the formation of the urogenital ridge. We aimed to characterize the regulation of *Lhx9* in the developing gonad and to investigate it's role in oocyte maturation and fertility.

*Methods*

In order to investigate Lhx9 function, we used chromatin immunoprecipitation sequencing (ChIP-seq) to identify Lhx9 target genes. To investigate regulation of *Lhx9* gene expression by Notch signaling, we are using *in situ* hybridization to demonstrate overlapping expression patterns of *Lhx9* and Notch pathway genes. This will be followed by analysis of *Lhx9* expression after inhibition of the Notch pathway using explant cultures.

Analysis of the role of *Lhx9* in oocyte quality and fertility in will be undertaken using a heterozygote mouse. Changes in gene expression of several cell-type markers will be assessed in both later stage embryonic gonad and adult gonads.

*Results*

Pathway analysis of ChIP-seq results revealed Lhx9 target genes regulate processes such as sexual differentiation and cell migration. Additionally, we found that $Lhx9^{+/-}$ adults, while initially fertile, develop gonadal abnormalities as they age.

*Conclusions*

*Lhx9* is implicated as having a vital role in developmental pathways that may be relevant to disorders of sex determination and infertility.

**id #666**

## Understanding the Batten disease associated protein CLN5

**Jade JR Yip[2, 1, 3], Hollie E Wicky[2, 1, 3, 4], Hannah L Best[2, 1, 3], Peter D Mace[1], Stephanie M Hughes[2, 1, 3, 4]**

*1. Department of Biochemistry, University of Otago, Dunedin, New Zealand*

*2. Brain Health Research Centre, University of Otago, Dunedin, New Zealand*

*3. Genetics Otago, University of Otago, Dunedin, New Zealand*

*4. Brain Research New Zealand, University of Otago, Dunedin, New Zealand*

The neuronal ceroid lipofuscinoses (NCL, Batten disease) are a group of autosomal recessive neurodegenerative lysosomal storage diseases that typically result in loss of vision, epilepsy, loss of motor function and cognitive decline. Mutations throughout the *CLN5* gene predominantly cause a late-infantile variant of NCL. The CLN5 protein is a soluble lysosomal protein of unknown function, with no strong sequence identity to any previously characterised protein. Here we aim to better understand the normal role of the CLN5 protein by investigating its structure and potential function. Using secondary structure prediction, sequence profile matching, and homology modelling, we have found that CLN5 shares weak homology with proteases that utilise a cysteine histidine catalytic dyad. Based on this, the predicted catalytic cysteine was mutated via site directed mutagenesis to investigate whether this mutant can rescue disease-associated phenotypes present in CLN5$^{-/-}$ affected cells. Prior to testing for phenotypic rescue, we will compare the trafficking and secretion of mutant and wildtype CLN5.

Complementary to functional studies, an optimised expression construct for CLN5 has been designed for purification and crystallisation studies. HEK293FT cells have been modified using lentiviral transduction to overexpress CLN5. CLN5 protein has been collected from the media of these cells and purified using nickel affinity chromatography. Future work is aimed at determining the structure of CLN5.

Combined, this work will lead to a better understanding of the normal function of CLN5, and as to why mutations in the gene lead to NCL.