id #25787

The face of a killer: how temperature, bacterial density and genome instability affect pathogenicity of a virulent Wolbachia strain.

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Commensal endosymbionts impose few deleterious effects on their host as the fitness of their host determines their own. To minimize the effect on their host, most endosymbionts establish a minimal infection density that ensures their transmission (typically maternal) to subsequent generations, but not high enough that it imposes too great a fitness cost to their host that prevents their transmission. Most strains of *Wolbachia*, a common endosymbiont of insects, establish low densities in their host imposing mild to weak fitness costs, with some providing fitness benefits during periods of nutritional stress or protection against microbial infections. However *w*MelPop (also known as *popcorn*) is unusual, as it over-replicates within all host tissues and halves adult lifespan. Determining the genetic basis of over-replication has been the focus of intense speculation with different models proposed; the favoured model links a region of genome instability to the strength of pathology, while a second suggests other regions of the genome are responsible. Absent from both studies are what effect does temperature play on genome instability, as shifts in temperature have clearly been shown to have an effect on pathology. Here we discuss what effect temperature has on genome instability, the over-replication phenotype and host pathology.

id #25789

Chromosome 21 in Down Syndrome and Leukemias

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id #25801

Evolution and selection in the ruminants – the view from the sheep's back

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The high quality draft reference genomes of sheep, cattle, goat and yak appear to provide the ideal resources for a comparative analysis of their genomes to understand the evolution, and recent selection, of ruminants. However, the most interesting regions of their genomes from these perspectives are frequently poorly assembled due to gene amplification and loss, copy number variations (CNV) and other genome rearrangements. Thus the genomes are just the starting point and access to detailed transcriptome information and genome sequences from other individuals of a species is required.

In ruminants the mammalian epidermal differentiation complex of genes contains a ruminant-specific amplification of a variant of the *SPRR2* family (*PRD-SPRRII-like* genes) highly expressed in the rumen. In addition, very high expression in the rumen of a gene (*TCHHL2*) present in the genome of most mammals, but not previously reported to be expressed, was observed. These genes are predicted to encode proteins involved in the cornification of the surface epithelium of the ruminant-specific rumen.

The beta globin locus in the ruminant lineage has undergone a number of duplications and other rearrangements. In sheep two quite distinct haplotypes are present, one with a 37kb deletion flanked by regions with much lower than expected sequence identity (~87.5%). Similarly the ruminant specific *MYADML* locus, which contains a large array of duplicated *MYADML* genes, appears to have two quite distinct haplotypes in sheep with a large deletion flanked by regions of low identity between the two haplotypes. The variants of both of these regions affect blood parameters and both appear to have been under selection during domestication.

Amplification of two genes encoding monoacylglycerol O-acyltransferases also appears to have occurred at the base of the ruminants. Unexpectedly *MOGAT3* does not appear to be expressed in the gastrointestinal tract and both *MOGAT2/3* have apparently novel expression in the skin. The *MOGAT3* region is dynamic with quite different structures in cattle and sheep and is a CNV in sheep, although no phenotype has been assigned.

These examples demonstrate the breadth of evolutionary processes in the ruminants and the power of comparative genomics to elucidate them.

id #25805

Beta globin geneevolution in the ruminants: evidence for an ancient origin of sheep haplotype B

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Domestic sheep (*Ovis aries*) can be divided into two groups with significantly different responses to hypoxic environments, determined by two allelic beta globin haplotypes. Haplotype A is very similar to the goat beta globin locus, whilst haplotype B has a deletion spanning four globin genes, including beta C, which encodes a globin with high oxygen affinity. We surveyed the beta globin locus using re-sequencing data from 70 domestic sheep from 42 worldwide breeds and three *Ovis canadensis* and two *Ovis dalli* individuals. Haplotype B has an allele frequency of 71.4% in *O. aries* and was homozygous (BB) in all five wild sheep. This shared ancestry indicates haplotype B is at least 2 to 3 million years old. Approximately 40 Kb of sequence flanking the ~37 Kb haplotype B deletion had unexpectedly low identity (~87.5%) between haplotypes A and B. Phylogenetic analysis showed that the divergent region of sheep haplotype B is remarkably distinct from the beta globin loci in goat and cattle, but still groups with the Ruminantia. We hypothesize that this divergent ~40 Kb region in haplotype B may be from an unknown ancestral ruminant and was maintained in the lineage to *O. aries*, but not other Bovidae, evolving independently of haplotype A. Alternatively the ~40 Kb sequence in haplotype B was more recently acquired by an ancestor of sheep from an unknown non-Bovidae ruminant, replacing part of haplotype A. Haplotype B has a lower nucleotide diversity than haplotype A, suggesting a recent bottleneck; while the higher frequency of haplotype B suggests a subsequent spread through the global population of *O. aries*.

id #25874

A novel literature-based approach to identify genetic and molecular predictors of survival in glioblastoma multiforme: Analysis of 14,678 patients using systematic review and meta-analytical tools

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Glioblastoma multiforme (GBM) has a poor prognosis despite maximal multimodal therapy. Biomarkers of relevance to prognosis which may also identify treatment targets are needed. A few hundred genetic and molecular predictors have been implicated in the literature, however with the exception of IDH1 and O6-MGMT, there is uncertainty regarding their true prognostic relevance. This study analyses reported genetic and molecular predictors of prognosis in GBM. For each, its relationship with univariate overall survival in adults with GBM is described. A systematic search of MEDLINE (1998–July 2010) was performed. Eligible papers studied the effect of any genetic or molecular marker on univariate overall survival in adult patients with histologically diagnosed GBM. Primary outcomes were median survival difference in months and univariate hazard ratios. Analyses included converting 126 Kaplan–Meier curves and 27 raw data sets into primary outcomes. Seventy-four random effects meta-analyses were performed on 39 unique genetic or molecular factors. Objective criteria were designed to classify factors into the categories of clearly prognostic, weakly prognostic, non-prognostic and promising. Included were 304 publications and 174 studies involving 14,678 unique patients from 33 countries. We identified 422 reported genetic and molecular predictors, of which 52 had P2 studies. IDH1 mutation and O6-MGMT were classified as clearly prognostic, validating the methodology. High Ki-67/MIB-1 and loss of heterozygosity of chromosome 10/10q were classified as weakly prognostic. Four factors were classified as non-prognostic and 13 factors were classified as promising and worthy of additional investigation. Funnel plot analysis did not identify any evidence of publication bias. This study demonstrates a novel literature and meta-analytical based approach to maximise the value that can be derived from the plethora of literature reports of molecular and genetic factors in GBM and other diseases. Caution is advised in over-interpreting the results due to study limitations. Further research to develop this methodology and improvements in study reporting are suggested.

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id #25881

Transcriptome analyses using human and zebrafish brain data reveal hypoxia as an important element in Alzheimer's disease

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A wide variety of observations point to the importance of hypoxia in Alzheimer's disease pathology. These include that hypoxia increases γ-secretase activity, *BACE1*, *PSEN1*, *PSEN2* and *APP* transcript levels and production of Aβ peptides. Hypoxia also

stimulates inflammatory responses, inhibits correct protein folding and increases oxidative stress by stimulating production of reactive oxygen species (ROS). Since brain microvasculature is sensitive to ROS and Aβ the potential exists for damaging positive feedback loops to arise where hypoxia leads to vascular damage that further reduces the supply of oxygen. We have performed gene regulatory network analysis of publicly available Alzheimer's disease brain transcriptome data and have compared this to analysis of data from the brains of zebrafish exposed to hypoxia. This reveals remarkable overlap in the patterns of gene transcription in these systems supporting that hypoxia is an important element in Alzheimer's disease pathology. In related work we are analysing the brain transcriptomes of young adult zebrafish (recently sexually mature) into which we have engineered familial Alzheimer's disease-like mutations. This is indicating distinct changes in gene regulation that may mirror changes in familial Alzheimer's disease brains before development of overt histopathology. Hypoxia response signatures are also evident. This work may reveal the initial cellular stresses caused by familial Alzheimer's disease mutations that ultimately result in disease.

id #25897

Experimental evidence for mitochondrial genomic adaptation to climate

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Mitochondria are key components of cellular metabolic processing, providing most of the cellular energy required for survival. These metabolic processes are regulated by a series of enzyme complexes within the mitochondrion, which are sensitive to heterogeneity in the prevailing temperature, resulting to thermal effects on mitochondrial and whole organism performance. While it was traditionally thought that the mitochondrial genome exhibits little capacity to respond adaptively to natural selection, thermal sensitivity of mitochondrial functioning, coupled with the observation that mitochondrial haplotype frequencies tend to associate with latitude or altitude, suggests that thermal selection may play a role in shaping the molecular architecture of the mitochondrial DNA. Here, we present experimental support for this contention. We describe two major mitochondrial haplotypes in *Drosophila melanogaster*, which exhibit opposing patterns of clinal variation along the Australian eastern seaboard. The haplotypes are delineated by 12 synonymous SNPs, with one haplotype predominantly found in the north of Australia, the other in the south. We extracted each of these haplotypes from two latitudinally-different populations and placed replicates of each inside a single isogenic nuclear background, using a powerful breeding design that unambiguously disentangles effects attributable to mitochondrial genetic variation from cryptic effects of residual nuclear variation. We then assayed flies of each haplotype for their capacity to withstand an extreme heat and cold event, and investigated patterns of copy number variation and gene expression of mtDNA protein-coding genes across the haplotypes. We find the northern haplotype confers greater heat resistance, although suffers when recovering from chill induced coma. The underlying SNPs involved in this phenotypic response lie unambiguously in the mitochondrial genome, and affect the level of codon bias and mitochondrial gene expression. Thus, we have uncovered a new-found role for SNPs that were previously considered to be completely non-functional, inside a genome that was likewise traditionally considered to be devoid of functional segregating allelic variation. Finally, our results strongly indicate that mitochondrial genomic variation can be entwined in the dynamics of thermal adaptation.

id #25911

MEIOTIC PAIRING, SEGREGATION AND EPIGENETIC REGULATION OF ACHIASMATE SEX CHROMOSOMES IN MAMMALS: A COMPARED VIEW FROM MARSUPIALS TO EUTHERIANS

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Homologous chromosome pairing/synapsis, recombination and segregation are the hallmarks of meiosis, the specialized kind of cell division leading to the production of gametes. Additionally to these events, meiosis is characterised by a precise epigenetic regulation, which is, in turn, related to the regulation of meiotic features such as recombination and transcriptional activity. While the features that govern the meiotic behaviour of autosomes are well conserved in distant species, sex chromosomes usually present many features that depart from these patterns. In mammals, sex chromosomes (X and Y) are different in size and gene content. This precludes them from performing a normal meiotic pairing in the heterogamentic sex and leads to a specific epigenetic program of meiotic sex chromosome inactivation (MSCI). In many species sex chromosomes still share a small region of homology (PAR) that allows them to synapse, recombine and segregate properly. However, there are a number of species in which sex chromosomes have achieved an extreme degree of differentiation and share little or none homology. This is the case of most marsupials and some eutherian species. In these species sex chromosomes do not synapse or recombine (are achiasmate), though they manage to properly segregate during first meiotic division. In the last years we have uncovered that the mechanisms that ensure the transmission of sex chromosomes under these circumstances rely on the modification of the synaptonemal complex, a meiotic specific structure that mediates chromosome synapsis. Interestingly, similar modifications of this structure appear from the most basal marsupials to gerbils and voles, indicating that synaptonemal complex may act as a backup system for the segregation of achiasmate chromosomes throughout mammalian evolution. We have also investigated the epigenetic regulation of sex chromosomes during meiosis and found that although the general patterns of MSCI are widely conserved, some remarkable deviations are present in species with achiasmate sex chromosomes. These differences mainly occur in the timing of incorporation and release of inactivation factors, like histone H3 methylation or H2AX phosphorylation. We will discuss all these topics in an evolutionary context related to sex chromosomes transmission, evolution and inactivation.

id #26083

Evolution of gene regulation in 20 mammals

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10. *Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, United Kingdom*

The mammalian radiation has corresponded with rapid changes in the non-coding genome, but we lack a comprehensive understanding of regulatory evolution in mammals. While comparison of whole-genome sequences across many mammalian species has revealed evolutionarily constrained non-coding elements (many of which are likely regulatory elements), experimental binding locations of transcription factors and histone marks have been shown to evolve rapidly, suggesting that there is no clear correspondence between non-coding sequence conservation and regulatory activity.

To experimentally dissect the evolution of regulatory regions in mammalian liver, we tracked the evolution of promoters and enhancers active in liver across twenty mammals from six diverse orders by profiling genomic enrichment of H3K27 acetylation and H3K4 trimethylation. We report rapid evolution of enhancers as a universal feature of mammalian genomes: across all study species half of all liver enhancers are unique to a single species. Most of these recently-evolved enhancers arise from exaptation of ancestral DNA, and not from lineage-specific expansions of repeat elements. In contrast, almost all liver promoters are partially or fully conserved across our study species. Recently-evolved enhancers can be significantly associated with genes under positive selection, demonstrating a powerful approach to annotating potential regulatory adaptations in newly sequenced genomes. These results provide unprecedented insight into the functional genetics underpinning mammalian regulatory evolution.

id #26170

Marsupial genomes to tackle contagious cancer, Chlamydia and multi-drug resistant bacteria

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The sequencing of the tammar wallaby, Tasmanian devil and koala genomes have allowed us to take a comparative approach to better understand immunity and disease in marsupials and are allowing us to make biomedically relevant discoveries. I will focus on the use of genomics to better understand a contagious cancer that is decimating Tasmanian devils and an intracellular bacterium (Chlamydia) that is affecting koalas. I will focus specifically on the power of genomics for elucidating host immune responses to novel diseases in threatened species. I will also talk about the discovery of expansions of antimicrobial peptide genes in marsupial genomes which we have shown to have broad spectrum activity against a range of gram positive and gram negative bacteria, including multi-drug resistant golden staph. These peptides evolved to protect immunologically naive young marsupial young but now show promise as novel antibiotics to combat the ever increasing threat from multi-drug resistant bacterial strains.

id #26242

Characterisation of major histocompatibility complex class I genes at the fetal-maternal interface in a marsupial (*Macropus eugenii*)

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Major Histocompatibility Complex class I molecules (MHC-I) are expressed at the cell surface and are responsible for the presentation of self and non-self antigen repertoires to the immune system. Eutherian mammals express both classical and non-classical MHC-I molecules in the placenta, the latter of which are thought to modulate the maternal immune response during pregnancy. Marsupials last shared a common ancestor with eutherian mammals such as humans and mice over 160 million years ago. Since, like eutherians, they have an intra-uterine development dependent on a placenta, albeit a short-lived and less invasive one, they provide an opportunity to investigate the evolution of MHC-I expression at the fetal-maternal

interface. We have characterised MHC-I mRNA expression in reproductive tissues of the tammar wallaby (*Macropus eugenii*) from the time of placental attachment to day 25 of the 26.5 day pregnancy. For placental samples we sequenced 10 PCR clones amplified from universal MHC-I primers at days 18, 19, 21, 24 and 25 of pregnancy. Putative classical MHC-I genes were expressed in the chorio-vitelline placenta, fetus and gravid endometrium throughout the whole of this period. MHC-I classical sequences isolated from placenta were phylogenetically most similar to the *Maeu-UC* (50/100 clones) and *Maeu-UA* genes (7/100 clones). Expression of three non-classical MHC-I genes (*Maeu-UD*, *Maeu-UK* and *Maeu-UM*) were also present in placental samples. The results suggest that expression of classical and non-classical MHC-I genes in extant marsupial and eutherian mammals may have been necessary for the evolution of the ancestral therian placenta and survival of the mammalian fetus.

id #26250

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

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The olive flounder Paralichthys olivaceus, is one of the most economically important marine aquaculture species, with a selective breeding program to increase aquaculture production in Korea. However, a paucity of information is available regarding the olive flounder genome. Bacterial artificial chromosome (BAC) library is an important tool in genomic research. We constructed two BAC libraries, with large, high quality inserts and deep coverage, for P. olivaceus as a crucial part of the olive flounder genome project. The libraries were constructed in the EcoRI and HindIII sites of the vector CopyControl pCC1BAC. The two libraries contain a total of 66,816 BAC clones arrayed in 174 384-well microtiter plates and correspond to 14.18 haploid genome equivalents based on olive flounder genome size of 550Mb. A random sampling of 335 BACs indicated an average insert length of 118 kb with a range of 80 to 180 kb, and 1.5% of the BACs do not contain inserts. The combined libraries have a greater than 99% probability of containing any single-copy sequence. The BAC library has been arranged in three-dimentional pools allowing screening with various PCR-based markers. BAC colonies were screened with PCR-generated gene-specific probes for the DMRT1 and 2 positive clones covering fully this gene were identified. We generated a total of 26,824 high-quality BAC-end sequences (BES), 96.5% of which were paired-BES. The average read length was 676 bp, representing 20.8 Mb of genomic DNA in total or 3.8% of the genome. This database of BAC-end sequences is useful for the assembly of the complete olive flounder genome sequence and is important for identification in functional genomics experiments.

id #26304

New tools for the classification of environmental DNA sequences in Geneious.

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The ability to efficiently classify unknown DNA sequences by species is central to studies of environmental DNA and metagenomics. The use of next-generation sequencing in such studies is becoming common-place allowing for high-throughput monitoring of environmental biodiversity and detection of indicator species. Geneious R8 provides new tools for high-throughput classification of environmental DNA using curated reference databases for comparisons. The 16S Biodiversity tool is a cloud-based service that classifies 16s rRNA amplicons using the RDP database. Results are presented as interactive pie charts allowing you to visualize the biodiversity present in your sample. The Sequence Classifier plugin provides added flexibility for environmental DNA studies, enabling you to taxonomically identify an unknown sample using any genetic marker. This tool performs pairwise sequence comparisons between your sequence(s) of interest and your own in-house database to classify sequences at whatever taxonomic level you choose. The use of custom databases and user-defined parameters allow the tool to be fully customized to your study. Geneious also provides a full suite of tools for NGS sequence trimming, filtering, pairing and assembling, enabling the entire analysis pipeline to be carried out in a single interface.

id #26313

The Koala Genome Consortium – the utilization of *de novo* genome and transcriptome sequencing for applied conservation genomics of an iconic Australian marsupial

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The koala, *Phascolarctos cinereus*, is a biologically unique and evolutionarily distinct Australian arboreal marsupial that is frequently regarded as an 'iconic symbol of conservation' due to a range of threatening processes such as disease and habitat loss. The Koala Genome Consortium is a multi-disciplinary collaboration utilizing both genomic and transcriptomic data to investigate conservation in this species via a genomics approach.

This presentation will report *de novo* koala transcriptome and genome sequencing, assembly, annotation and analysis and the unique molecular attributes we have discovered for this iconic, monotypic marsupial. Two geographically separate koalas, have been RNA sequenced for 15 tissue types, to create the first comprehensive catalog of annotated transcripts for this species, which we estimate represents approximately 15,000 koala genes and also detected the presence of the Koala retrovirus (KoRV) in the transcriptome data from both animals.

Comparative analyses using other marsupial transcriptome data were used to investigate potential gene duplications and we report evidence for copy number expansion of the alpha amylase gene, and of an aldehyde reductase gene. Further, we will

report how we are now using these data to develop a SNP array to investigate koala population genomics, immune gene diversity, disease and retroviral status across the Australian east coast koala distribution as well as in captive animals.

In addition, these koala transcriptome data are being used to validate and annotate the full koala genome sequence assembly in preparation for koala genome release draft version 1 in 2015. Version 1 of the genome release will form the foundation of evolutionary genetic studies of marsupials in general, thorough analysis of koala immune genes and importantly the opportunity for genome level investigation of the koala retrovirus.

id #26315

Estimating the sensitivity of eDNA detection

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Environmental DNA (eDNA) is increasingly being used as a survey tool to infer species distributions, yet the sensitivity of the technique still requires careful evaluation. Imperfect sensitivity, or imperfect detection, is a feature of all survey methods and needs to be accounted for when interpreting survey results. We present a framework to estimate the sensitivity of both the field and laboratory components of eDNA survey methods and show how these can be combined to estimate the overall sensitivity. We apply this framework to a species-specific eDNA survey to estimate the sensitivity, or probability of detection, across seasons for three invasive aquatic species present in Australia; *Perca fluviatilis*, *Cyprinus carpio*, and *Misgurnus anguillicaudatus*.

Our results show that detection sensitivities for the three invasive species varied greatly according to season: spring surveys resulted in higher detection sensitivities compared to autumn surveys, possibly as a result of increased activity levels in the target species. We use the sensitivity estimates to explore different field survey designs, by varying the number of water samples and number of PCR aliquots per sample, to achieve a desired detection sensitivity.

For eDNA to be used as a routine management tool, the sensitivity of eDNA detection surveys must be estimated and accounted for. The framework we have developed allows researchers to quantify the overall sensitivity of a particular eDNA survey method and optimise sampling regimes to improve detection sensitivities. This has the potential to improve detection of low-density species to assist in the management of both endangered and invasive taxa.

id #26404

Evaluating the accuracy of DNA tests for specimen identification: a bandicoot case study

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DNA detection of species from environmental samples is an effective means of studying rare and cryptic wildlife, and diagnostic DNA tests are increasingly applied to management questions. Taxon-specific DNA tests rely upon PCR primers that selectively amplify DNA from target species. Where DNA from several species might be amplified, or where false positives have important management implications, DNA sequencing is also required. To ensure the accuracy of DNA tests for specimen identification it is important to develop appropriate reference sequence databases for each study system, and to assess potential sources of error.

We have developed a test to detect bandicoot DNA from trace samples. Bandicoots have declined dramatically since European settlement and are at risk from introduced predators. We designed primers that amplify 134bp of the ND2 gene, targeting sequences specific to the six extant species of Isoodon and Perameles. To evaluate the limitations of these primers and the likelihood of erroneous species assignments, we conducted a series of tests in silico, in vitro and in vivo. Specifically, we sequenced the ND2 gene for all Australian bandicoots and, using a distance-based analysis, determined that this amplicon sequence provided sufficient resolution to distinguish among all species, except for some ambiguity between I. auratus and some mainland Australian I. obesulus. We also determined that these bandicoot-specific primers did not amplify DNA from 42 other mammal species, demonstrating substantial specificity. Finally, we used the bandicoot-specific primers to test DNA from 22 predator scats collected in Tasmania. DNA from the eastern barred bandicoot (P. gunnii) was detected from two scats, which were previously shown to originate from Tasmanian devil and cat.

id #26408

Evolution of virulent viruses in honey bees exposed to a vector

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Prior to the arrival of a parasitic mite, *Varroa destructor*, the Western honeybee (*Apis mellifera*) was host to a number of asymptomatic RNA viruses, suffering occasionally from mild, seasonal outbreaks. Mid 20th century, *Varroa* jumped from its native host, the Eastern hive bee (*A. cerana*) to *A. mellifera*. In the decades following, *Varroa* has spread throughout much of the beekeeping world, with the notable exclusion of Australia. In many countries including New Zealand, the arrival of *Varroa* was accompanied by a dramatic increase in the virulence of RNA viruses and widespread colony collapse. *Varroa* transmits viruses directly between bees as it feeds on the haemolymph of pupae and adults, where previously, viruses were transmitted passively by feeding and reproduction. The occurrence of highly virulent viral strains apparently fulfilled theoretical predictions that virulence increases when pathogens are transmitted via a vector: host survival is no longer required to ensure transmission. However in other countries such as South Africa, honeybee populations do not suffer the same colony losses even though *Varroa* has been present for more than a decade. Furthermore, in the US and the

Netherlands, *Varroa*-tolerant honeybee strains have been selected for. This suggests that the host-virus-vector interaction varies, depending on factors such as host genotype, prevalence and diversity of viruses, and level of *Varroa* mite infestation.

Using a next-generation sequencing approach, we have compared the viral diversity and prevalence in four populations of honeybees that vary in their response to *Varroa.* While the viral species present in our populations were similar, we see dramatically different viral titres and sequence polymorphisms in multiple honeybee viruses. Our study has also revealed two novel honeybee viruses. Our ultimate goal is to assess virulent viruses in *Varroa*-naïve Australian hosts to tease apart the confounding variables that contribute to the evolution of virulence.

id #26410

Ecotypes, ecological speciation and escargot

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Adaptation to the local environment may drive the formation of ecotypes, which may be the first step towards the origin of new species (Mallet 2008). Edible *Placostylus* snails are found in a wide range of environments throughout New Caledonia (Brescia 2011). Using geometic morphometrics we found that shell shape varied more among populations than within, and had a strong relationship with some environmental factors. To determine whether this morphological variation was plastic we studied mtDNA and nuclear SNP datasets (>3,000 loci). In sympatry *P. fibratus* and *P. porphyrostomus* maintained genetic and morphological differences, suggesting a genetic base to phenotypic variation. The genetic variation, in contrast to shell shape, did not correlate with climatic conditions. Some populations in contrasting environments were morphologically distinct but genetically indistinguishable for neutral markers. However, convergence of shell shape was observed in adjacent populations that were genetically isolated but exposed to similar habitat and climatic conditions.

We infer that morphological divergence in the *Placostylus* snails of New Caledonia is mediated by adaptation to the local environment as required by the ecological speciation model. Where soft tissue anatomy distinguishes two taxa (Neubert et al. 2009) we can differentiate five genotypic clusters, with concordance between shell shape and genetic assignment.

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id #26420

Step-wise Duplication of MHC-I Genes in the Evolution of Eutherian MHC Region: A Batty Perspective

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Bats are natural reservoirs to a variety of viruses that are highly pathogenic in other species (such as Ebola, SARS-like Coronaviruses, Nipah and Hendra viruses) but rarely cause disease in bats. The mechanisms responsible for the control of viral replication in bats remain unknown. Towards understanding the potentially unique features of the bat immune system, we describe the first characterisation of the MHC-I region of the Australian black flying-fox (*Pteropus alecto*). The bat MHC-I region is highly condensed, yet relatively conserved in organisation and is unusual in that MHC-I genes are present within only one of the three highly conserved class I duplication blocks. With reference to other mammalian MHC-I genomic maps, we propose the duplication of MHC-I genes occurred in a step-wise manner across the class I region. MHC-I genes moved first into the β block in bats, subsequently into the κ block in Laurasiatherian mammals, and finally into the α block in rodents and primates. The bat MHC-I region provides evidence for the duplication and amplification of class I genes within the MHC region of eutherian mammals, filling an important phylogenetic gap in the evolution of the MHC.

id #26432

Genomic imprinting in honey bees: motive, mechanism and evidence for inter-sexual conflict

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Because of haplo-diploidy and polyandry, social insect colonies are ripe with the conditions that can generate intersexual genomic conflict. In particular, if males could imprint genes in their spermatozoa such that their female offspring are more likely to become reproductive workers or develop as a queen, then they could considerably enhance their inclusive fitness. We will

present three complementary lines of evidence that imprinting occurs in the honey bee as a result of reproductive conflicts between rival males and between queens and males. First, when we made reciprocal crosses between two subspecies, *Apis mellifera capensis* and *A. m. scutellata* to produce genetically identical offspring workers reared in the same colony, workers with a Capensis father has significantly larger ovaries, with 1/3 more ovarioles, than workers with a Scutellata father. Second, patterns of DNA methylation vary significantly between eggs, spermatozoa, and adult male honey bees, suggesting that males may imprint certain genes, possibly in ways that benefit the reproductive success of their daughters. Finally, we will show that genome-wide methylation patterns differ between diploid embryos that arise from fertilized eggs and eggs that arise via thelytokous parthenogenesis. Thelytokous eggs have two sets of chromosomes derived from a female, whereas sexual eggs have one set of chromosomes derived from a male, and one from a female. Queen-laid bi-parental embryos showed a significantly greater frequency of methylated sites and genes than did worker-laid uni-parental embryos providing support for the hypothesis that honey bees imprint their genomes via DNA methylation.

id #26434

Post-transcriptional regulation of mitochondrial gene expression in the model amoeba *Dictyostelium discoideum*

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Mitochondrial genomes encode proteins involved in oxidative phosphorylation for the production of energy. Therefore, the regulation of mitochondrial gene expression is essential for normal cell function. Mitochondrial gene expression is primarily controlled at the post-transcriptional level where transcripts are subject to modifications such as processing, methylation, splicing and editing. While this has been widely studied in plants and animals, the regulation of mitochondrial gene expression in protozoa remains poorly understood. We have investigated this process in *Dictyostelium discoideum,* an amoeba recognised by the national institute of health as a non-mammalian model for biomedical research and other aspects of eukaryotic cell biology. We have shown that the mitochondrial genome of*D. discoideum* is transcribed into eight major polycistronic RNAs (named A-H) that undergo processing events to produce mature mRNAs, tRNAs and rRNAs. Transcription across the intervening regions of transcripts A-H suggest they are processing intermediates that are derived from a single larger transcript. Analysis of the 5' ends of these polycistronic RNAs reveal that only transcript A is derived from transcription initiation while transcripts B-H are the result of intermediate processing events that are then further processed into mature RNA molecules. These processing events are facilitated by PtcE, which differentially regulates tRNA and non-tRNA processing. Homologs of PtcE are found in specific protozoan and algal lineages acquired via horizontal gene transfer, but are lacking in plants and animals, representing a unique form of regulating mitochondrial gene expression in protozoa and algae. Studies in other protists are beginning to reveal similar features in mitochondrial transcription and transcript processing and the important role that horizontal gene transfer has played in this process. Therefore, it appears that the regulation of mitochondrial gene expression has evolved differently in protists and is significantly expanding our knowledge of a process essential to mitochondrial biogenesis and energy production.

id #26446

Henry Bennett: from mathematical geneticist to historian of science

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Henry Bennett (1927-2015) began his career in geneticist with a novel and powerful approach to multilocus linkage disequilibrium, and made several further contributions on this theme. He worked on many of the major topics of mathematical genetics of his era and also engaged in practical data analysis, especially in regard to kuru, the strange neurological disease of the New Guinea highlands. He was greatly influenced by R. A. Fisher, and after Fisher's death devoted himself to making Fisher's work available in an accessible and scholarly form. The talk will describe this transition, with details drawn from the author's own interactions with Bennett as student and colleague.

id #26453

Does genetic meltdown of *Carsonella* symbionts leave *Eucalyptus* psyllids in need of new microbial partners?

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Psyllids (Hemiptera) are plant-sap feeding insects that are obligately associated with their co-evolved primary symbiont, *Carsonella*. Such obligate symbioses are often exemplars of the phenomenon of extreme genome reduction, where gene loss has resulted in incomplete metabolic pathways. Metagenomics shows that bacterial diversity in individual psyllid species is very low, but secondary symbionts, including *Wolbachia*, *Sodalis*-like and *Arsenophonus*-like bacteria have been detected and may play important complementary roles, e.g. in symbiont-based nutrient acquisition.

Psyllids of the genus *Cardiaspina* are lerp-forming *Eucalyptus* specialists, and recent devastating outbreaks of taxonomically not fully resolved *Cardiaspina* species in Australia highlighted the need for understanding their taxonomy, host plant associations and symbioses. Using genomics and transcriptomics we investigated the ecological and evolutionary relationships of a group of *Cardiaspina* spp. with their obligate symbiont *Carsonella*, and their dominant secondary symbionts. Targeting the bacterial symbionts housed in the bacteriome with NGS, the *Carsonella* genomes from six *Cardiaspina* host species associated with different *Eucalyptus* species, as well as other more distantly related gall-forming and free-living psyllids from Australia, were sequenced and assembled. Comparative genomics indicated, as reported in other psyllids, that genetic meltdown of the

conserved *Carsonella* genome has occurred. Further analysis of the genomes of secondary symbionts, which most closely resemble *Arsenophonus* spp. in all but one of the six *Cardiaspina* psyllids, will demonstrate the extent metabolic complementarity enables co-occurring symbionts and host to thrive.

id #26457

Dpp/TGF-β signalling in autophagy dependent programmed cell death

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An integral part of animal development and adult survival is the maintenance of cellular and tissue homeostasis that requires programmed cell death (PCD). The main mode of PCD is caspase-dependent apoptosis with alternative cell death mechanisms such as autophagy having important context specific functions. Autophagy is the catabolic process of cellular self-digestion through the action of lysosomal enzymes and is rapidly induced in response to stress. Numerous signalling pathways converge on autophagy, yet how these specific signals are integrated to regulate autophagy during cell death is poorly understood. To understand the mechanism and regulation of PCD during development we have been using *Drosophila* as a model. Our studies have shown that removal of the larval midgut is not dependent on the apoptotic machinery, but requires autophagy. We have shown that the signals that regulate starvation-induced autophagy, including TOR and PI3K, are also required to regulate autophagy during midgut removal. In our ongoing studies to dissect the regulation of autophagy-dependent cell death we identified the *Drosophila* transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP) homologue, Decapentaplegic (Dpp). TGF-β signalling pathways are highly evolutionarily conserved and mediate transcriptional reprogramming in a wide variety of cells/tissues to regulate numerous biological processes including cell growth, differentiation and cell death. We have found that sustained Dpp signalling prevents midgut removal and blocking Dpp signalling induces premature autophagy and midgut degradation. In addition, our studies reveal that the Dpp pathway interacts with TOR and autophagy pathways during midgut histolysis. Furthermore, persistent Dpp signalling in the midgut prevents hormone-mediated events and suggests interplay between these pathways. Thus our studies suggest that the regulation of midgut removal is complex requiring the integration of numerous signalling pathways and demonstrate an unanticipated role for Dpp signalling in autophagy-dependent cell death.

id #26463

Drivers of population differentiation: A natural experiment with the Australian house sparrow.

id #26465

Drivers of population differentiation: A natural experiment with the Australian house sparrow.

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The house sparrow (*Passer domesticus)* was introduced to Australia in the 1860's by Acclimatisation societies. Over the next hundred years the species spread across all of eastern Australia from the main introduction points around Melbourne and Adelaide. The sparrow has successfully invaded the full range of Australian climates from Tasmania, through the arid centre and to tropical Queensland. This introduction provides an excellent opportunity to study invasion biology and adaptation in one of the world's most broadly distributed avian species. Because the house sparrow is an obligate commensal species, they have jumped from settlement to settlement creating a fragmented meta-population across the sparsely populated areas of Eastern Australia. This meta-population allows for comparisons between populations that have varied climate conditions, establishment times and levels of isolation. Using microsatellites we have found strong genetic differentiation across the Australian distribution (26 sample populations, n = 1248). Genetic data indicate strong structure across the NSW/QLD boarder and some substructure within the North and South populations. This population structure can mostly be explained by founder effects, isolation by distance and independent human introductions that were identified through our review of the invasion history. As the house sparrow has colonized new climates, we would expect to find adaptations to new conditions. We have already identified phenotypic differentiation in body size across Australia that is consistent with Bergmann's rule that predicts smaller body size in warmer climates. Body size has been shown to be highly heritable in house sparrows but could also be strongly affected by the developmental environment. Our immediate plans are to use genome-wide SNP data to determine the extent to which phenotypic divergence amongst populations might be attributed to divergence in functional regions of the genome.

id #26466

The breakdown of self-incompatibility during a range expansion

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It is commonly observed that for species that vary in their mating system among populations, range margins are enriched for increased selfing rates. This has often been attributed to a response to selection under mate and/or pollinator limitation. However, previous theoretical studies have shown that a reduction of inbreeding depression could facilitate the breakdown of self-incompatibility (SI) and therefore the invasion of self-compatibility (SC); we might thus expect that, during a range

expansion (a series of founder events) SC could be favoured by selection in populations in which inbreeding depression has been purged. Here, we present results from a spatially explicit individual-based simulation model of a 2-dimensional range expansion, in which both inbreeding depression and the number of S-alleles at an SI locus can evolve, and in which there are recurrent mutations to SC. We find that under certain conditions (low recombination and migration rates), SC evolves and completely displace SI. Under other conditions, SC and SI are able to coexist in the expanded metapopulation, with spatial segregation of SC and SI populations. Importantly, our results indicate clearly that evolution of inbreeding depression during a range expansion can be entirely sufficient for the evolution of SC at range margins, and that mate or pollinator limitation is not necessary (though it might be important, too).

id #26470

Multiple, independent and convergent evolution of sex chromosome in Reptiles

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Sex chromosomes are the most dynamic entity in any genome and have evolved many times throughout vertebrate evolution. They differ from autosomes in that the two homologues typically differ in morphology and gene content. The comparison between species with evolutionarily young sex chromosomes is ideal for gaining insight into the mechanisms driving sex chromosome evolution. For example, sex chromosomes in reptiles display tremendous diversity in morphology, even within the races of the same species, implying rapid evolution of sex chromosomes and thus represent different evolutionary stages. Therefore, reptile sex chromosomes provide unique opportunity to capture evolution in action through comparative analysis of sex chromosome sequences among distantly related taxa. Here, we combined chromosome microdissection with next generation sequencing and cytogenetic mapping of repetitive sequences to understand the origin and evolution of sex chromosomes in divergent group of reptiles (including lizards, snakes and turtle). Comparative sequence analyses of X, Y, Z and W sex chromosomes from several candidate taxa revealed multiple and independent origin of sex chromosomes. However, mapping of repetitive sequences among candidate species identified homologies among sex chromosomes in several taxa, implying convergent evolution. Therefore, our data provide evidence of multiple, independent and convergent evolution of sex chromosomes in reptiles.

id #26473

Silencing the marsupial X: functional similarity of Rsx and Xist

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Publish consent withheld

id #26475

Can local selection maintain stasis in the face of gene flow? Exploring shell shape variation in time and space.

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4. *Ecology Group, Massey University, Palmerston North, New Zealand*

Genetic analysis allows an estimate of recent population structure and gene flow, but inferences about persistence and form change comes from fossils. We combined morphological, genetic and spatial information from extant and sub fossil landsnails (*Placostylus ambagiosus*) to explore patterns of phenotypic variation between populations over the last 5 thousand years.

The endangered New Zealand flax snail *Placostylus ambagiosus* is restricted to a region less than 50 km^2 in sizebut local fragmented populations are phenotypically distinct. Geometric morphometrics can discriminate many distinct extant populations suggesting populations are adapted to their local environment or microhabitat. Analysis of mtDNA sequence data supports population structure within a single species (Buckley *et al*. 2011).

Using a modified version of the double digest restriction-site associated DNA sequencing method (ddRAD; Peterson *et al*. 2012) we have developed a reduced genome library from multiple populations of *Placostylus ambagiosus* snails. Although we have data for few individuals, the large number of loci (>1000) suggests that gene flow among extant populations is currently low and was also low in the recent past when forest habitat was more continuous.

Sub fossil snail shells of *Placostylus ambagiosus* have been recovered from 13 horizons (and carbon dated) spanning a short timeframe (~5000ybp), with isolated fossils dating to ~40,000ybp. These sub fossil deposits are found close to two extant, phenotypically distinct populations. Stable local environments may result in stasis even where intraspecific gene flow is occurring. We used geometric morphometric analysis of shell shape from extant and subfossil populations and compared the fit of three models of morphological evolution (random walk, directional, stasis) using a maximum likelihood approach (Hunt 2006). If local selection is strong we expect some morphological traits to show stasis, even when gene flow from distinct populations is likely.

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id #26476

SNP discovery and variation across the rock lobsters *Jasus edwardsii* and *Sagmariasus verreauxi* based on ddRAD-seq data

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3. *Victoria University of Wellington, Wellington, New Zealand*

Jasus edwardsii and *Sagmariasus verreauxi* are lobster species that have a relatively broad distribution in Australia and New Zealand, spanning considerable temperature and ecological gradients. These species represent valuable fisheries in both countries and are subject to heavy exploitation. Therefore determining the temporal and spatial scales of dispersal and gene flow across these species' ranges is essential for effective conservation and management. In order to improve fishery management, genetic parameters could be used to investigate divergence, diversification and dispersal in exploited lobster populations. However, due to the lack of a reference genome for either species (or closely related species) and the reduced amount of species-specific genetic markers, the current methods for detection of fine scale population differentiation have poor resolution[1-5]. In this study, we combined Next-Generation Sequencing technology with a cross-species SNP discovery by applying a reduced representation genomic approach based on Restriction site Associated DNA sequences (ddRAD-seq)[6]. This allowed examining genome wide variation without any prior genome knowledge. Reads from ddRAD-seq libraries, generated from 42 samples of *J. edwardsii* (4 locations) and 55 samples of *S. verreauxi* (3 locations), were trimmed, de-multiplexed and discarded where the Phred score < 33. Sequences were assembled and the ddRAD loci that were shared, or variable, within (at least 10 individuals) and across both species (at least 20 individuals) were identified using *pyRAD*[7]. *De novo* assembly was also performed under less restricted parameters using *Geneious* software to check and remove putative paralogous loci. Overall, 2,390 loci (~140 bp length) were identified, from which 123 were shared across the two species and 2,267 were intra-specific (1,048 in *S.verreauxi* and 1,219 in *J. edwardsii*). A total of 11,527 SNPs were discovered, with an average of 4.82 SNPs per locus and global Minor Allele Frequency (MAF) of 0.11. SNPs with a depth <5, MAF <0.05 and significant deviations from Hardy-Weinberg equilibrium (P<0.05) were discarded. The 4,406 remaining SNPs were catalogued for future downstream analyses based on target-capture sequencing for fine scale genetic studies across all *Jasus* species. The outcomes of this study will open new avenues, with direct application to the lobster fisheries management.

1. Iacchei, M. et al. Combined analyses of kinship and FST suggest potential drivers of chaotic genetic patchiness in high gene-flow populations. Mol. Ecol. 22, 3476–3494 (2013). 5. Thomas, L. & Bell, J. J. Testing the consistency of connectivity patterns for a widely dispersing marine species. Heredity (Edinb). 111, 345–54 (2013).
2. Morgan, E. M. J. et al. Investigation of genetic structure between deep and shallow populations of the southern Rock Lobster, Jasus edwardsii in Tasmania, Australia. PLoS One 8, e77978 (2013). 4. Iacchei, M. et al. Combined analyses of kinship and FST suggest potential drivers of chaotic genetic patchiness in high gene-flow populations. Mol. Ecol. 22, 3476–3494 (2013).
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4. Thomas, L. & Bell, J. J. Testing the consistency of connectivity patterns for a widely dispersing marine species. Heredity (Edinb). 111, 345–54 (2013).
5. Palero, F. et al. Genetic Diversity Levels in Fishery-Exploited Spiny Lobsters of the Genus Palinurus (Decapoda: Achelata). J. Crustac. Biol. 30, 658–663 (2010).
6. Peterson, B. K. et al. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7, e37135 (2012).
7. Eaton, D. a R. PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics 30, 1844–1849 (2014).

id #26479

SNP discovery and variation across the rock lobsters *Jasus edwardsii* and *Sagmariasus verreauxi* based on ddRAD-seq data

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Jasus edwardsii and *Sagmariasus verreauxi* are lobster species that have a relatively broad distribution in Australia and New Zealand, spanning considerable temperature and ecological gradients. These species represent valuable fisheries in both countries and are subject to heavy exploitation. Therefore determining the temporal and spatial scales of dispersal and gene flow across these species' ranges is essential for effective conservation and management. In order to improve fishery

management, genetic parameters could be used to investigate divergence, diversification and dispersal in exploited lobster populations. However, due to the lack of a reference genome for either species (or closely related species) and the reduced amount of species-specific genetic markers, the current methods for detection of fine scale population differentiation have poor resolution[1-5]. In this study, we combined Next-Generation Sequencing technology with a cross-species SNP discovery by applying a reduced representation genomic approach based on Restriction site Associated DNA sequences (ddRAD-seq)[6]. This allowed examining genome wide variation without any prior genome knowledge. Reads from ddRAD-seq libraries, generated from 42 samples of *J. edwardsii* (4 locations) and 55 samples of *S. verreauxi* (3 locations), were trimmed, de-multiplexed and discarded where the Phred score < 33. Sequences were assembled and the ddRAD loci that were shared, or variable, within (at least 10 individuals) and across both species (at least 20 individuals) were identified using *pyRAD*[7]. *De novo* assembly was also performed under less restricted parameters using *Geneious* software to check and remove putative paralogous loci. Overall, 2,390 loci (~140 bp length) were identified, from which 123 were shared across the two species and 2,267 were intra-specific (1,048 in *S.verreauxi* and 1,219 in *J. edwardsii*). A total of 11,527 SNPs were discovered, with an average of 4.82 SNPs per locus and global Minor Allele Frequency (MAF) of 0.11. SNPs with a depth <5, MAF <0.05 and significant deviations from Hardy-Weinberg equilibrium (P<0.05) were discarded. The 4,406 remaining SNPs were catalogued for future downstream analyses based on target-capture sequencing for fine scale genetic studies across all *Jasus* species. The outcomes of this study will open new avenues, with direct application to the lobster fisheries management.

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5. Palero, F. et al. Genetic Diversity Levels in Fishery-Exploited Spiny Lobsters of the Genus Palinurus (Decapoda: Achelata). J. Crustac. Biol. 30, 658–663 (2010).
6. Peterson, B. K. et al. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7, e37135 (2012).
7. Eaton, D. a R. PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics 30, 1844–1849 (2014).

id #26482

Searching for rabbit genes under virus-driven selection

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We are studying natural host-parasite co-evolution between the European rabbit (*Oryctolagus cuniculus*) and the rabbit haemorrhagic disease virus (RHDV). European rabbits were introduced to Australia in the 18th century. They quickly became a mammalian pest causing extensive ecological and economic problems. RHDV was imported into Australia as biocontrol agent. It escaped from quarantine in 1995, and was afterwards also deliberately released to reduce rabbit numbers. Initially, RHDV caused mortality rates of around 95%. Now, about 20 years after, rabbit numbers have increased and genetic resistance to RHDV in wild Australian rabbits has been observed. Our aim was to identify genes contributing to this increased resistance against RHDV. Using SNPs detected by the genotyping-by-sequencing (GBS) we have screened the genomes of wild rabbits from South Australia, and detected several loci that are likely targets of RHDV-driven selection.

id #26483

Avian phylogenomic analyses revealed the macroevolution patterns of bird genomes

Characterization of genomic biodiversity through comprehensive species sampling has the potential to change our understanding of evolution. To study evolution across a major vertebrate class, dissect the genomics of complex traits, and resolve a centuries-old debate on the avian species tree, we formed a consortium focused on sequencing and analyses 48 bird genomes covered all 30 neognath orders, representing a wide range of avian evolutionary diversity. The phylogenomic analyses with full genome data produced a highly supported avian order phylogeny that resolves many debates on the timing and topology of their radiation. Whole genome comparison for all bird species with other vertebrate species revealed several distinct macroevolution patterns of avian genome. The small genome size of bird was a consequence of massive loss of repeat elements and thousands of functional genes in bird ancestral stage.

id #26484

Mapping Red Velvet: chromosome evolution in a novel tumour in Tasmanian Devils

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Fragile sites and their involvement in chromosome evolution is a controversial area of study, with two conflicting hypotheses being debated. There are regions of the chromosome particularly susceptible to breaking. The debate is centred on whether these occur randomly along the genome, or if there are hotspots that have been conserved in marsupial evolution, and Tasmanian Devils provide us with an excellent model for research.

A novel tumour in a Tasmanian Devil individual has been found not to be a strain of the well characterised Devil Facial Tumour Disease (DFTD). This potential amelanotic melanoma is known as “Red Velvet”. Karyotyping suggested it had chromosome rearrangements in two of the most rearranged chromosomes in DFTD; chromosome 1 and 5. These are also two of the most rearranged chromosomes amongst marsupials, suggesting that they are prone to breakage. We are characterising the Red Velvet tumour to determine whether there are shared breakpoints between the Red Velvet tumour and DFTD, and if these coincide with evolutionary breakpoints on marsupial chromosomes. Bacterial artificial chromosomes (BAC) clones are being mapped to Red Velvet chromosomes by fluorescence in situ hybridisation (FISH). These same BACs have been used to map and characterise DTFD and normal devil chromosomes, allowing for direct comparisons to be made. We are focussing on chromosomes 1 and 5, mapping at least 10 BACs for each. Interestingly, preliminary mapping results suggest that it is chromosome 2 rather than chromosome 1 that is rearranged in Red Velvet. In addition to FISH mapping, immunostaining will be used to compare methylation patterns between Red Velvet, DFTD and normal devil chromosomes to determine whether changes in methylation patterns align with fragile sites, repeats, evolutionary breakpoints or tumour breakpoints. This will enable us to determine whether there is a correlation between tumour and evolutionary breakpoints, and genomic and epigenomic features.

id #26486

The evolution of embryonic-maternal communication in reptiles and mammals

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In live bearing amniotes (reptiles, birds, mammals) communication during embryonic development occurs across placental tissues, which form between the uterine tissue of the mother and the chorioallantoic membrane (CAM) of the embryo in eutherian mammals and all viviparous reptiles. Embryonic communication to the mother can be achieved by the production of hormones to coordinate structural and functional changes to the placenta. To understand the evolution of placental hormone production, we examined the expression of genes involved in hormone synthesis, metabolism, and hormone receptivity in the chorioallantoic membrane of species across the amniote phylogeny. We collected transcriptome data for the chorioallantoic membranes of the chicken (oviparous), the lizards*Lerista bougainvillii* (both oviparous and viviparous populations) and *Pseudemoia entrecasteauxii* (viviparous), and the horse *Equus caballus* (viviparous). The viviparous taxa differ in their mechanisms of nutrient provisioning; *L. bougainvillii* is lecithotrophic, but *P. entrecasteauxii* and the horse are placentotrophic (embryos are nourished via placental transport). Of the 423 hormone-related genes that we examined, 91 genes are expressed in all studied species, suggesting that the chorioallantoic membrane ancestrally had an endocrine function. No genes are expressed only in viviparous species, suggesting that the evolution of viviparity has not required the recruitment of any specific hormone-related genes. Finally, we found that the expression of ten hormone-related genes has been lost in species that exhibit substantial nutrient transport across the placenta, suggesting the loss of gene expression might be an important mechanism that occurs during the evolution of novel phenotypes such as placentotrophy.

id #26488

Population histories of Australian animals

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Around the LGM much of Australia was a dusty, hyper-arid landscape; rainfall was up to 30% less than current levels. Currently there is little knowledge about how or where the Australian biota survived this period. We have reconstructed the demographic history of a subset of Australian animals in order to understand the response of endemic species to these conditions because they are similar to many of those predicted under current climate change models for large parts of the continent (albeit with different drivers). It is imperative that we quickly discover which species and ecosystems were most adversely impacted during the LGM and examine the major responses that contributed to the adverse outcomes, as well as identify the location and nature of the differing habitats in which species survived.

id #26493

Broad methylation patterns in a contagious and evolving cancer, devil facial tumour disease

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Devil facial tumour disease (DFTD) is an evolving contagious cancer adversely affecting Tasmanian devil populations; at least four DFTD clonal strains have been identified based on minor karyotypic differences. Management efforts on devils and DFTD should reflect evolutionary trajectories of DFTD. An important facet of cancer evolution includes epigenetic factors, which play an important role in gene regulation and genome stability. One of the most well-known epigenetic factors is DNA methylation. Using immunofluorescence staining based techniques, broad patterns of DNA methylation have been observed in Tasmanian devils and in DFTD cell from multiple time periods and strains. Chromosome end regions are heavily methylated in DFTD and normal Tasmanian devils. The DFTD karyotype is composed of shattered and rearranged X chromosome material; atypical areas of methylation in DFTD may be related to methylated inactive X chromosome material. In relation to other non-contagious cancers which are known for rapid changes and evolution, undetectable changes in DFTD broad methylation patterns over time and between strains may reflect a stabilisation in DFTD evolution.

id #26494

Sex in Dragons: evolution of sex chromosomes in Australian dragon lizards

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In contrast to mammals and birds, sex chromosomes in reptiles are morphologically highly variable. For example, in lizards, sex chromosomes display tremendous diversity in morphology, even within the races of the same species, implying rapid evolution of sex chromosomes. Extant forms can be considered to represent different evolutionary stages. In Australian dragon lizards, genotypic (GSD) and temperature dependent sex determination (TSD) have evolved multiple times within the last 24 million years. There are ~ 70 species currently described in Australia. Both GSD and TSD occur within the same genus, and in at least one species, the central bearded dragon (*Pogona vitticeps*), there is an interaction between genotype and egg incubation temperature in sex determination. Therefore, this species as well as the group represent an ideal model to study and understand evolution of sex chromosomes and evolutionary transitions between modes of sex determination. We performed comparative mapping of *P. vitticeps* sex chromosome BAC clones in selected Australian dragon lizards, representing both GSD and TSD species. Our data revealed that chromosome rearrangements involving fission, fusion and repeat amplification are responsible for the evolution of ZW sex microchromosomes in atleast one species of dragon lizard, *P. vitticeps*. In addition, our comparative gene mapping of amniote sex chromosome genes in *P. vitticeps* revealed that chromosome 2 of this species represents an ancestral synteny, harboring genes from both chicken Z and platypus sex chromosomes, suggesting possibility of common origin of XY and ZW sex chromosomes in amniotes.

id #26496

Anchoring dragon lizard sequence to chromosomes to trace the evolution of reptile genomes

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Squamates (lizards and snakes) are a speciose lineage of reptiles displaying considerable karyotypic diversity, particularly among lizards. Understanding the evolution of this diversity requires comparison of genome organisation between species. Although several squamate species have now been sequenced, only the green anole lizard has any sequence anchored to chromosomes and there is only limited gene mapping data available for five other squamate species. This makes it difficult to reconstruct the events which have led to the observed squamate karyotypic diversity. By using two different approaches to map large, conserved blocks of genes, we were able to anchor almost 40% of the recently sequenced central bearded dragon (*Pogona vitticeps*) genome to chromosomes. We constructed comparative maps between dragon, anole and chicken genomes to show that squamate macrochromosomes are well conserved between species, supporting findings from previous molecular cytogenetic studies. Macrochromosome diversity between species is generated by intrachromosomal and a small number of interchromosomal rearrangements. Like birds, most karyotypic diversity between squamate species is due to microchromosome rearrangements. The possession of microchromosomes may facilitate the generation of karyotypic diversity in reptiles.

id #26497

De novo assembly and identification of cytokine genes in *Pogona vitticeps*

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Cytokines are small proteins that play an important role in the immune response in vertebrates. Extensive study of cytokine genes in mammals has demonstrated the importance of their time and place of expression to achieve an appropriate immune response. Similar analyses in divergent taxa, such as the central bearded dragon, *Pogona vitticeps* (*P. vitticeps*), can provide important insight into the essential molecular mechanisms and evolution of cytokine regulation. The genome of *P. vitticeps* was recently sequenced, but many immune genes were not annotated. Automated annotation techniques are not sensitive enough to detect rapidly evolving genes, such as cytokines, and they must be manually curated. Consequently, key cytokines have not yet been characterized in *P. vitticeps*. In order to characterize cytokine expression and regulation in the immune response in *P. vitticeps*, we first need to determine the complete nucleotide sequences of key cytokine genes. We constructed full length transcripts from short paired-end read RNA sequencing from activated spleen cells using the Trinity platform. De novo transcript reconstruction and analysis allowed us to identify candidate cytokines that are missing from the *P. vitticeps* assembly, including interleukin 2, for which transcriptional activation evens have been well documented in mouse and human. Identification of key cytokines in *P. vitticeps* will reveal genes that are evolutionarily conserved and thus likely to be essential to the vertebrate immune response. In addition, the nucleotide sequences of key cytokines in *P. vitticeps* will enable us to characterize the immune response for comparison with mammals.

id #26502

Evolution of the Major Histocompatibility Complex of wild pigs

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The Suidae family (suids, pigs or hogs) and their related taxa Tayassuidae (tayassuids or peccaries) are mostly wild animals which play important roles in their natural environment. The well known member of the former family is the domestic pig (*Sus scrofa*) which is important in agriculture. However, a number of their wild relatives play a role in emerging and zoonotic diseases. Some of them such as the sub-Saharan African bushpig and common warthog appear to be asymptomatically infected by the African swine fever virus. Thus it is possible that they may have developed some local adaptation and can potentially serve as a veterinary model. In addition, the wide distribution of some of these species and their populations in different environments provide a unique opportunity to investigate the links between polymorphisms and environmental phenotypes such as pathogen pressure, temperature, altitude among others. To better understand the adaptive immune system of these taxa, we generated Major Histocompatibility Complex (MHC) resources for 11 wild species of Suidae and Tayassuidae, by applying DNA capture for targeted sequencing, using the *S. scrofa* MHC Hp1a.1 haplotype as a reference sequence. Capture products for 86 individuals were subsequently Next Generation Sequenced. We will describe the effect of species on capture parameters and present some preliminary analyses of the MHC consensus sequences generated for each species and some differences found between the MHC of sub-Saharan African and Eurasian suids.

id #26514

The Tasmanian devil microbiome

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The Tasmanian devil (*Sarcophilus harrisii*) is currently endangered due to the spread of Devil Facial Tumour Disease, which has decreased the devil population size by over 86 % in the past 18 years. The Save the Tasmanian Devil Program has established an insurance population, currently consisting of around 700 devils in 21 zoos or free range enclosures throughout Australia, with the aim to retain genetic diversity of devils in captivity until it is safe to re-introduce them back into the wild. Recent whole-genome sequencing studies have significantly increased our understanding of the genomics and genetics of the species. However, the "second genome" of the devil – its microbiome – has remained a major gap in our knowledge. As increasing evidence demonstrates that the microbial community plays a crucial role in the health and welfare of humans, livestock and companion animals, it is believed that it also has important implications for wildlife conservation.

In this study, we characterised the devil microbiome in 26 wild and 8 captive individuals. A total of 59 microbiota samples, including 17 gut (faecal), 9 oral, 15 skin and 18 pouch, were sequenced at bacterial 16S rDNA V1-V3 region on a Roche 454 GS FLX System. A total of 1,279,034 sequences with the average length of 487.6 bp were generated, which clustered into 69,306 operational taxonomic units (OTUs) that were classified to 39 bacterial phyla spanning 245 orders and 939 genera. Devil gut microbiota is dominated by Firmicutes (53.6%), Proteobacteria (17.1%) and Fusobacteria (15.5%). The pouch has similar microflora composition as abdominal skin, with Firmicutes and Proteobacteria being the co-dominant phyla, together comprising 70.6% of the pouch and 74.3% of the skin bacterial community. The oral cavity of devils is inhabited by Proteobacteria (20.5%), Bacteroidetes (18.9%), Firmicutes (17.9%), Fusobacteria (15.4%), and a large proportion of unclassified bacteria (26.7%). Significant differences in the microbiome were found between devils from captivity and the wild, which provides useful information for future strategic planning for devil conservation and management.

id #26515

Designing powerful RNA-seq experiments

Erica V Todd[1], Mik Black[1], Neil J Gemmell[1]

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RNA sequencing (RNA-seq) is a powerful tool in transcriptomics that enables whole transcriptome analysis *de novo*, and is quickly replacing microarrays for differential gene expression (DE) studies in non-model organisms. However, utilising powerful technology is not the same as having statistical power to address a research question. Technological advances do not eliminate biological variation, or circumvent the need for biological replication in establishing statistical significance of DE tests. Real-world budget constraints necessitate a trade-off between sequencing depth and sample size in RNA-seq experimental design. However, the field lacks clear experimental design guidelines and many published studies utilise few (2-3) biological replicates. We synthesise recent progress in statistical power analysis and sample size calculation for RNA-seq experiments examining DE, and derive 'rules-of-thumb' for RNA-seq experimental design. Sequencing more replicates at lower depth achieves the greatest statistical power for DE analysis. Deeper sequencing gives diminishing returns on power once read depths are sufficient to overcome Poisson counting error (av. 10 mapped reads per gene). Additional biological replicates have a far greater impact on power, although required sample sizes depend critically on the biological variance and fold change of the data. We evaluate power of our own RNA-seq datasets for detecting sex-specific gene expression in the brain and gonad of sequentially hermaphroditic wrasse (*Thalassoma bifasciatum*), as examples of ecological datasets with characteristically high

biological variance. With modest replication (3-5 replicates per condition), only the very largest expression differences (≥ 3 fold, typical of male versus female gonad) are detectable with high statistical confidence (≥ 80% power). Detecting more subtle expression differences (typical of male versus female brain), requires very large sample sizes. Current technology costs still limit the feasibility of large sample sizes in RNA-seq, and we suggest ways of maximising the utility of RNA-seq technology for ecological and evolutionary research.

id #26516

Regulation of autophagy-dependent cell death in *Drosophila melanogaster*

Shannon Nicolson, Tianqi Xu, Donna Denton, Sonia Dayan, Sharad Kumar

Programmed cell death is an important process required for eliminating damaged cells, maintaining homeostasis, and removing obsolete tissues and cells during development. Included amongst the cell death modalities are apoptosis, dependent on the action of proteases termed caspases, and autophagy. Autophagy is a highly conserved process responsible for degrading and recycling the cytoplasmic contents of cells. Autophagy acts as a cell death mechanism in specific contexts including during *Drosophila melanogaster* metamorphosis. Here it is required for the removal of obsolete larval tissues such as the larval midgut and salivary glands. While removal of both tissues is triggered by developmentally timed pulses of the steroid hormone ecdysone, midgut removal is not dependent on caspases but requires autophagy. In salivary glands however, both autophagy and caspase-dependent apoptosis are necessary for complete salivary gland removal. These tissues thus serve as useful *in vivo* models in which to investigate the regulation of autophagy-dependent cell death.

Using the *Drosophila* midgut as a model system, we have identified the Dpp signalling pathway as a novel regulator of autophagy-dependent cell death. The Dpp pathway is the homolog of the TGFβ/BMP pathway responsible for a broad array of functions in *Drosophila* such as defining cell fate and maintaining stem cell number. To investigate whether the Dpp pathway has a broader role in regulating developmental cell death, we examined the role of the Dpp signalling pathway during *Drosophila* salivary gland cell death. Interestingly, the Dpp signalling pathway does not regulate programmed cell death of the salivary gland as it does in the midgut. Although active Dpp signalling occurs during salivary gland cell death, genetic activation or inhibition of the Dpp signalling pathway does not prevent cell death of the salivary glands. This suggests a tissue-specific role of the Dpp pathway in regulating autophagy-dependent cell death during *Drosophila* development.

id #26517

Designing powerful RNA-seq experiments

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RNA sequencing (RNA-seq) is a powerful tool in transcriptomics that enables whole transcriptome analysis *de novo*, and is quickly replacing microarrays for differential gene expression (DE) studies in non-model organisms. However, utilising powerful technology is not the same as having statistical power to address a research question. Technological advances do not eliminate biological variation, or circumvent the need for biological replication in establishing statistical significance of DE tests. Real-world budget constraints necessitate a trade-off between sequencing depth and sample size in RNA-seq experimental design. However, the field lacks clear experimental design guidelines and many published studies utilise few (2-3) biological replicates. We synthesise recent progress in statistical power analysis and sample size calculation for RNA-seq experiments examining DE, and derive 'rules-of-thumb' for RNA-seq experimental design. Sequencing more replicates at lower depth achieves the greatest statistical power for DE analysis. Deeper sequencing gives diminishing returns on power once read depths are sufficient to overcome Poisson counting error (av. 10 mapped reads per gene). Additional biological replicates have a far greater impact on power, although required sample sizes depend critically on the biological variance and fold change of the data: datasets with higher biological variance or smaller fold changes require larger sample sizes to overcome higher measurement error. With modest replication (3-5 replicates per condition), only the very largest expression differences (≥ 3 fold) are detectable with high statistical confidence (≥ 80% power), unless biological variance is very low (i.e. typical of cell lines and inbred animal strains). Detecting subtle expression differences (< 2 fold change) requires very large sample sizes (> 10 replicates per condition). More powerful (well-replicated) study designs remain prohibitively expensive with current technology, especially for med-high variance datasets. We suggest a pilot sequencing approach in order to estimate power, and establish the feasibility of larger experimental designs.

id #26519

Evolution and diversity of the Complement system in Crocodilians

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The complement system plays an important role in the innate immune response in higher vertebrates. It consists of about thirty proteins grouped in five major gene families which encodes for distinct plasma proteins that react with one another forming three activation cascades (alternative, lectin and classical) and converge in a single terminal pathway. The Complement system is involved in cell lysis and initiation of phagocytosis by opsonization of pathogens and induction engages cells of immune system to trigger processes leading to inflammation. This system appears to be highly conserved in vertebrates. However there

is a gap in the knowledge in regards to evolution and diversity of the Complement system in Crocodilians. To address this, here we investigated a large number of Complement system genes in the recent available genomes of three crocodilian species (*Alligator mississippiensis, Crocodylus porosus, and Gavialis gangeticus*), and compare these with that from other vertebrates including reptiles, birds and mammals. To further understand the extent of conservation and differentiation of the complement system among the three extant crocodilian families, we have surveyed 25 exons representing seven genes across 20 species of crocodilians including alligators, crocodiles and gharials. Preliminary analyses show that the Complement system of Crocodilians has the necessary gene repertoire for the potential activation of the three pathways, there are relatively high frequencies of substitutions among species and most of the genes form orthologous clades.

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id #26523

Preliminary analyses of the diversity in platypus genomes

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The platypus is endemic to Australia and exhibits a fascinating suite of characteristics, being a specialised semi-aquatic, fossorial, carnivorous and egg laying mammal. It occurs naturally in freshwater streams, rivers and lakes of eastern Australia, including Tasmania and King Island. Mitochondrial and nuclear DNA has revealed the existence of least three evolutionary significant units within the platypus and existent of discrete genetic populations at the regional and river basin level with very limited gene flow. In addition, platypus genome and transcriptome data analyses have provide significant insights into the evolution of the species including the role of different selective pressures on gene expression. Given this and the position of the platypus as part of the most basal mammal group, this species is ideal for investigating the evolution of genetic factors underlying different biological processes in mammals. This includes whether genetic structuring and differences between platypus populations have resulted in gene, and potentially genome-wide differences.. Here we assess the genetic diversity of genes associated with metabolism and electroreception using genome sequences from platypuses from New South Wales. This is the first whole-genome sequencing project to look at the landscape of coding genes being part of a multi-institutional project in which platypus population structure and history, and fine-scale recombination rates are being studied.

id #26524

Is there a cost to biparental inheritance of mitochondria?

Julia Dowe[1], Julie Lim[1], Madeleine Beekman[1]

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In sexually reproducing organisms, genetic material is passed on to offspring from both parents, each parent contributing half of their nuclear DNA. In contrast, almost all organisms inherit their cytoplasmic organelles, the mitochondria and chloroplasts, exclusively from one parent (uniparental inheritance). The near ubiquity of uniparental inheritance suggests that there is an evolutionary advantage to inheriting only one organelle lineage rather than two. According to the conflict hypothesis, mitochondria are inherited uniparentally to avoid competition within the cell between two mitochondrial lineages. Smaller mitochondrial genomes replicate faster, and the more copies present within a cell, the more likely a lineage is to be transmitted to the next generation. Hence, competition selects for smaller genomes, but smaller mitochondrial genomes might negatively affect cell and organismal fitness.

The slime mould (*Physarum polycephalum*) is an ideal model organism to test the conflict hypothesis. Crossings of certain combinations of strains can result in either uniparental or biparental inheritance of mitochondria. Some strains of slime mould also carry a plasmid, a small, independently replicating genome, which is always transmitted to offspring regardless of which parent transmits mitochondria. We can manipulate mitochondrial inheritance experimentally and measure the effect on fitness in relation to the number of mitochondrial lineages present. We can also measure if the presence of the plasmid negatively affects organismal fitness. As well as measuring the effect of the number of mitochondrial lineages on fitness, we can also study if increased competition amongst mitochondria leads to a reduction in genome size. In its vegetative stage, the slime mould is basically one huge cell filled with millions of mitochondria, the ideal environment for competition among genomes. If competition among unrelated genomes is shown to cause decreased organismal fitness and a reduction in genome size, this will provide strong support for the conflict hypothesis.

id #26525

A tale of bees, their ectoparasite and vectored viruses

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Classic epidemiological theory predicts that virulence increases when pathogens are transmitted via a vector. When a pathogen requires its host to remain mobile, so that the pathogen can spread to naïve hosts, selection should act against pathogens that kill or immobilise their host too quickly. Thus, over evolutionary time, pathogens are predicted to become less virulent. The arrival of a vector changes the dynamic, as now a pathogen can spread even when its presence debilitates the host. Implicit in these predictions is the presence of a trade-off between virulence and replication rate of the pathogen; the faster a pathogen replicates, the more severe the effects on the host. We investigate the effect of mode of transmission on the evolution of virulence and determine the relationship between replication rate and virulence using RNA viruses of honeybees.

Many RNA viruses are vectored by the ectoparasitic mite *Varroa destructor.* Originally found on the Asian hive bee *Apis cerana*, *Varroa* jumped species to infect the Western honey bee *A. mellifera* some time in the 1980s and is now present on all continents apart from Australia and Antarctica. A range of positive-sense single strand RNA viruses of the *Dicistroviridae* and *Iflaviridae* families (Picornavirales) present covert infections in *Varroa*-naïve colonies of honeybees, with occasional and often seasonal outbreaks. The introduction of *Varroa* sees a marked increase in viral titre, a decrease in viral diversity, and colonies in collapse. We use an experimental evolution approach to tease apart the role of mode of transmission, the role of the vector itself and the role differences in resistance of the bees to viral infections play in the evolution of virulence of RNA viruses. We performed serial transmission experiments, determined if viral titres increased and tested the virulence of evolved viruses on adult *Varroa*-naïve bees.

id #26527

Towards marsupial pluripotent stem cells for species preservation and research.

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The Tasmanian devil (*Sarcophilus harrisii*) is headed towards extinction in the wild within our lifetime, mainly due to the contagious facial tumour that has now spread throughout Tasmania[1]. Production of pluripotent stem cell lines from this endangered marsupial could provide a guarantee against extinction, a valuable resource for studying the facial tumour and an inexhaustible source of gametes for species preservation by assisted reproduction; including artificial insemination, *in-vitro* fertilisation and therapeutic cloning[2]. We have utilized induced pluripotent stem (iPS) cell technology[3] in combination with species specific stem cell growth factors to derive pluripotent-like stem cells from the skin fibroblasts of one male and one female Tasmanian devil. These stem cell growth factor dependent cell lines have normal karyotypes after 20 passages or 6 months in cell culture, express alkaline phosphatase and other molecular markers of pluripotency and can differentiate into multiple cell lineages *in-vitro*.

We have further utilzed this approach to generate pluripotent-like stem cell lines from another dasyurid marsupial, the fat tailed Dunnart (*Sminthopsis crassicaudata*) indicating that this methodology might be successfully applied in other marsupial species. The pluripotent-like marsupial stem cells generated in this study represent a new resource for the elucidation of conserved pluripotency and differentiation gene regulatory networks and possibly a new kind of "extinction insurance" for the Tasmanian devil.

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id #26528

PETAL LOSS, a boundary gene that helps define perianth architecture in *Arabidopsis*

David Smyth[1]

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The floral blueprint is defined early in flower development. It depends on the sites of organ initiation, and the occurrence of boundaries between them. In *Arabidopsis*, one boundary gene involved in the perianth blueprint is *PETAL LOSS* (*PTL*). This encodes a trihelix transcription factor that is specifically expressed in four zones between sepal primordia before and as they arise. Genetic and cytological experiments reveal that PTL acts to inhibit cell division radially in these zones. Its wild type function also allows appropriate space for petal primordia to arise just inside. Loss of PTL function results in overgrowth

between sepal primordia, causing blockage to the auxin signalling of petal initiation. PTL also acts later to repress the basal outgrowth of sepal margins, and more widely in other lateral organs including leaves. The gene occurs throughout flowering plants except for grasses, but not in more basal land plants. It may have been recruited from a duplication in the trihelix family (otherwise known to control light and stress responses) (1) to define the outer perianth whorl, and to help sculpt the shape of all above-ground organs (2).

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id #26530

Impacts of drift and selection on the genetic diversity of threatened species

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Preserving population genetic diversity is at the core of conservation genetics. For the most part, research targeting this challenge has been approached with neutral genetic markers, such as microsatellites, or a small set of functional loci, typically MHC. In this talk, I draw on a series of recent works in which we have examined how selection influences diversity of other putatively functional immune genes, toll-like receptors (TLR), in a range of threatened bird populations. We have investigated the types of selection operating on TLR diversity, at the phylogenetic level as well as within populations. We have also examined whether drift can outweigh selection, and how neutral and selective loci provide different perspectives on population genetic characteristics. This research stream leads into my more recent work investigating the neutral and selective processes that influence genetic diversity of Tasmanian devil, especially in the large, intensively managed insurance population. Importantly, these findings have implications for the conservation management of threatened species, a theme I emphasise throughout my talk. In collaboration with conservation partners, including the Department of Conservation in New Zealand, the Zoo and Aquarium Association and Save the Tasmanian Devil Program in Australia, and San Diego Zoo Global in the USA, research into the underlying population genetic processes that influence genetic diversity of threatened species can reveal management implications for threatened populations throughout Australasia and the world.

id #26531

Heterozygosity-fitness correlations and inbreeding depression in the Tasmanian devil

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The risk of extinction in fragmented and declining populations is often elevated due to a decrease in genetic diversity and an increase in inbreeding. This interlocking of processes is important to understand, as extinction is rarely due to a stand-alone effect, but rather an accumulative effect of many variables. In the endangered Tasmanian devil (*Sarcophilus harrisii*), the emergence of Devil Facial Tumor Disease (DFTD) has lead to a severe population decline of nearly 90%. A decrease in heterozygosity has been observed due to the elevated occurrence of inbreeding post-disease relative to pre-disease. This research aims to identify the presence and/or severity of inbreeding depression in Tasmanian devils, identifying which population parameters exert the greatest magnitude on species survival.

In 2014, 97 Tasmanian devils were captured in a free-range breeding facility, located in New South Wales and are currently being genotyped for 36 microsatellite loci. The microsatellite markers being used consist of 11 previously published microsatellites and 25 newly isolated microsatellites. Phenotypic data was also collected, examining morphological, physiological and life history measurements including: body condition, body weight, reproductive success, parasitic load, lymphocyte count, asymmetry, testis size and survival. This research will examine if heterozygosity-fitness correlations (HFCs) exist in the Tasmanian devil.The application of HFC research into conservation management is underdeveloped. The research here will explore the practical implications of HFCs, by assessing how the information provided can be incorporated into current conservation management practices.

id #26532

Does inbreeding affect Thoroughbred racetrack performance?

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The Thoroughbred racehorse population has been closed and highly selected for racing performance for over 300 years. However, average levels of inbreeding have increased in recent years due to the introduction of the commercial stallion. Consequently, the potential effects of inbreeding depression on the population must be considered.

In this study, we tested whether high levels of inbreeding are correlated with decreased levels of racing performance in Thoroughbreds. The levels of inbreeding were determined using pedigree and single-nucleotide polymorphism data and correlated with racetrack success. The results of this study may assist in breeding decisions not only for Thoroughbreds, but also for other animal populations for which information to conduct such studies is not available.

id #26534

Developmental cell death of *Drosophila* larval midgut requires autophagy

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Programmed cell death (PCD) is critical for multi-cellular organisms during development and in the maintenance of cellular homeostasis. Although most PCD during animal development occurs by caspase-dependent apoptosis, autophagy dependent cell death is also important in specific contexts. Previous studies in our laboratory have established that PCD of the *Drosophila* larval midgut tissue is dependent on autophagy and can occur in the absence of the main components of the apoptotic pathway. As autophagy is primarily a survival mechanism in response to stress such as starvation, it is currently unclear if the regulation and mechanism of autophagy as a pro-death pathway is distinct to that in survival. To establish the requirement of the components of the canonical autophagy pathway during cell death, we examined the effect of systematically knocking-down components of the autophagy machinery on autophagy induction and timing of midgut PCD. We found that there is a distinct requirement for the individual components of the autophagy pathway in a pro-death context. Furthermore, we found that TORC1 is upstream of autophagy induction in the midgut indicating that while the machinery may be distinct the activation may occur similarly in PCD and during starvation-induced autophagy signalling. Our data reveal that while autophagy initiation occurs similarly in different cellular contexts, there is a tissue/function-specific requirement for the components of the autophagic machinery. The next stage of our study is to further dissect out the regulatory machinery of autophagy-dependent cell death. A targeted screen based on proteomic analysis is being conducted.

id #26535

Novel insights into the evolutionary genomics of *Bemisia tabaci* cryptic invasive species using the nextRAD genome-wide scans approach

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Publish consent withheld

id #26537

A developmental and evolutionary genetics explanation for a cytogenetic problem: the age-related decline in meiosis in human females

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The explanation of why women from about age 37 have an accelerated rate of reproductive decline and increasing rate of trisomic offspring has been elusive. That disturbances of the fidelity of mitosis in peripheral blood lymphocytes occurs at the same ages suggests that the mediator of the meiotic problems may not originate from the ovary. Similar, accelerated rates of decline in meiosis with ageing are not observed in animal models and the phenomenon appears to be linked to the evolution of menopause.

Menopause is known to be linked to evolution within the CYP17A1 gene (cytochrome P450c17 protein) and the concurrent co-localisation of P450c17 and CytB5 activity in a specialised region of the adrenal cortex, the adrenal reticularis (AR), limiting its function to the synthesis of the hormone DHEA. During development, the AR cells arise from the mesonephros along with the Sertoli cells in males and theca (ovarian cells) in females, and all three cell types share the ability to synthesise DHEA. DHEA has major roles in androgen and estrogen sythesis and in stimulating the peroxisome proliferator PPARα, which is a major regulator of fatty acid metabolism.

Ageing of cells in the adrenal cortex occurs relatively early. No TERT activity has been detected and cells cease replication by about age 40 in both males and females. This particularly affects the AR which ceases DHEA synthesis at the same time. As reproduction declines and after menopause, DHEA is only synthesised by the ovarian theca cells.

An hypothesis is presented that proposes that whole body changes occur in the late 30 age group as a result of cessation of DHEA synthesis by the AR. Some DHEA synthesis by the theca cells continues but it is insufficient to maintain optimal oocyte maturation. Both meiotic and mitotic fidelity reduce as a result of changes in hormones and the lipid composition of cellular membranes, secondary to loss of DHEA synthesis by the AR. In the ovary, follicular apoptosis accelerates with increasing levels of palmitic acid. Age specific changes in DHEA-S and fatty acids from adipose and lens tissue are presented to support the hypothesis.

id #26538

Current medical genetic strategies for amyotrophic lateral sclerosis gene discovery

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Amyotrophic lateral sclerosis (ALS, also known as motor neuron disease, MND) is a fatal neurodegenerative disease characterised by progressive death of motor neurons. Ten percent of cases are familial, with the remaining 90% considered sporadic. To date, the only known causes of ALS are gene mutations. Known mutations only account for 60% of familial ALS. We aim to identify remaining ALS genes. The identification of novel ALS genes will increase our knowledge of disease biology and provide tools for diagnosis and long-term therapeutic discovery.

We apply both unbiased and hypothesis driven strategies for disease gene discovery to the analysis of whole exome sequence data from Australian familial ALS cases. We have completed exome capture and sequencing of 111 individuals from 74 ALS families. Custom bioinformatics analysis of multi-generational families has produced small lists of novel candidate gene mutations. All sequence variants were prioritized for functional studies to examine potential pathogenicity.

Our strategies for mutation discovery also include the identification and analysis of both functional candidate genes and recently published ALS genes. These genes are rapidly screened for mutations by interrogating the exome dataset using bioinformatics scripts to identify known or novel ALS mutations.

The penetrance of mutation-linked disease in familial ALS varies substantially, ranging from classic Mendelian inheritance to apparently sporadic disease. As such, familial ALS genes were also examined in apparently sporadic ALS cases. Rapid sequencing of 624 sporadic ALS cases using Fluidigm Access Array and Illumina MySeq sequencing is underway to identify variation in known ALS genes, including a novel gene identified by our laboratory.

To date, mutation analysis provides the only conclusive diagnostic test for ALS. The identification of additional novel ALS genes will increase the power of diagnosis in ALS, and can be used for pre-clinical screening of unaffected individuals within ALS families.

id #26539

Evaluation of complement system genes for disease association studies in the saltwater crocodile *(Crocodylus porosus)*

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Lymphoid proliferation/ Vasculitis/ Encephalitis (LVE) is a new syndrome that has caused high mortalities amongst captive juvenile saltwater crocodiles and has been shown to be associated with Herpesviridae. In some instances, susceptibility to viral infection within individuals can be associated with limited diversity of major histocompatibility complex (MHC, a component of the adaptive immunity) class I genes or distribution of certain MHC super-types. Recently, we have found a preferential distribution of a MHC class I exon 3 variant in LVE-symptomatic animals when compared with apparently healthy animals. We have considered however, that additional data from other immune components are needed to complement these preliminary findings. Thus assessing other immune genes for polymorphisms that could be used for this is required. Given that antibacterial and antiviral properties and effectiveness have been attributed to the complement system of crocodilians, we investigate the diversity of the terminal components C8a and C8b in 24 yearling saltwater crocodiles, hatched at a crocodile farm in the Northern Territory. Preliminary sequence diversity analyses show these genes have some of substitutions, which could potentially be used for complementing the MHC findings.

id #26541

Unraveling the secrets of diabetes mellitus in Burmese cats

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Cats are among the most common household pets. They share the same environments and lifestyles as humans and this exposes them to many of the same risk factors for diabetes. The incidences of obesity and diabetes in cats are increasing for the same reasons as they are in humans and have become a serious veterinary problem. Feline diabetes is a metabolic disease characterized by insulin resistance, defective insulin secretion and beta-cell loss. Risk factors include excessive body weight, gender, age and breed. While any cat can develop diabetes, a significantly higher prevalence has been estimated among purebred Burmese cats from Australia, New Zealand and the UK. Although the underlying mechanisms predisposing Burmese cats to diabetes are as yet unknown, there is clear evidence for a genetic aetiology. We have applied a whole-genome association mapping for diabetes in 10 diabetic and 57 non-diabetic Burmese cats. DNA samples were genotyped with the Infinium iSelect 63K Cat DNA genotyping array. Our analysis identified two regions that are significantly associated with diabetes in Burmese cats and we are currently investigating two positional candidate genes known to be involved in the absorption and metabolism of lipids. Feline heredity diseases such as feline diabetes mellitus serve as important animal models and could have a great impact on the understanding of diabetes mellitus in other species, with both veterinary and medical applications.

id #26542

Torso-like controls secretion of the growth factor Trunk to pattern the *Drosophila* embryonic termini

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Drosophila terminal patterning represents a paradigm for studying the spatial activation of Receptor Tyrosine Kinases (RTKs) by growth factors. Patterning of the *Drosophila* embryonic termini is achieved by localised activation of the Torso receptor tyrosine kinase by the cysteine knot growth factor Trunk. Trunk is expressed ubiquitously in the embryo and has been long proposed to be ubiquitously secreted from the embryo and cleaved and activated in the extracellular space only at the embryo poles. Governing localised activation of Torso is the perforin-like protein Torso-like, which is localised to the extracellular space at the embryo poles and has been proposed to promote localised proteolytic activation of Trunk. However, a protease involved in terminal patterning had not been identified, and the role of Torso-like remained obscure. We have recently shown[1] that while Trunk must be cleaved in order to bind and activate the Torso receptor, this process occurs independently of Torso-like function. We have now also shown that Trunk is cleaved by Furin proteases intracellularly prior to its secretion, and not extracellularly as previously proposed. Finally, by visualising Trunk localisation in live embryos we have found that the role of Torso-like is to control secretion of Trunk, thus providing the mechanism for local activation of Torso. Together, these data solve a long standing question in early *Drosophila* embryo patterning, and define a new role for perforin-like proteins in eukaryotes.

1. Henstridge et al., (2014). *Nat. Commun.* **5,** doi:10.1038/ncomms4419 (2014).

id #26544

Regulation of oogenesis in honey bee workers via programed cell death

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Currently little is known about the proximate mechanisms that underlie the 'sterility' of the worker caste of social insects. Studies into a mutant 'anarchistic' strain (in which the workers activate their ovaries and lay eggs) of honey bee, *Apis mellifera*, identified a promising candidate gene for regulating oogenesis. Ovarian expression of *Anarchy*, a peroxisomal membrane protein, predicts the ovary state of workers with 88.2% accuracy and its expression is sensitive to the presence of the queen. When we knocked down expression of *Anarchy* in the ovary using RNAi we altered the expression of *Buffy*, a gene that regulates programmed cell death. Whole-mount multiplex fluorescent *in situ* hybridization show that *Anarchy* transcripts localize to degenerating oocytes within the ovary. Our results suggest that *Anarchy* is involved in the regulation of oogenesis via programmed cell death (PCD). We then investigated oogenesis in the young adult honey bee worker ovary in the presence of queen pheromone and in its absence. In the presence of queen pheromone workers continually produce oocytes but these are aborted at an early stage. The degeneration of the germ cells has the morphological hallmarks of PCD. We also found that workers exposed to queen pheromone have higher levels of caspase activity (a quantitative measure of PCD) in the ovary than those not exposed. Therefore the mechanistic basis of 'worker sterility' relies in part on the regulation of oogenesis via programmed cell death.

id #26545

Understanding the evolution of Stomatopoda using molecular data

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Mantis shrimps (Stomatopoda) are an ecologically and economically significant crustacean group, acting as dominant predators in coastal ecosystems, and serving as an important fishery resource in many coastal communities. Despite their importance, much remains to be discovered about their evolution and phylogenetic relationships. Accurate taxonomic and systematic knowledge underpin our ability to manage and conserve biodiversity, but many stomatopod species await description. Although phylogenetic analyses of mantis shrimp have been conducted in the last two decades, these were based primarily on morphological data. Therefore, previous phylogenetic hypotheses have not yet been tested using molecular methods. To address this issue we are performing the first molecular phylogenetic study of the largest stomatopod family, Squillidae, using mitochondrial and nuclear markers. We are describing new squillid species using morphological and molecular data, and assessing the monophyly of genera. We are also examining the timeframe for the evolution of Stomatopoda as a whole, through the use of fossils and molecular clock analyses. These analyses will provide significant insights into when/how traits such as 3D vision, as well as prey "smashing" and "spearing" evolved in this group, and lead to an improved understanding of the systematics of stomatopods.

id #26555

Expression profiling of cerebellar abiotrophy genes in Australian working kelpie dogs

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The Australian Kelpie is a dog breed developed for livestock herding. Cerebellar abiotrophy (CA) is a movement disorder which results in early onset ataxia, and was first documented in Australian Kelpie (AK) and Australian Working Kelpie (AWK) dog breeds in 1989. The cerebellum in these dogs is characterised by the loss, or developmental failure of cerebellar Purkinje and granular cells, which affects movement and coordination, but with varying degrees of severity. We have completed a genome-wide association and putatively mapped CA in working Kelpies to five associated regions across four chromosomes: 3, 22, 35 and X, containing seven candidate genes. Some of the identified loci contain candidate genes that are known to be associated with cerebellar ataxia in humans. However, a small number of dogs identified as CA affected remain unexplained by the mapping analysis. The results suggest that CA in the AWK is a complex Mendelian disorder. The associated regions are currently being investigated for sequence variants using whole genome sequence data (Illumina HiSeq 2000) from three CA affected AWK, five unaffected Kelpies and forty-three dogs from ten other canine breeds. Further work is underway to investigate gene expression patterns in the cerebellum and frontal cortex of six CA affected AWK dogs (Affymetrix Canine 1.0ST gene arrays).

id #26556

Discovery of a deleterious mutation in the Hungarian Puli that causes disease similar to Bardet Biedl Syndrome in humans

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Bardet Biedl Syndrome is ciliopathy characterized by retinopathy, obesity, polydactyly, renal dysfunction, mental retardation and hypogonadism. For the first time, we report the natural occurrence of the corresponding syndrome in the domestic dog. To identify regions that potentially contain a causative mutation for the disease, we conducted a genome wide association study using 170K SNP genotyping data from 2 cases and 12 controls produced from Illumina's CanineHD BeadChip array. Due to a lack of any statistically significant markers, we interrogated whole genome sequencing data from a parent-affected offspring trio, an additional case and 46 control dogs of other breeds. We called variants within all regions of interest and then filtered them according to our expectation that: the disease was recessive; monogenic; and the causative allele was rare. This left us with a single nonsense SNP in *BBS4*. We validated and genotyped this SNP for 3 affected and 42 non-affected Hungarian Puli using PCR and Sanger sequencing and accepted genotypes called from whole genome sequencing data that passed quality filtering in dogs of other breeds. With a total of 75 genotyped dogs, we find that the SNP has a significant association with disease ($P_{CHISQ}=2.837^{-18}$). By using the genotyping array data to infer haplotypes, we noticed that the mother of an affected individual appears homozygous for the risk allele despite being heterozygous for the SNP. This suggests that this deleterious mutation arose relatively recently. From this study, we have shown that the technique of combining various types of data and utilizing parent-offspring samples has enabled the identification of a relatively new mutation using a modest number of affected samples. This is especially important for mapping rare disease genes where samples are often limited. Also, breeders are now able to test and avoid propagation of this disease throughout populations.

id #26557

Characterizing the transcriptome of a transmissible cancer

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Devil Facial Tumour Disease (DFTD), is a contagious cancer that threatens the long-term survival of the world's largest marsupial carnivore, the Tasmanian devil (*Sarcophilus harrisii*). Since the emergence of the disease in the mid 1990's, four karyotypically distinct Devil Facial Tumour (DFT) strains or variants have been described. In the present study, we compare the gene expression profiles of DFTD variants using transcriptome sequencing to investigate whether gene expression differences or expressed mutations could drive phenotypic differences between tumour strains. We found no significant differences in the expression of protein-coding genes between strains. We also report investigations into the possibility of non-synonymous strain-specific mutations. The transcriptome sequence also provides an important resource of expressed genes, which we have used to identify highly expressed genes with oncogenic and angiogenic potential, including BCL2, NDRG1 and protein kinases, which provide targets for development of therapeutic agents.

id #26560

Species boundaries and geographic structure in Grey Box (*Eucalyptus moluccana*)

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Grey box (*Eucalyptus moluccana*) plays an important ecological role as foundation species in the woodlands of eastern Australia. Throughout its wide distribution from NSW central coast to SE QLD populations are severely impacted by extensive land clearing, fragmentation, ecosystem degradation and dieback due to outbreaks by lace lerp insects (psyllids). Understanding the genetic diversity and structure of this foundation species is essential to determine whether populations with depleted genetic diversity are more susceptible to insect attack. Morphological studies suggest grey box has three varieties whereas others suggest hybridization with close relatives potentially obscuring the interpretations of phylogenetic relationships in this group. However none of these hypotheses have been corroborated. Most phylogenetic studies have so far not included grey box. Moreover, phylogenetic studies of the genus *Eucalyptus* using molecular markers show poor resolution of relationships in the group of box species. This could be due to processes such as reticulate evolution and recent divergence of the species. In this study we aim 1) to determine phylogenetic relationships of grey box and its close relatives, 2) to determine the genetic structure of grey box populations, and 3) to test whether reticulate evolution could be a common force shaping the evolution of this complex group. Several populations of grey box encompassing its whole distribution as well as close relatives representing different taxonomic hierarchies within Section *Adnataria* were investigated. DNA extractions were performed from leaves and cambium. Successful samples were amplified for hypervariable chloroplast regions. Bayesian phylogenetic analyses were performed for concatenated chloroplast sequences. Our work shows populations of grey box are partially structured however species boundaries are not defined. Reticulate evolution might occur in this complex group at different taxonomic hierarchies. We are testing nuclear markers to further corroborate these findings.

id #26561

Loss of reproductive parasites during biological invasions

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Many insects are associated with maternally inherited microbial endosymbionts such as *Cardinium* and *Wolbachia* bacteria; both can manipulate host reproduction. Their role in biological invasions by insects is still poorly understood. We have characterised the genetic and endosymbiont diversity of the invasive Kelly's citrus thrips, *Pezothrips kellyanus*, an important pest of citrus in Australasia and the Mediterranean region. Our analyses of mitochondrial and nuclear markers revealed that *P. kellyanus* originated from Australia from where New Zealand and the Mediterranean region were independently colonised. Australian populations had a high prevalence for *Cardinium* and *Wolbachia* infections. In New Zealand and Mediterranean populations, *Cardinium* was fixed while *Wolbachia* was mostly absent. This could be due to stochastic loss of *Wolbachia* prior to the establishment of invasive populations. Alternatively, *Wolbachia* was selected against by environmental factors, or lost due to a possible role as a reproductive parasite that constrains invasiveness of infected populations. We tested this by establishing infected and uninfected laboratory populations. Reciprocal crossing experiments revealed that *Wolbachia* is the more aggressive reproductive manipulator than *Cardinium*. Both bacteria induced cytoplasmic incompatibility (CI) between infected males and uninfected females with different outcomes in this haplodiploid host system: *Wolbachia* CI resulted in female embryonic mortality and some larval mortality, while *Cardinium* CI resulted in conversion of some fertilised embryos into male offspring without affecting larval development. Furthermore, *Wolbachia* occurred at higher titres than *Cardinium*, which indicates that the different modes of host manipulation may be dependent on bacterial density. We conclude that *Wolbachia* was lost from the invasive populations due to its overall more severe effects of host manipulations while *Cardinium* was more benign. These findings support the enemy release hypothesis which predicts that invaders experience less natural enemies in a new environment and thus are able to increase their abundance and distribution.

id #26568

The genomic basis to climatic adaptation in Drosophila - a multi-species view

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There is increasing interest in linking genomic data to ecological contexts and Drosophila provide prime candidates for exploring such links. The dark-eyed fruit flies (*repleta* group in genus *Drosophila*) display the highest tolerance to heat and desiccation stress of all *Drosophila* species groups (Kellerman *et al*. (2012) *PNAS* **109**, 16228-16233; Kellermann *et al*. (2012) *Evolution* **66,** 3377-3389). Of these, *D. hydei* displays a moderate to high tolerance to heat and desiccation with *D. repleta* displaying a slightly weaker tolerance, while *D. buzzatii*, *D. aldrichii* and *D. mojavensis* are all highly thermally tolerant and are cactophilic species. To identify the genomic characteristics leading to this varied thermal tolerance within the same group, we have assembled and annotated the genomes of three of these species. We have developed high throughput and multiple evidence based ortholog scan pipelines that can help ascertain lineage bias in genome evolution, reducing the number of false positive candidate genes we can then subsequently analyze. We use this pipeline to explore the nature of the major

genomic changes in this species groups and potential links to thermal/desiccation tolerance. The approach developed in this study should help in analyzing and interpreting the results of similar comparisons in other groups of related species.

1. Kellerman et al. (2012) PNAS 109, 16228-16233
2. Kellermann et al. (2012) Evolution 66, 3377-3389

id #26570

Comparative analysis of gene expression in two intertidal snails in response to temperature stress

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Understanding how species respond to temporal and spatial changes in their abiotic environments is a central goal in evolutionary and ecological genomics. *Nerita melanotragus* and *N. albicilla* are widespread intertidal molluscs, distributed across a number of temporally and spatially fluctuating environmental gradients, including abrupt changes in temperature over a tidal cycle. These two species differ in their ecology and geographic ranges, as *N. melanotragus* is a mid littoral species, which ranges from temperate to subtropical climes, while *N. albicilla* is a low littoral species found in tropical and subtropical regions. The colonisation of different littoral areas in the intertidal zone means that *N. melanotragus* (mid littoral) is more likely to sustain longer periods of temperature stress than *N. albicilla* (low littoral). Consequently, these two species present an interesting case to examine differences in their patterns of gene expression in response to the same temperature conditions. In this experiment, nine individual samples from each of *N. melanotragus* and *N. albicilla* were randomly allocated into three treatments (14 °C, 22 °C and 31 °C) with three biological replicates in each treatment. After three hours at the treatment temperature, animals were euthanized and RNA was extracted. RNA from each individual was sequenced using 90 bp paired end chemistry on an Illumina Hiseq where each individual received a minimum of 30 million reads. Sequence reads from each species were *de novo* assembled and differential gene expression patterns were determined for each species across the treatment temperatures. The two species had highly divergent patterns of gene expression under the treatment conditions. Few differentially expressed genes (~20) were observed in *N. albicilla*, and these were dominated by molecular chaperones. More differentially expressed genes (~100) were observed in *N. melanotragus,* but no dominant class of genes was observed. This data suggested that *N. albicilla* had a more significant stress response to the temperature treatments used in this experiment. Overall this supports the idea that low littoral species undergo thermal stress at lower temperatures than mid littoral species.

id #26587

Engaging Undergraduate Genetics Students with Scientific Literature and the Peer Review Process - a Journal Club Learning Activity for Large Groups.

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As science educators, one of our primary concerns is to develop teaching and learning strategies that ensure students graduating from our teaching programs have knowledge and capabilities that are readily transferrable to a variety of postgraduate careers. In the health science professions, such as medicine, nursing, clinical genetics and biomedical research, evidence-based practice forms the foundation of postgraduate training and professional development. Postgraduate teaching programs in nursing, medicine and other health-related professions often incorporate journal club learning activities[1, 2]. In a journal club learning activity, participants discuss and critique relevant recent research journal articles with their peers and mentors, enabling them to improve their understanding of current practice, research design, methodology and statistics[3]. Critical appraisal of published research carried out in a journal club activity can help develop student's capabilities in the transferable skills of reading comprehension, numeracy, critical thinking, writing, public speaking and teamwork. Here we describe a journal club learning activity that was designed, implemented and evaluated in a class of 90 third year Genetics undergraduates. This activity takes place over a four week period, incorporating 3 two hour tutorials and culminating in 15 minute oral presentations by groups of 4 students to their peers and mentors.

1. Sheehan, J. (1994). A journal club as a teaching and learning strategy in nurse teacher education. J Adv Nurs, 19(3), 572-578.
2. Ahmadi, N., McKenzie, M. E., Maclean, A., Brown, C. J., Mastracci, T., & McLeod, R. S. (2012). Teaching evidence based medicine to surgery residents-is journal club the best format? A systematic review of the literature. J Surg Educ, 69(1), 91-100.
3. Deenadayalan, Y., Grimmer-Somers, K., Prior, M., & Kumar, S. (2008). How to run an effective journal club: a systematic review. J Eval Clin Pract, 14(5), 898-911.

id #26588

Sex reversal and frequency-dependent selection can drive transitions and innovation in sex determining mechanisms in reptiles.

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Vertebrates display contrasting strategies ranging from complete genetic control of sex (genotypic sex determination -- GSD) to environmentally determined sex (e.g. temperature-dependent sex determination -- TSD). Phylogenetic analyses suggest frequent evolutionary transitions between GSD and TSD in environmentally sensitive lineages, including reptiles. These transitions are thought to involve a genotypic system becoming sensitive to temperature, with sex determined by gene-environment interactions. Most mechanistic models of transitions invoke a role for sex reversal. Here we show that sex reversal in the dragon lizard coupled with Fisher's frequency-dependent selection acting on resultant skewed sex ratios can drive rapid transition from GSD to TSD. Apart from climatic shift, there is no need to invoke adaptive advantages of TSD over GSD, though such advantage may consolidate the transition. As the frequency of sex reversal becomes more prevalent, frequency dependent selection will favour any mechanism that results in over-production of the rarer sex in some individuals, including selection for novel genetic sex determining mechanisms. Sex reversal under climatic shifts, coupled with frequency-dependent selection may provide an engine for transitions between TSD and GSD in part responsible for the great diversity we observe in the mechanisms of sex determination in reptiles.

id #26591

Conservation of entire chromosomes over long evolutionary periods in insects

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Comparison between the genomes of different Drosophila species has shown that the genomes are based on six different chromosomal elements, the so-called `Muller elements'. There are 5 major elements (A – E) and one minor element (F). In *Drosophila melanogaster*, the major elements are respectively chromosomes X, 2L, 2R, 3L and 3R.

Assembly of the Queensland fruit fly (Qfly – *Bactrocera tryoni*) has previously produced several thousand scaffolds, ranging is size from 3Mb down to 1kb or less. We have used GBS mapping to assign 1,774 scaffolds to five linkage groups, representing approximately 25% of the estimated total genome length. The five autosomal chromosomes, corresponding to Muller elements A – F, are shown to be chromosomes previously designated in Qfly, and also medfly, as 5, 3, 4, 6 and 2.

More than 90% of genes in *D. melanogaster* and *B. tryoni* are present on their homologous chromosome, indicating high conservation at the chromosome level, over two evolutionary lines estimated to have diverged up to100 mYr ago. Within chromosomes, however, the order of genes has been highly scrambled by inversions or other translocation events.

This stability at the chromosome level is in stark contrast to other groups such as mammals, plants, birds and fish, where chromosome numbers and organization vary enormously among species that have diverged over much shorter periods of time. The large number of intra-chromosomal rearrangements argues that cis-acting gene interactions are unlikely to explain this stability. We suggest a simpler explanation. The insects in question lack telomerase, which may be needed to facilitate the formation of new chromosomes.

id #26598

Genomic explorations of regressive evolution using blind subterranean diving beetles

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The loss of morphological traits over evolutionary time (regressive evolution) occurs repeatedly across phylogenetically distant lineages, yet convincing empirical support for theoretical explanations remain sparse. Selectionist (direct and indirect natural selection) and neutral theories evoke very different genetic mechanisms and create polarised scientific debate. The current study investigates a group of water beetle species (Dytiscidae) that evolved by independent colonisation of subterranean aquifers of the Western Australian desert, from surface water bodies during the late Miocene/Pliocene. The subsequent legacy is represented by hundreds of blind species that are physically isolated within these calcrete formations. This physical system is unique in that it permits an unrivalled opportunity to explore the regressive loss of vision in a comparative phylogenetic manner that is statistically robust. We have used transcriptomes from five representative species to design an in-solution hybrid capture method to track the molecular evolution of eye-genes, comparing ancestral surface lineages with their blind underground descendants. Here we present initial insights from opsin visual transduction genes, derived from the transcriptomic phase of the project; explain the RNA bait design that is currently being used to capture genomic DNA across a much wider species assemblage and preview the preliminary results.

id #26612

Sex chromosomes of Queensland fruit fly and their relation to the dot chromosome 4 of Drosophila

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The X and Y chromosomes of Queensland fruit fly (Q-fly – *Bactrocera tryoni*) are well differentiated, X being one of the largest chromosomes and Y being the smallest. Both X and Y are heavily staining and both do not polytenise, indicating that they are highly heterochromatic. It has been shown that the Y chromosome lacks any fertility factors, and the only sex-determination factor genetically located to the Y is the *Dominant Male Determiner*, *M*. Shearman (Genetica 116: 25-43, 2002) postulated that Muller's F element, the dot chromosome 4 of *Drosophila melanogaster*, was likely to be the origin of the X and Y chromosomes in *B. tryoni*, in part due to the homology of the other Muller elements, A to E, with *B. tryoni* autosomes 2 - 6. More recent evidence has supported a connection between the X chromosome of various Diptera and *D. melanogaster* chromosome 4, based on two-fold genomic sequence coverage of female-derived versus male-derived DNA for genes homologous to *D. melanogaster* chromosome 4 (Vicoso and Bachtrog, Nature 499: 332-335, 2013).

Mapping of *B. tryoni* genes now provides direct support. Around two thousand genes have been mapped both to *D. melanogaster* chromosome arms and to *B. tryoni* chromosomes. Of these, at least four genes with *D. melanogaster* chromosome 4 homology have been mapped to the *B. tryoni* X chromosome, but no genes from other Drosophila Muller elements have been located to this chromosome. However, 15 *D. melanogaster* chromosome 4 genes have been mapped to various *B. tryoni* autosomes, and this correlates with equal female-derived and male-derived coverage.

id #26634

Combining traditional and environmental DNA (eDNA) based monitoring to improve the management of native and invasive fish species.

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Detailed species distribution data is essential prior to undertaking any management actions. However, this is often challenging for invasive fish species since traditional monitoring methods (i.e. electrofishing, fyke netting, bait trapping) are often biased and unable to accurately determine the extent of the invasion front (Porreca *et al*., 2013; West *et al*., 2007). Environmental DNA (eDNA) based species detection has significantly improved our ability to detect aquatic vertebrates at low densities and is subsequently an extremely valuable monitoring method prior to deciding on appropriate management actions (Goldberg *et al*., 2014). Here we present the results of both traditional (i.e. electrofishing and bait trapping) and eDNA based monitoring surveys conducted in Blakney Creek (NSW). Within this system, the continued spread of the invasive redfin perch (*Perca fluviatilis*, L. 1758) is threatening one of only three self-sustaining populations of the endangered Southern pygmy perch (*Nannoperca australis*, G. 1861). The results show that eDNA based monitoring is able to determine the distribution of the invasive redfin perch with high resolution. Overall the combined results of the traditional and eDNA based monitoring survey shows that redfin perch are widely established in Blakney Creek but absent from one of its tributaries (i.e. Urumwalla Creek). Consequently, management action should focus on the Urumwalla Creek population to conserve one of the last remaining Southern pygmy perch populations in NSW.

id #26695

Talking Genetics

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The central role of genetics in many disciplines of science, particularly health and conservation, gives it a wide exposure to the general public. Talking with the general public about genetic concepts is an important skill for students, including veterinary science students. We run a video assignment in which students research a clinical-based scenario and role-play vet-client conversations and vet-geneticist conversations. This year we are building on the assessment of the communication competency through multi-disciplinary collaboration. While the concept appears simple, the assignment requires problem-solving within the scenario, the use of language appropriate to the situation, and clear explanations of sometimes complex genetic scenarios.

id #26768

An innovative approach towards promoting and teaching genetics through real-world applications to the next generation of scientists

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Providing secondary students with the opportunity to learn genetics by conducting research with real-world applications is likely to encourage them to follow a career in this field. The excitement and challenge of applying their knowledge and understanding

along with the possibility of new discoveries provides a strong motivational platform for learning. Therefore, part of the challenge for teachers is to deliver programs and curricula that foster student interest in the biological world and exposes them, through their own involvement, to the work and practices undertaken in current research. This presentation describes an approach taken by a secondary school that aims to raise the profile of genetics and molecular science at the secondary school level through the involvement of students in hands-on research projects, conducted at the school, in collaboration with both a tertiary institution and commercial enterprise. This approach has resulted in the development of a self-sufficient research laboratory within the school, enabling students to work on projects both in and out of the classroom. To my knowledge this is a unique innovation in a secondary school environment. I believe that this approach goes beyond improving the delivery of subject content and has been successful in assisting students to make stronger connections in their learning and apply their knowledge to a broader context resulting in a shift towards independent thinking and improved application of problem solving skills.

id #26776

The RNA binding protein Quaking regulates formation of circRNAs

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Circular RNAs (circRNAs), formed by non-sequential back-splicing of pre-mRNA transcripts, are a widespread form of non-coding RNA in animal cells. However, it is unclear whether the majority of circRNAs represent splicing by-products without function, or are produced in a regulated manner to carry out specific cellular functions. We have found that hundreds of circRNAs are regulated during human epithelial-mesenchymal transition (EMT) and find that the production of over one-third of abundant circRNAs is dynamically regulated by the RNA binding protein, Quaking (QKI), which itself is regulated during EMT. Furthermore, by modulating QKI levels we found the effect on circRNA abundance is dependent on intronic QKI binding motifs. Critically, the addition of QKI motifs is sufficient to induce *de novo* circRNA formation from transcripts that are normally linearly spliced. These findings demonstrate circRNAs are both purposefully synthesised and regulated by cell-type specific mechanisms, suggesting they play specific biological roles in EMT. Because some of the most highly expressed circRNAs are among those that are regulated in EMT, our findings strongly suggest that certain circRNAs have EMT-related functions, and thus may affect mesenchymal cell properties such as migration, invasion and the propensity for cancers to metastasise[1].

1. Conn et al. (2015) Cell 160: 1125-1134

id #26783

Insights into monsoonal tropics biogeography through comparative genomics of Carlia skinks

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Our understanding about the biodiversity across the monsoonal tropics of northern Australia has only recently started to become clearer through the application of molecular approaches. Next-generation sequencing coupled with exon-capture is enhancing our knowledge of the true species diversity, particularly in cryptic groups like reptiles. Here we highlight how targeted capture for widespread rainbow skinks (*Carlia*) identifies areas of refugia, biogeographic barriers, cryptic species and an evolutionary framework to start to identify areas of significance for conservation. Together with growth in computational analyses, this provides a valuable example of how genomics in non-model organisms can now be used to improve our understanding of regions of Australia where we lack detailed historical climatic information.

id #26807

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

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Previously, we hypothesized that an RNA-based pathogenic pathway has a causal role in the dominantly inherited unstable expanded repeat neurodegenerative diseases. In support of this hypothesis we, and others, have characterized rCAG.rCUG100 repeat double-strand RNA (dsRNA) as a previously unidentified agent capable of causing pathogenesis in a Drosophila model of neurodegenerative disease. Dicer, Toll, and autophagy pathways have distinct roles in this Drosophila dsRNA pathology. Dicer dependence is accompanied by cleavage of rCAG.rCUG100 repeat dsRNA down to r(CAG)7 21-mers. Among the “molecular hallmarks” of this pathway that have been identified in Drosophila, some [i.e., r(CAG)7 and elevated tumor necrosis factor] correlate with observations in affected people (e.g., Huntington’s disease and amyotrophic lateral sclerosis) or in related animal models (i.e., autophagy).TheTollpathwayisactivatedinthepresenceofrepeat-containingdsRNAand toxicity is also dependent on this pathway. How might the endogenously expressed dsRNA mediate Toll -dependent toxicity in neuronal cells? Endogenous RNAs are normally shielded from Toll pathway activation as part of the mechanism to distinguish “self” from “non-self” RNAs. This typically involves post-transcriptional modification of the RNA. Therefore, it is likely that rCAG.rCUG100 repeat dsRNA has a characteristic property that interferes with or evades this normal mechanism of shielding.

We predict that repeat expansion leads to an alteration in RNA structure and/or form that perturbs RNA modification, causing the unshielded repeat RNA (in the form of its Dicer-cleaved products) to be recognized by Toll-like receptors (TLRs), with consequent activation of the Toll pathway leading to loss of cell function and then ultimately cell death. We hypothesize that the proximal cause of expanded repeat neurodegenerative diseases is theTLR recognition (and resultant innate inflammatory response) of repeat RNA as "non-self" due to their paucity of "self" modification.

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id #26817

Mapping cancer-related genes in devil facial tumour disease

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Devil facial tumour (DFT) disease, a contagious cancer, has caused a dramatic decrease in Tasmanian devil numbers in the wild. The unusual feature of this disease is that the tumour cells themselves are the infectious agent. The tumour arose in a female devil and has since spread throughout the population by devils biting each other during social interactions. The key genes involved in DFT tumourigenesis have not yet been determined. Genome sequencing approaches have identified a handful of putative candidates with amino acid substitutions and several predicted rearrangements/deletions but are limited at this stage in their ability to detect structural mutations because of the fragmented nature of the reference genome assembly. We have used an alternative approach to identify candidate genes and pathways. We selected genes commonly implicated in tumour pathways in human cancers to determine whether they were rearranged in DFTD and therefore likely to have contributed to tumourigenesis. Bacterial artificial chromosome (BAC) clones containing candidate genes were mapped to normal and tumour chromosomes by fluorescent in situ hybridization (FISH). Many cancer-related genes were rearranged in DFT and several have at least one extra copy in the tumour, such as the "guardian of the genome" *TP53* and *ERBB3*, a member of the epidermal growth factor receptor family of receptor tyrosine kinases implicated in proliferation and invasion of tumours in humans. Our mapping results have highlighted pathways that may be perturbed in DFT and provided strong candidates, not previously detected by sequencing DFT genomes, for future analysis.

id #26826

Time to acknowledge the elephant in the room? How the illegal wildlife trade contributes to extinction, biosecurity risk, and organised crime

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Publish consent withheld

id #26836

Sugar gliders in Tasmania: an introduced predator or an elusive native?

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The sugar glider (*Petaurus breviceps*) is widely distributed through Australia and Papua New Guinea and is found on a number of Indonesian islands. Historical records suggest that the species was introduced to Tasmania in 1835, as pets, from Port Phillip in Victoria. The population is now widespread but their provenance has not been investigated. Recently Tasmanian sugar gliders have been implicated in the predation on the highly endangered swift parrot (*Lathamus* discolour) causing nest failures of close to 100%. Without intervention to prevent this predation *L. discolor* is predicted to go extinct within two decades. To determine appropriate management of this presumed novel predator, there is an urgent need to establish the provenance of Tasmanian *P. breviceps*. Here we use sequencing of mitochondrial ND4 and ND2 genes to show that Tasmanian *P. breviceps* comprise only two sequences separated by just a single base pair. These sequences are identical to sequences obtained from *P. breviceps* sampled in Victoria. Genetic divergence is much lower than would be expected for a species isolated for around 10kya from the Australian mainland since the Australia-Tasmania land bridge disappeared in the last glacial period. These data suggest that *P. breviceps* in Tasmania have been introduced recently and have since filled a new ecological

niche as an apex predator. Management of the species around the breeding habitats of the swift parrot can now move forward with suggestions of humane lethal traps and inventive forestry management.

id #26839

Youth is wasted on the young: telomere manipulation in painted dragons

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Telomeres are an important structural feature found at the ends of chromosomes; they are involved in gene protection and controlling cellular senescence. Recent research strongly suggests their involvement in the ageing process of organisms. However, most research into telomere dynamics has focussed on correlations between various biological markers and telomere length. In order to determine whether telomeres are truly having an effect, or are simply a proxy for some other cause, we need to be able to manipulate their lengths. In our most recent study we have investigated a possible method of telomere manipulation using TA-65, a telomerase activator, to stimulate telomere elongation in painted dragon lizards (*Ctenophorus pictus*). Telomeric attrition was significantly slowed in younger lizards, while no effect was observed in older individuals.

id #26841

Tracking selection signatures in the chickpea genome by whole genome re-sequencing of 69 chickpea accessions

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Next-generation sequencing (NGS) technology offers a cheap and high-throughput genotyping option to discover genome variation and selection signatures in less utilised crop species, such as chickpea. Chickpea (*Cicer arietinum* L., 2n=16), a self-pollinated diploid species with a genome size of ~700 Mb, is the second most widely grown pulse in the world (FAOSTAT, 2012).

We performed whole genome re-sequencing (WGRS) of 64 Australian chickpea varieties (released from 1978 to 2013), four Indian landraces, and one wild chickpea species (*Cicer reticulatum*) with 5-15X coverage. Alignment of 1.2 billion Illumina paired-end reads to the draft Kabuli genome sequence of chickpea resulted in the identification of over 800,000 SNPs. To handle the high error rate of NGS data, allele frequencies were estimated using site frequency spectrum as prior leading to improved inference of population genetic parameters[1]. Population structure analysis reveals distinct groups among varieties and narrow genetic diversity in recently released varieties. Several regions of the chickpea genome are under positive selection based on Tajima's D test [2]. Both Wright's fixation index, Fst genome scan and genome-wide association studies (GWAS) identify a 100kb region on chickpea chromosome 4 that is significantly associated with ascochyta blight resistance, a disease that severely impacts the chickpea production in Australia and other regions of the world. This region is co-located in a large QTL interval of 7Mb~30Mb confirmed previously by three different mapping populations genotyped at low density with SSR or SNP markers. This 100kb region has been validated by GWAS of another 132 genotypes with WGRS data. In total, 13 predicted genes are located in this region including NBS-LRR receptor-like kinase (common class of disease resistance genes in plants), wall-associated kinase, zinc finger protein and serine/threonine protein kinase. One significant SNP located in the coding sequence of a predicted gene leads to amino acid substitution. All this information will shed light on understanding natural and artificial selection in chickpea.

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id #26856

Sex and speciation: did sex chromosome degradation and turnover trigger mammalian divergence?

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Here I propose that the evolution of the sex determining *SRY* gene and definition of a novel XY chromosome pair in therian mammals ~166 MYA imposed a reproductive barrier with the ancestral population of mammal-like reptiles, and triggered the speciation event that led to the evolution of therian mammals. I also propose that more recently (~145 MYA), Robertsonian fusion of the therian XY pair with an autosome posed a reproductive barrier that promoted divergence of eutherian (placental) mammals and marsupials.

Humans and other therian mammals share a sex chromosome pair composed of a highly conserved X and a small Y chromosome, which progressively degenerated and specialised. The male-dominant *SRY* gene on the Y diverged from its X-borne partner *SOX3* about 166 MYA, the time that therians diverged from prototherian mammals (monotremes such as platypus). In reptiles, and even monotremes, this XY pair is represented by autosomes. The bizarre $X_1X_2X_3X_4X_5Y_1Y_2Y_3Y_4Y_5$ in

monotremes, with homology to the bird ZW, suggests that the sex chromosomes of reptile-like mammals were terminally degraded. Translocation with autosomes stabilised this system in monotremes, whereas the evolution of *SRY* led to sex chromosome turnover in therians. Hybrids between animals with the ancient system and the new *SRY* system would have had high frequencies of sex reversal, intersex development, and infertility, ensuring divergence of prototherian and therian mammals.

Several modern rodent lineages have variant sex determining systems (including complete loss of the Y), suggesting that rodents are undergoing a new explosion of speciation driven by Y chromosome degradation and sex chromosome turnover.

This theory conflicts with the long-prevailing paradigm that speciation results from accumulation of small mutational differences in isolated populations, but receives support from the many groups of fish and reptiles in which closely related species have different sex determination mechanisms, and interspecies hybrids are infertile.

id #26865

Opening ancient human genome and microbiome data for medical research

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Whole genome and microbiome sequence data can be generated for ancient human remains, covering different key evolutionary time periods, but to date there has been limited release of such data in an organised fashion for reuse by the medical research community to perform potentially informative comparisons. As genomics and microbiome data are becoming routinely generated for ancient samples, it becomes crucial to keep track of raw data, processed data, and metadata in an organised and searchable fashion that enables multidisciplinary access and reuse. The Australian Centre for Ancient DNA (ACAD) at the University of Adelaide has collaborated with the University of Adelaide Libraries and eResearch SA to deliver open access to ancient genomics and microbiome data, through a project funded by the Australian National Data Service (ANDS). In May 2015 the project will launch the Online Ancient Genome Repository (OAGR), a world-first, open access database for ancient human DNA, integrating all layers of available genomic and microbiome data and associated metadata in a searchable format. Using OAGR's initial phase of whole genome data, we have investigated the genetic modification in the chromosome 12 purinergic receptor 7 gene (P2RX7) over 50,000 years of hominin evolution. We find that Neandertal (50,000 years ago), Denisovan (30,000) and early modern human hunter-gatherer individuals possessed rare (in current modern populations) gain-of-function variants that promote increased pathogenesis, which transitions to the less pathogenic alleles during the introduction of European farming cultures between 8,000-5,000 years ago. Preliminary findings suggest that the increased pathogen load associated with the densely populated, sedentary farming lifestyle has exerted strong selection pressure towards altered pathogenicity, potentially linking with susceptibility to multiple sclerosis (perhaps associated with the altered inflammation response) and mood disorders. By combining genomic variant analysis with microbiome data obtained from sequencing ancient dental calculus, OAGR will enable researchers to address key genetic and microbial changes over human evolution, and the potential relationships to modern health – such as the endemic modern 'western' diseases. Through establishing data repositories, we aim to contribute to a culture of open data and facilitate the development of novel Bioinformatics tools for data analysis.

id #26872

Pharmacogenetic analysis of nicotinic acetylcholine receptor biology *in vivo*

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Identification of the receptors that underlie target site resistance to spinosad and neonicotinoid insecticides in *Drosophila melanogaster* has provided us with probes to discriminate between at least two receptor subtypes. The simple variation of exposure levels allows us to qualitatively measure response phenotypes. This sensitivity is useful for analysis of both receptor function directly and in detecting perturbations in neural signaling of nAChR pathways. We are using these compounds in a variety of assays to understand how nAChRs function in *D. melanogaster* and to detect other proteins that are important in their role in neural signaling.

id #26876

Changing the landscape of the science laboratory: A move to inquiry-based learning

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The science laboratory has traditionally been seen as a critical part of an undergraduate science degree. In this space, students apply knowledge gained through the lecture series and participate in laboratory activities designed to test concepts taught but which often result in a predicted outcome. The step-by-step nature of traditional laboratories limits the opportunity for students to develop critical thinking and analysis skills both fundamental to research science. Inquiry-based laboratories have been shown to result in a deeper understanding of scientific content, increase confidence in understanding and performing science and improve students' attitudes towards science (Gormally *et al.* 2011; Weaver *et al.* 2008; Wood 2009) . To improve the learning outcomes of our large first year biology cohort, the laboratories were redeveloped into guided-inquiry with educators facilitating teams of students to design and carryout an experiment.

To evaluate the impact of the redevelopment on student satisfaction and learning outcomes, students were surveyed and multiple choice exam data was compared before and after the redevelopment. An analysis of the survey questions indicated

that students thought the laboratories improved the quality of their university experience, helped them to understand the major concepts of the topics, challenged them intellectually and helped to develop their data analysis skills. Overall, there was a significant improvement in student answers to exam questions (paired t-test p=0.001). The exam questions were further classified as content related or laboratory related, determined by whether the material examined by the question was covered during one of the laboratory sessions. There was a significant improvement in exam questions identified as content related before the redevelopment and laboratory related after the redevelopment (paired t-test p=0.0001). Overall these findings suggest that the move to inquiry-based learning has improved the learning outcomes of first year biology students at Flinders University.

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id #26877

Tumour suppressor WWOX moderates the mitochondrial respiratory complex

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Common chromosomal fragile site *FRA16D* is a frequent site of DNA instability in various types of cancer, with the *WWOX* gene that spans it often being perturbed. WWOX has been shown to be able to suppress tumour growth, however the mechanism of this suppression has not yet been fully delineated. WWOX contains two WW domains as well as a short-chain dehydrogenase/reductase enzyme, with little currently known in regards to its SDR enzyme activity. It has previously been demonstrated that WWOX participates in pathways involving aerobic metabolism and regulation of reactive oxygen species. An *in vivo* genetic analysis was performed in *Drosophila melanogaster* to identify functional interactions between WWOX and metabolic pathways. WWOX was found to have an effect on the maintenance of cellular homeostasis in cells with mitochondrial deficiencies, with altered WWOX levels able to modulate variable cellular defects (including cellular outgrowths) caused by genetic deficiencies of components of the mitochondrial respiratory complexes. This modulation requires the SDR enzyme active site of WWOX and the defective respiratory complex-induced cellular outgrowths were also shown to be mediated by reactive oxygen species, dependent upon the Akt pathway and sensitive to levels of autophagy and hypoxia-inducible factor. WWOX is known to contribute to homeostasis by regulating the balance between oxidative phosphorylation and glycolysis. This data demonstrates that reduction of WWOX levels results in diminished ability to respond to metabolic perturbation of normal cell growth. Thus the ability of WWOX to facilitate escape from mitochondrial damage-induced glycolysis (Warburg Effect) could be a plausible mechanism for its tumour suppressor activity.

id #26893

Functional deficit of IQSEC2 with missense mutations disrupts normal dendritic spine morphogenesis.

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There is considerable genetic and phenotypic heterogeneity associated with intellectual disability (ID), specific learning disabilities, ADHD, autism and epilepsy. Our laboratory has been involved in identifying genetic causes of ID, focusing on genes of the X-chromosome including the IQ motif and SEC7 domain containing Protein 2 (*IQSEC2*) gene. The disease spectrum due to mutations in *IQSEC2*continues to expand with more than 15 distinct mutations contributing to non-syndromic ID though to early onset seizure phenotypes in affected males, and in some cases, female patients. The pathogenesis underpinning these mutations is not known. Here we report our investigations on the role of *IQSEC2* on the plasticity of dendritic spines. A lentiviral shRNA approach achieved a 57% ablation of *Iqsec2* expression in primary hippocampal cell cultures from mice, modeling partial loss-of-function mutations. Investigating gross morphological parameters after eight days of *in vitro* culture (8DIV) identified a ~ 32% reduction in primary axon length, and 27% increase in the number and 31% increase in complexity of dendrites protruding from the cell body. Focusing on the development of dendritic spine structures at 15DIV there was an increase of 34% in the number of protrusions per dendritic segment compared to control with the proportion of immature filopodia to mature spines similar across all treatments. By 21DIV the number of dendritic spines had normalised between the controls and ablation groups but showed a reduction in the number of immature spines with Iqsec2 ablation. In contrast to this increased complexity and spread of dendritic neurons with ablation of Iqsec2, overexpression of IQSEC2 WT leads to neurons with shorter axons that are more compact and display simpler dendritic branching. These observations provide evidence of dosage sensitivity for this gene that normally escapes X-inactivation in females and links these disturbances in expression with alterations in the morphology of developing neurons.

id #26894

Transcriptomic response to to climate stress in a generalist and a specialist *Drosophila* species.

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Drosophila serrata and *D. birchii* (sister species of the *montium* subgroup) show marked differences in their ability to tolerate desiccation stress. *D. serrata* (a habitat generalist) has both a greater desiccation resistance than *D. birchii* (a rainforest restricted species), as well as a stronger acclimation response in which an initial moderate desiccation produces a higher resistance in subsequent challenge. Here, we explore the transcriptomic contribution to these phenomena through RNA sequencing. *D. serrata* and *D. birchii* adults were exposed to desiccation stress both with and without a period of acclimation. A total of six time points were sampled, three during the stress and three during the subsequent recovery. Unstressed flies were also sampled at all six time points to control for the effect of circadian expression patterns. Under both stress conditions (naive or acclimated), many more genes were determined to be differentially expressed in *D. serrata* compared to *D. birchii*. When comparing the naive to the acclimated conditions, only a few genes showed a differential response in *D. birchii* compared with hundreds in *D. serrata*. These results suggest that changes in gene regulation contribute strongly to the resistance and acclimation phenotypes in these species.

id #26895

Subterranean Pool Party: Determining trophic links between subterranean invertebrates in a groundwater ecosystem in Western Australia.

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The Yilgarn Region of Western Australia contains a rich diversity of subterranean invertebrates, and comprises hundreds of physically isolated calcrete aquifers which resembles a subterranean archipelago. Each calcrete has a unique combination of aquatic species including diving beetles (Dytiscidae), crustaceans (Isopoda, Amphipoda, Copepoda, and Ostracoda) and worms (Oligochaeta), but little is known about how the species within a calcrete interact trophically. Understanding the food webs in this aquifer system will provide valuable information for conservation management of these aquifer ecosystems, some of which are currently threatened by groundwater extraction and mining of the calcrete for mineral resource processing. This project focuses on one calcrete (Sturt Meadows) within the Yilgarn and aims to use Next Generation Sequencing (NGS) techniques (including metabarcoding) to identify the food web of its subterranean ecosystem. The project also aims to determine the primary source of energy for the ecosystem and determine if the energy comes from external sources of carbon or whether it's produced directly via chemoautotrophic bacteria. Finally, the project will combine metabarcoding and stable isotope analysis to provide insight into trophic interactions within the ecosystem. In this poster, we present the preliminary analyses and development of a reference barcode database of all the stygofauna and flora associated with the Sturt Meadows calcrete. These data will be used as the basis of primer design for the future metabarcoding analyses. Results supported previous studies showing that the stygofauna comprises three dytiscid beetle species and three amphipod species, but further revealed the presence of five copepod species and at least six species of oligochaetes. Results from recent field collections, that assessed the distribution of species and the degree to which taxa are spatially associated, will also be presented.

id #26908

Investigating the evolution of replication timing and monoallelic expression in orthologs of genes subject to genomic imprinting in therian mammals

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Genomic imprinting, the parent-of-origin dependent expression of a few dozen genes in eutherian and marsupial species, has so far not been observed in the earlier diverged monotreme mammal lineage or in birds. Interestingly, however, asynchronous replication, a hallmark of monoallelically expressed genes, has been observed in therian mammals and also in birds (Kitsberg et al. 1993; Dünzinger et al. 2005; May et al. 2008). In this study, we use fluorescence in situ hybridisation (FISH) and transcriptome analysis to assess the replication and expression status of platypus orthologs on a group of eutherian imprinted

genes, including *Igf2*, *Igf2r*, *Mest*, *Wt1*, *Copg2*, and *Ube3a*. The FISH results reveal that asynchronous replication is conserved in all mammalian lineages, and also in birds, at orthologs of imprinted genes. The transcriptome results provide evidence that some of these genes are transcribed from both alleles, whereas RNA-FISH experiments in fibroblast cells show that a proportion only expressed a single allele. Together these findings provide evidence that platypus orthologs of imprinted genes are expressed from one allele in many cells, but also indicate that there is mutually exclusive expression of the alleles (i.e. expression is from different alleles in different cells). We propose that this random monoallelic expression could be the precursor to parent-of-origin dependent expression of imprinted genes in therian mammals.

1. Dünzinger U, Nanda I, Schmid M, Haaf T, Zechner U. 2005. Chicken orthologues of mammalian imprinted genes are clustered on macrochromosomes and replicate asynchronously. Trends in Genetics 21: 488-492.
2. Kitsberg D, Selig S, Brandeis M, Simon I, Keshet I, Driscoll DJ, Nicholls RD, Cedar H. 1993. Allele-specific replication timing of imprinted gene regions. Nature 364: 459-463.
3. May A, Reifenberg K, Zechner U, Haaf T. 2008. Asynchronous replication dynamics of imprinted and non-imprinted chromosome regions in early mouse embryos. Exp Cell Res 314: 2788-2795.

id #26927

Apoptotic regulators in the auditory system: key factors in hearing loss

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Background: Acquired hearing loss (including age-related, noise-induced and drug-induced loss) is a significant public health issue. The molecular mechanisms resulting in this condition are poorly understood. However, it is clear that programmed death (apoptosis) of cells within the cochlea is often involved. Through better understanding of the regulation of this process we hope to identify therapeutic targets for prevention and treatment of acquired hearing loss.

Objectives: To understand the molecular regulation of cell death in the ear so as to identify drug targets for prevention and treatment of hearing loss.

Method: A range of functional genomics approaches are being applied to elucidate the regulation of auditory apoptosis. Primarily, a panel of engineered mouse strains harbouring mutations in apoptotic regulators is being assessed for hearing loss, to better understand cell death in the auditory system.

Results: Mutations at particular points of the intrinsic pathway of apoptosis have a profound effect on the auditory system. Deficiencies of certain apoptotic regulators can disrupt development of the auditory system or result in hearing loss in the adult.

Conclusion: Tightly regulated apoptosis is required for both development and maintenance of hearing. Targeting of apoptotic regulators will likely prove useful in prevention of cell death and resultant hearing loss in the ear. We are actively pursuing this as a strategy for development of novel therapies for acquired hearing impairment.

id #26932

DNA detection probabilities for the remote sampling of wildlife: the case of foxes in Tasmania

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PCR-based diagnostic tests are increasingly being applied to the analysis of wildlife for the identification of illegally traded or otherwise handled species and for many aspects of wildlife management. The application of these diagnostic tests requires rigorous verification commensurate with the cost of being wrong. Specifically, the risk of obtaining a false negative result (sensitivity) needs to be balanced against the risk of obtaining a false positive result (specificity) in the context of the question to be answered. There are particular risks surrounding the accurate assignment of DNA sequences to known taxa when amplifying from environmental samples that contain a pool of poorly characterised sequences. Here we report on an experimental “blind” analysis of a sequential PCR-based test developed to detect fox (Vulpes vulpes) DNA in predator scats. We show that this simple test involving an initial PCR-based screening step followed by sequencing for part of the cytochrome b gene, is highly accurate with a specificity of ~99.6% and a sensitivity of ~ 84%. We also show, using an in silico analysis of barcoding efficacy of sequences from 74 vertebrates, that this same short sequence of cytochrome b can provide high specificity for the assignment of vertebrate species likely to be encountered in Tasmania. We highlight the importance of developing appropriate reference sequence databases for each environmental system and of evaluating the potential for mis-identification of sequences from different taxa when undertaking wildlife studies.

id #26934

Retrotransposons and Genome Evolution

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Mammalian genomes are plastic and subject to constant change from a variety of processes, including retrotransposon insertions and retrotransposon mediated duplications and deletions. How does this contribute to evolution in the long term, and can we identify genomic regions less likely to be altered by retrotransposons. Because retrotransposons can account for the majority of the genome sequence in eukaryotes their accumulation and clade specificity have been implicated in speciation, regulation of gene expression, exaptation and structural variation. Understanding the mechanisms that govern retrotransposon distribution and replication are thus of fundamental importance.

We have carried out comprehensive genome analyses of repetitive DNA sequences, including retrotransposons in 10 species: human, mouse, rat, horse, cow, sheep, dog, elephant, opossum and platypus. We have also found that the ancestral, LINE L2 retrotransposos and their derived SINE Mirs - inactive in eutheria and marsupials - have persisted in conserved regions of shared synteny. Other recently derived (clade specific) SINEs or horizontally transferred elements such as BovB tend to accumulate more outside of these regions, hinting that these regions are resistant to incursion of currently active or horizontally transferred.

Our results suggest that aspects of mammalian genome structure are conserved either through natural selection or as a result of other constraints on retrotransposon insertion.

id #26935

Evolution of *IGF2/WSB1* interchromosomal interactions in mammals and mammalian hybrids

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Long-range interactions between loci on different chromosomes allow for the co-regulation of genes that are not locally clustered. Many of these interactions rely on CTCF, a highly conserved zinc-finger protein, which together with cohesin, has a genome-wide role in higher-order chromatin organisation and regulation. We investigated the interchromosomal interaction beween the imprinted *IGF2*gene, a potent mitogenic growth factor involved in embryo-fetal growth and development, and the non-imprinted *WSB1* gene, a WD-protein subfamily member thought to be part of an E3 ubiquitin ligase complex. The IGF2 gene is imprinted in therian mammals but not monotremes and we asked if this difference is also reflected in *IGF2* interchromosomal interaction. We investigated the conservation of the interchromosomal *IGF2/WSB1* interaction across amniotes using BAC clones in DNA FISH to observe interaction frequency in a cell population at single nuclei resolution. We found the *IGF2/WSB1* interaction conserved across mammalian evolution, although the interaction frequency was lower in chicken, platypus and bovine compared to that observed in mice. We conclude that interchromosomal interactions predate the evolution of genomic imprinting in mammals.

Changes in DNA methylation and imprinted gene expression have also been linked to abnormal hybrid growth phenotypes. Using a well-characterised bovine hybrid model we investigated if differences in *IGF2* interaction occur in *Bos primigenius indicus* (Brahman) and *Bos primigenius taurus* (Angus) bovine hybrids, where reciprocal crosses have distinctly different birth weights. We used DNA FISH and 3C techniques to investigate these interactions at both single nuclei and cell population level respectively. Preliminary data so far indicates a decrease in interaction frequency correlates with a higher birth weight in male BxA hybrids.

id #26940

Transcriptomic response to to climate stress in a generalist and a specialist *Drosophila* species.

Stephen Pearce[1], Madeleine Gane[1, 2], Rahul V Rane[1, 2], Ary A Hoffmann[2], John G Oakeshott[1]

1. *CSIRO, Canberra, ACT*
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Drosophila serrata and *D. birchii* (sister species of the *montium* subgroup) show marked differences in their ability to tolerate desiccation stress. *D. serrata* (a habitat generalist) has both a greater desiccation resistance than *D. birchii* (a rainforest restricted species), as well as a stronger acclimation response in which an initial moderate desiccation produces a higher resistance in subsequent challenge. Here, we explore the transcriptomic contribution to these phenomena through RNA sequencing. *D. serrata* and *D. birchii* adults were exposed to desiccation stress both with and without a period of acclimation. A total of six time points were sampled, three during the stress and three during the subsequent recovery. Unstressed flies were also sampled at all six time points to control for the effect of circadian expression patterns. Under both stress conditions (naive or acclimated), many more genes were determined to be differentially expressed in *D. serrata* compared to *D. birchii*. When comparing the naive to the acclimated conditions, only a few genes showed a differential response in *D. birchii* compared with hundreds in *D. serrata*. These results suggest that changes in gene regulation contribute strongly to the resistance and acclimation phenotypes in these species.

id #26942

The evolution of endemic crustaceans from arid zone groundwater habitats

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Groundwater-dependent ecosystems in the Australian arid zone contain highly diverse populations of endemic crustacean taxa with complex evolutionary histories. Recent phylogenetic studies have, furthermore, helped to reveal potential evolutionary connections between geographically disparate endemic crustaceans from springs of the Great Artesian Basin in South Australia and subterranean calcrete aquifers in central Western Australia. Research incorporating larger multi-locus datasets and a broader geographic coverage is needed, however, to unravel the details of these historic links and to better understand the relationships amongst taxa. For this project, we will utilise next-generation sequencing technologies to explore the evolution

and systematics of amphipods (Chiltoniidae) and aquatic isopods (*Haloniscus*) from groundwater-dependent ecosystems in the Australian arid zone and to further assess evolutionary connections between taxa from these regions. Transcriptomes of chiltoniid and *Haloniscus* taxa have been sequenced and assembled for use in orthology prediction through the recently developed Orthograph pipeline. This pipeline is a graph-based approach that reciprocally blasts RNA-seq contigs against official gene sets derived from the annotated genomes of reference species. This approach produces a specialised set of orthologous markers that can be used to develop baits for target enrichment. Here we present details on the methodological processes we have undertaken to begin identifying new phylogenetically useful markers that will allow us to assess our aims for non-model crustacean species.

id #26943

Application of DArTseq molecular marker platforms in phylogeographic and evolutionary genetics research of vertebrates at different geographic ranges and evolutionary timescales.

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Next-generation sequencing (NGS) approaches are increasingly being used to generate multilocus data for phylogeographic and evolutionary genetics research. Several genome complexity reduction methods that allow only a subset of the genome to be sequenced have been described. We detail the applicability of an Australian genome complexity reduction approach using restriction enzymes (DArTseq) in vertebrate study systems at different evolutionary and geographic scales. DArTseq has been applied across numerous plants species, due to high throughput capabilities, genome coverage and inter-specific transferability; however, it has not yet been widely used in animal systems. We present the results of two case studies using data from the DArTseq molecular marker platform: SilicoDArTs ("presence/absence" (dominant) markers) and SNPs in fragments present in the representation. Firstly, we used DArTseq in a large phylogeographic study of the agamid lizard *Ctenophorus caudicinctus* for 91 individuals, spanning the geographic range of this species across the Australian arid-zone. A lower density DArTseq assay resulted in ~100,450 SilicoDArt markers and 28,900 SNPs. Secondly, we applied this approach to an evolutionary genetics study of a classic frog hybrid-zone (*Litoria ewingii – L. paraewingi*) in Victoria across 94 individuals, which resulted in 48,117 SNPs for a lower density and 67,060 SNPs in a high density assay. We analysed SNP data using multiple approaches including data-reduction PCAs, Bayesian coalesence approaches (SNAPP), and population genetics (e.g., fastSTRUCTURE). We show that results from DArTseq are comparable to traditional approaches, when compared to a multi-gene study of *C. caudicinctus* (mtDNA & 5 nuclear genes) and a *Litoria ewingii – L. paraewingi* hybrid study (microsatellites), but with a significant increase in resolution. We conclude that DArTseq is a platform that can be utilised successfully in phylogeographic and evolutionary genetics research of vertebrates at different geographic ranges and timescales, and provides a cost-effective alternative to other traditional and NGS approaches.

id #26944

Victorian Venom Bank - the first bite.

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Animal venoms have two clear roles in human medicine – (i) the starting point for the production of effective antivenoms to neutralise toxicity, and (ii) the development of therapeutic leads and pharmaceutical tools from natural products. Progression of these two research pathways will be most effective when underpinned by a robust understanding of the diversity of the constituent venom toxins, and a taxonomically complete collection of venoms with matching whole animal specimens, including adequate representation of intraspecific geographic variation.

The Victorian Venom Bank aims to initiate a new resource for research into antivenoms, pharmaceutical leads, and taxonomy. The Venom Bank contains matched samples from each specimen of venom, venom gland, liver and heart tissue and the vouchered animal, stored at Museum Victoria. Published sequence data from these specimens will be available for research worldwide. We are not aware of any such resource existing outside of antivenom manufacturers or commercial suppliers worldwide.

Our 'pilot' animals for the Victorian Venom Bank are snakes from the *Notechis* lineage. The tiger snakes and related lowland and highland copperheads are the most common large elapid snakes in Victoria and responsible for the majority of human-snake interactions and bites. We collected specimens and milked them for venom, which was stored fresh frozen. We euthenised the animals 5 days later, to maximise venom production in the glands, then extracted the venom gland, and took liver and heart tissues samples which were stored in the Museum Victoria Biobank facility. Specimens were preserved and registered into the Museum Victoria herpetology collection. Transcriptome libraries were produced from venom glands and liver tissue and the sequence transcripts we produced were translated and Blasted against the Toxprot database to identify venom constituents, where available. We make preliminary comparisons of the most common transcripts between tissue types and among species.

id #26946

Finding a good pair of genes: Do lizards choose mates based on their immune gene diversity?

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Disease is an increasing conservation concern. Sociality adds complexity to disease dynamics, where group living may increase the spread of parasites through a population. Genetic diversity acts as a buffer to disease. In particular, immune genes are critical to species persistence; diversity of these genes may be a means of host defence against novel parasites. Further, immune gene diversity may influence mate choice, where females may choose a male with 'good genes' to enhance the fitness of her offspring. Our study species, the rocky crevice dwelling *Egernia stokesii* (gidgee skink), forms stable family groups, has high levels of monogamy, and delayed dispersal. Genetic clustering of this species at both a population and social group scale reflects the spatial patterns of social groups and habitat availability. Despite social and habitat constraints, inbreeding is absent suggesting behaviours such as kin avoidance may play a role in mate choice. This is supported by evidence of *E. stokesii* ability to recognise kin/non-kin. In this context, we ask: Do these lizards choose mates based on their diversity at immune genes? Using blood samples from a previous population level study of *E. stokesii*for which paternity and non-breeding status of individuals is known, next-generation DNA sequencing techniques (Illumina MiSeq, paired end) were applied to derive genotypes for the major-histocompatibility complex (MHC). MHC genes involved in both intracellular (Class I) and extracellular (Class II) disease resistance were sequenced. Individual MHC diversity measures were used to test for the significance of mate MHC diversity compared with other candidate mates, while controlling for relatedness (derived from microsatellite genotypes) and spatial proximity. We present preliminary results and outline future work on immune gene diversity in this social lizard.

id #26947

Transcriptome analyses of cumulus and granulosa cells of the murine ovary

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Cumulus cells (CC) and granulosa cells (GC) of the ovary play a vital role in supporting the growth and development of the oocytes. CC and GC play functionally distinct roles in the mature follicles and comparing their transcriptomes (mRNA and small RNA) at a global and systems level will elucidate molecular mechanisms underlying their functional diversity. Several microarray and qPCR studies have revealed mRNA signatures that are characteristic to these cells but there has been no unbiased sequence dataset of the mRNA repertoire of the supporting cells in mice.

Murine CC and GC were isolated just prior to ovulation at 11.5 hours post human Chorionic Gonadotropin treatment and were deep sequenced. Our study provides the first genome-wide overview of specific features of the murine CC and GC coding transcriptomes using next generation sequencing. We identified 40 genes upregulated and 22 genes downregulated (CC/GC) using stringent settings. We found *Has2*, *Ptx3, Tnfaip6, Lox* and *Btc* to be upregulated in the cumulus while *Amh, Grem1, Col3a1* and *Ihh* were upregulated in the granulosa cells. We then performed network analyses on the list of differentially expressed genes and found gonadotropins (FSH and LH), TGFβ and EGF superfamilies highly associated indicating that these networks are triggered to bring about cumulus expansion and ovulation in line with literature.

We also found genes with no known functions in these cells including *Nupr1*, *Egfl7*, *Cotl1*, *Muc16* and *Msln* to be differentially expressed. In order to gain insights into the expression levels of these genes during ovulation, we performed TaqMan assays to monitor mRNA levels from 0h (hCG stimulation) to 16h (post ovulation). This analysis provided novel insights into the molecular changes through ovulation especially in response to ovulatory inflammatory stress.

We are currently analyzing the small RNA datasets from these cells that could provide novel insights into the small RNA regulation of these cells.

id #26948

Investigating the transcriptome wide impact of expanded polyalanine tract mutations in *ARX* contributing to intellectual disability and seizures.

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Aristaless related homeobox *(ARX)* gene encodes a paired-type homeodomain transcription factor with critical roles in embryonic development. Mutations in *ARX* give rise to intellectual disability (ID), epilepsy and brain malformation syndromes. Over half of all mutations in *ARX* lead to expansion of two (of four) polyalanine tracts in the ARX protein. Our recent investigations in mice modelling the two most frequent polyalanine expansions (PA^E) seen in human patients clearly show a marked reduction in mutant Arx protein expression in the developing forebrain (1). While both mice models display similar reductions in mutant Arx protein, each displays a distinct phenotype that recapitulates the phenotypes seen in patients; $Arx^{(GCG)7}$ (PA1) mice have seizures in addition to learning and memory problems, with seizures not reported in the $Arx^{432\text{-}455dup24}$ (PA2)mice. To investigate the genetic mechanism(s) underpinning these phenotypic differences we chose a

transcriptome wide approach of RNA-seq to analyse gene expression in the developing (12.5dpc) forebrain of these mice. Our analysis has identified a total of 283 genes significantly dysregulated (Log2FC >+/-1.1, P-value <0.05) when both mutations are compared to wild-type (WT) animals. When each mutation is considered separately, there is a greater number of genes dysregulated in the more severe PA1 mice (825 genes) compared to WT than is noted in the PA2 animals (78 genes) when compared to WT. The majority of genes significantly dysregulated in PA1 mice are also perturbed in the milder PA2 mice, but fail to reach significance compared to WT at this early stage of development. A recent study has demonstrated estradiol administration during early postnatal development prevented spasms in infancy and seizures in adult Arx PA1 mutants (2). Our current analysis aims to identify key pathways disrupted in the mutant mice that may contribute to the phenotypes and transcriptional changes involved in the estrogen pathway that can be targeted for treatment. We conclude that genes commonly dysregulated in both mutant genotypes will contribute to the consistent clinical features of ID whilst genes that are disrupted preferentially (or earlier in development) or at an increased level in PA1mice contribute to the more severe phenotype of infantile epileptic seizures.

1. Lee et al., Hum Mol Genet 2014 23(4):1084-94
2. Noebels et al., Sci. Transl. Med. 6, 220ra12 (2014).

id #26951

Genomic Distributions of Interspersed repeats both within, and across mammalian species

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Transposable elements (TEs) are genomic segments of mobile DNA able to replicate and insert themselves elsewhere in the genome. TEs are grouped into families based on transposition mechanism, structure, and phylogeny. Some TE families are ancestral and shared across species (MIRs and L2s), whereas other TE families were more active after species divergence and have a large amount of clade-specific members (L1s, BovBs, and Alus). Moreover, TEs are a likely driver of mammalian genome evolution, as repetitive elements derived from TEs are ubiquities in mammalian genomes. Our aim is to identify underlying patterns of mammalian genome conservation of interspersed repeats in terms of both ancestral and clade-specific TE activity.

We separated each species' genome into 1.5 mbp bins of contiguous sequence and recorded each bin's number of interspersed repeats. Next, we performed an intra- and inter-species interspersed repeat analysis. For the intra-species analysis, we performed principal component analysis (PCA) and measured the weight of each repeat family in high-ranking principal components (PC1 and PC2) as a measure of their genomic impact. We then compared the results of each analysis to identify underlying patterns of mammalian genome repeat architecture. The inter-species analysis is designed to show whether underlying repeat patterns based on genome structure share homology at the sequence level. The analysis employs a novel method that involves using genome wide pairwise alignments to remodel a species TE content according to a reference. Making it possible to perform an across-species correlation analysis of TE content.

Intra-species analysis showed that both ancestral and largely clade-specific TEs often weighted highly in high-ranking orthogonal PCs. Suggesting that mammalian genomes are comprised of ancestral domains, marked by the accumulation/conservation of ancestral repeat families. Inter-species analysis revealed that various ancestral and clade-specific repeat families were highly correlated in corresponding genomic regions between mammalian species. However, we found that the distribution of clade-specific SINEs was quite variable across species. Therefore, it is likely, there is some level of underlying conservation of genome architecture according to the distribution of both ancestral and some clade-specific repeat families. Whereas, clade-specific SINEs may be an agent of genomic variation.

id #26952

Horizontal Transfer and Evolution of Transposable Elements in Animals

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Eukaryotic genomes contain an abundance of repetitive transposable elements (TEs) that influence the duplication and rearrangement of regulatory DNA. Because of their dynamic nature, many TEs are capable of horizontal transfer (HT) – the transmission of genetic material between non-mating species. Most reported cases of HT involve DNA transposons, long terminal repeat (LTR) retrotransposons or retroviruses. However, recent evidence shows that various non-LTR retrotransposons (e.g. BovBs) can also move between species, suggesting that horizontal transfer is much more widespread in vertebrates than previously believed.

BovB is a long interspersed element (LINE) about 3.2kb long. It is found scattered among a wide range of eukaryotic genomes including reptiles, insects, marsupials and some other mammals. Moreover, it appears to retain remarkable sequence similarity across very divergent host species. By extracting full-length BovB sequences from all publicly available genomes, we were able to extend the current BovB phylogeny to include bats and other vertebrates, and provide further evidence of horizontal transfer.

This extensive analysis of full-length BovBs led to the conclusion that BovB copy numbers vary drastically between species or clades. For example, ruminants and Afrotherian mammals contain thousands of BovB copies, while equids and bats are very BovB-sparse by comparison. So it seems the horizontal transfer process has two parts: effective insertion of the TE, then expansion throughout the genome. Now that we have identified species with BovB (indicating successful colonisation), we hope to discover why some species are more resistant to TE expansion than others.

Growing evidence suggests that HT of TEs can act as a catalyst for actively transforming eukaryotic genome structure and content. Further research over a wide range of different species and TEs is needed to determine how often this process occurs, and the extent of the subsequent genomic change.

id #26953

The impact of repetitive elements on gene expression evolution in monotremes and marsupials

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Transposable elements are discrete segments of DNA that can move within genomes. In rodents and primates, approximately 38% to 45% of the genome is made up of transposable elements (TEs), and nearly half of the human genome has been identified as comprised of TEs. In addition, advances in sequencing technologies have begun to reveal the substantial contribution of these elements to either in gene expression or genome evolution.

Monotremes and marsupials are two lineages not only special in their reproductive process, they have also diverged morphologically and physiologically compared with eutherians, due to evolutionary selection pressure. Therefore, studying the association between TEs and other genome features within these species can provide a useful comparative perspective with respect to mammalian gene expression and genome evolution.

We have designed a series of methods and programs for analyzing the association between TEs and changes in gene expression as a result of evolution. As a first step, we have carried out *ab* initio identification and annotation of transposable elements in monotremes (platypus and echidna) and marsupials (opossum).

We have found that about 21% of the opossum genome sequence comprised LINE-1, CR1 and RTE, compared with 26% in the previous study. And about 41% of the echidna genome contains TEs, >35% of the genome sequence were non-LTR retrotransposons.

id #26958

Puzzling mitochondrial heteroplasmy in Australian native bee, *Amphylaeus morosus*

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Mitochondrial heteroplasmy is the occurrence of polymorphic sequences within a single individual. Mitochondrial heteroplasmies are only sporadically reported in the literature, but are probably quite common in somatic tissue. We genotyped populations of the native bee *Amphylaeus morosus* from Victoria and Queensland, using Sanger sequencing of the COI 'barcode' region. We found clear heteroplasmy at multiple nucleotide sites, and these were consistent across specimens, though not every female had all heteroplasmies. All heteroplasmies were found in both Queensland and Victoria populations. Apart from these heteroplasmies, we found no other nucleotide variation in our sequences. We then cloned a single female and of the 16 sequences recovered, 15 had unique sequences. Of these, some, but not all, represented the heteroplasmies obtained from our Sanger sequence of the same specimen. For Sanger sequences, using alternative base pairs for heteroplasmic sites, and cloned sequences we found no mitochondrial stop codons, nor stop codons associated with heteroplasmies when using a nuclear coding scheme, so it seems unlikely our results are due to numts. The lack of sequence variation in our Sanger sequences could be explained by a massive and recent genetic sweep, perhaps due to the Wolbachia strains we found in our specimens. However, this would not explain the maintenance of conserved heteroplasmic sites across the wide range of this bee species. Extensive intraspecific barcoding of other native bee species by our lab has not revealed such consistency in heteroplasmies, so it seems unlikely that heteroplasmies in *Amphylaeus morosus* are sequencing artefacts. Our results present a major puzzle for interpretation. One possibility is that selection is maintaining polymorphism in COI for this species, but that presents severe challenges because of the mitochondrial population bottlenecks that occur during ovigenesis. We welcome discussions on our findings and suggestions for possible mechanisms that may explain our data.

id #26960

Avoiding the relatives – sleepy lizards do it too!

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Social organization is widespread; even largely solitary species must organize themselves to enable contacts with mates and reduce competition with conspecifics. Although the forms of social structure can be subtle in solitary species, understanding the factors that influence them may be important for understanding how different forms of social organization evolved. We investigated the influence of genetic relatedness and spatial structure on social associations in a solitary living Australian scincid lizard, *Tiliqua rugosa*. We derived the genetic relatedness of 46 lizards from analysis of genotypes at 15 microsatellite DNA loci, and described social networks from GPS locations of all the lizards every 10 min for 81 days during their main activity period of the year. We found that connected male dyads were significantly more related than expected by chance, whereas connected male/female and female/female dyads had lower relatedness than expected. Among neighbouring male/male and male/female dyads, the strongest social relationships were between lizards that were the least related. Explanations of this pattern may include the avoidance of inbreeding in male/female dyads, or the direction of aggressive behaviour towards less related individuals in male/male dyads. Observed social associations (inferred through synchronous spatial proximity) were generally lower than expected from null models derived from home range overlap, and many close neighbours did not make social contact. This supports our hypothesis for the presence of deliberate avoidance between some neighbouring individuals.

We suggest that lizards can discriminate between different levels of relatedness in their neighbours, directing their social interactions towards those that are less related. This highlights differences in how social associations are formed between species that are solitary (where associations form between unrelated conspecifics) and species that maintain stable social groups structured by kinship.

id #26962

Platypus global meiotic silencing shows recent origin of MSCI in therian mammals

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In therian mammals heteromorphic sex chromosomes are subject to meiotic sex chromosome inactivation during meiotic prophase I while the autosomes reactivate after a period of transient global transcriptional suppression. In response to sex chromosome silencing, retroposed autosomal copies of sex chromosome genes are required. In birds, global transcriptional silencing also occurs however no autosomal reactivation occurs and X-derived retrogenes have not evolved.Egg laying monotremes are the most basal mammalian lineage, feature a complex highly differentiated XY sex chromosome system with homology to the avian ZW, and also lack retrogenes originating from the sex chromosomes. In order to delineate the point of origin of sex chromosome specific silencing in mammals we investigated whether MSCI exists in platypus. Our results show that platypus sex chromosomes display only partial and transient colocalisation with repressive chromatin marks and surprisingly completely lack a hallmark meiotic epigenetic signature present in other mammals. Remarkably platypus instead feature an avian like period of generalised transcriptional suppression through pachytene with the sex chromosomes and the future mammalian X maintaining strong perinucleolar association.Our work demonstrates for the first time that 'mammalian' meiotic silencing evolved after the divergence of monotremes as a result of the differentiation of the therian XY sex chromosomes providing a novel evolutionary scenario from which the future therian X chromosome commenced the trajectory toward MSCI.

id #26968

Establishing the phenotypic deficits in a mouse modeling the most frequently expanded polyalanine tract mutation in *ARX* contributing to intellectual disability and seizures.

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Intellectual disability (ID) is a complex and debilitating disorder that affects approximately 1 in every 50 people worldwide. The *Aristaless-related homeobox gene* (*ARX*) is a frequently mutated gene on the X-chromosome contributing to X-linked intellectual disability, epilepsy and brain malformations. Over half of all mutations in *ARX* lead to expansion of two polyalanine (PolyA) tracts. Utilising mice modelling the two most frequently expanded PolyA expansion mutations our recent investigations have identified a marked reduction in Arx protein expression in the developing forebrain (1). Interestingly, both mouse models display similar reductions in mutant Arx protein. However, only one mouse model has been reported to recapitulate the phenotype seen in patients; $Arx^{(GCG)7}$ (PA1) mice have seizures in addition to learning and memory problems (2). The phenotypic data for the mouse modelling the more frequent PA2 mutation in patients has not been reported and constitutes a barrier to understanding the molecular mechanisms involved. Here we report functional analysis during postnatal life in $Arx^{432\text{-}455dup24}$ (PA2) mice in order to identify cognitive and behavioural deficits. Using a battery of behavioural tests both mouse models were found to have reduced activity, increased anxiety, impaired learning and memory and reduced sociability. In general, mice with the PA1 expansion mutation displayed greater behavioural deficits than the PA2 mice, in keeping with more severe phenotypes reported in patients. Both mouse models exhibited seizure characteristics from postnatal day 13, and an increased mortality rate from postnatal day 15, when compared to wild-type littermates. Taken together, this data indicates the $Arx^{432\text{-}455dup24}$ (PA2) mice display a phenotype that recapitulates the often milder clinical findings observed in human patients but spans the spectrum including comorbidities such as infantile spasms. Understanding the molecular and functional deficits that arise due to expanded PolyA tract mutations in Arx is a necessary step on the path to the development of intervention therapies for interneuropathies.

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2. 2) Kitamura et al., Hum Mol Genet 2009 18(19):3708-24

id #26973

Evolution of gene clusters involved in the inflammatory response

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Publish consent withheld

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id #26974

Why do they find it so hard? Using the Genetics Concept Assessment to characterise student misunderstanding.

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Learning genetics is challenging – the learner must reconcile concrete observations with abstract concepts, face cognitive overload, master a fundamental biological foundation, and deal with randomness and complexity (or is that just me?). As a teacher, I strive to guide students towards deep understanding by giving clear explanations, highlighting connections, and scaffolding problem-solving strategies. Why then, do they still give such weird answers to exam questions? The missing link is what the students are actually thinking. Approaches that reveal what students are thinking and how they think within a context should lead to more effective teaching and learning strategies[1]. Concept assessments (or inventories) are sets of multiple choice questions that indicate students' understanding of core concepts and principles. We have applied the Genetics Concept Assessment[2] to a second year genetics course this year, and compared student responses with previously published patterns[3]. In particular, we are interested in identifying concepts that are commonly misunderstood, especially those for which a high proportion of students give a common, incorrect answer. Such misconceptions have the potential to reveal much about how students think about genetics. We hypothesise that some concepts are commonly misunderstood together, and aim to identify these connections. We hypothesise that such connections between misconceptions can be aligned with specific cognitive principles[4]. By working to explain the underlying causes of misconceptions, we aim to suggest strategies to minimise the establishment of misconceptions, maximise their replacement with deep understanding, and thus maximise students' potential to learn and understand genetics.

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id #26979

The genetics of uterine vasculature

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Pregnancy is a complex process underpinned by genes that control nutrient transport, waste removal, gas exchange, tissue growth and remodelling, and immunological protection of developing embryos. Angiogenesis, the growth of blood vessels, is one of the most important changes that takes place during pregnancy to facilitate these functions. We have used transcriptomics to investigate the genetic control of uterine angiogenesis in viviparous amniotes, combined with qPCR to identify the relative importance of different splice variants of vascular endothelial growth factor for the evolution of pregnancy.

id #27154

A novel genetic method for generating inter-species Brassicaceae hybrids

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Wild relatives of crop plants often contain superior traits such as resistance to disease, pod shatter or tolerance to specific environmental conditions such as cold, drought or heat stress that are attractive for inclusion in commercial crop varieties. Often when crosses between different species or domesticated cultivars and wild relatives are made, hybrid embryo development aborts before seed maturity. Often embryo abortion is associated with parental conflict in the developing endosperm due to inappropriate allocation of maternal resources. We have identified a class of genes, wide-hybrid rescue (*whr*), that partial overcomes parental conflict barriers during hybrid endosperm development. We discovered that when *A. thaliana whr1* female mutants were crossed to *B. pinetorum,* hybrid seed maturity and subsequent germination was approximately ten times higher than the wild type control. Mutant *whr1* has excess cell division in the endosperm associated with increased transcription of protein coding genes and long non-coding RNAs. We will present ongoing research into the characterization of *A. pinetorum* hybrids and the identification of *B. rapa whr* mutants.

id #27155

Identification of long non-coding RNAs from developing seeds of *Arabidopsis thaliana*

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Using Illumina sequencing we identified 7,258 long non-coding RNAs (lncRNAs) from developing siliques of *Arabidopsis thaliana* ecotypes Columbia-0 and C24 1 day after pollination (DAP). Of these lncRNAs, 1,562 were intergenic and the remaining 5,696 partially overlapped with either exons or UTRs of annotated protein coding regions. Interestingly, we identified intronic lncRNAs that appear to initiate transcription in introns and extend into the adjacent exon both in the sense and antisense orientation of the annotated protein-coding gene. We also identified the intronic long non-coding RNA, COLDAIR, that is required for targeting the repressive PRC2 complex to the flowering time regulator *FLC*. In order to identify cell specific lncRNAs in the chalazal and peripheral endosperms and embryo nuceli, we developed *Arabidopsis* GFP marker lines to perform cell sorting of nuclei using the INTACT method. We are currently sequencing lncRNAs from these tissue specific nuclei. Our long-term aim is to undertake functional analysis of these lncRNAs.

id #27178

Apparent refutation of the Lewontin-Birch introgression hypothesis in Queensland fruit fly

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The two major native fruit fly pest species in Australia are Queensland fruit fly, *Bactrocera tryoni* (TRY) and Lesser Queensland fruit fly *Bactrocera neohumeralis* (NEO). TRY has invaded Southern fruit growing areas, and microsatellite analysis has shown that the strains of TRY involved, TRY(S), are genetically differentiated from TRY in its main range of Queensland.

Lewontin and Birch in 1966 proposed that TRY(S) might be derived from an introgression event of NEO into TRY. Sequencing of laboratory strains of wild-derived TRY, TRY(S) and NEO appeared to confirm this hypothesis, showing that TRY(S) and NEO are extremely close in a few regions, with 100% homology of assembled sequences that contain exons, introns and inter-gene regions.

Although numerous TRY sequences were involved in these comparisons, all of the NEO sequences were based on a single strain that had been held in laboratories for several years. It seemed possible, although unlikely from the data, that introgression in the opposite direction, TRY(S) into the NEO lab strain, could account for the results. Attempts to resolve this possibility using PCR on wild TRY(S) and NEO flies gave equivocal results.

We have now resolved the question using genome sequencing of a batch of wild NEO flies. The results are still being analysed in detail, but it is clear that in regions of close homology between the old NEO and TRY(S) strains, the new NEO differs substantially from TRY(S) and from the old NEO strain. It is difficult to escape the conclusion that the introgression event in question occurred in the laboratory, and not in the wild as postulated under the Lewontin-Birch hypothesis.

id #27191

RNA 5-methylcytosine is required for oxidative stress tolerance in *Arabidopsis thaliana.*

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Reactive oxygen species are produced in plant and animal cells in response to many different abiotic or biotic conditions, and their uncontrolled accumulation can lead to oxidative stress. While protein-coding genes have been demonstrated to play a role in oxidative stress tolerance, a role for post-transcriptional RNA modifications has not been demonstrated for oxidative stress in plants. Here we characterize TRM4B (Transfer RNA Methyltransferase 4B), which is an RNA methyltransferase that catalyzes RNA 5-methylcytosine (m^5C). We discovered *Arabidopsis thaliana trm4b* mutant seedlings are more sensitive to oxidative stress, but not high salt. Another RNA methyltransferase, TRDMT1 (tRNA aspartic acid methyltransferase 1) was found to not be involved in oxidative stress tolerance. We initially identified m^5C sites transcriptome-wide using RNA bisulfite conversion next generation sequencing (RBS-seq) on wild type and *trdmt1 trm4b* siliques. We discovered 48 potential m^5C sites in mRNAs and non-coding RNAs with at least 10% methylation. Currently we are identifying oxidative stress induced m^5C sites in wild type and *trm4b* seedlings using RBS-seq. We have identified 39 and 7 potential m^5C sites in transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), respectively. Twenty-four methylated nuclear tRNAs were detected, containing 39 potential m^5C sites, which occur at six structural positions. Of these 39 potential sites, 3 required TRDMT1, and 25 absolutely required or had reduced methylation in *trm4b* mutants. Interestingly, none of the 25 detected mitochondrial or chloroplast tRNA species contained methylated sites, which is consistent with an ancient cyanobacterial origin for chloroplasts. Together our results suggest RNA m^5C is a conserved and important post-transcriptional modification in plants.

id #27193

Identification of *pla/ubp14*, an *Arabidopsis* mutant that displays lengthened plastochron and larger organs

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A characteristic feature of plants post-embryonic growth is the initiation of leaf primordia from the shoot apical meristem (SAM) at regular time intervals. The time between the initiation of two successive leaf primordia from the SAM is referred to as the plastochron. Although vital in influencing overall shoot architecture, our current understanding of the underlying genetics of plastochron regulation is very limited. Here, we describe the identification of an *Arabidopsis* mutant, *pla* that affects plastochron index by lengthening the time taken for successive leaves to appear. Accordingly, at different stages of vegetative growth, the *pla* mutant consistently displays reduced number of leaves compared to wild-type plants. In addition, the mutant also displays pleiotropic phenotypes affecting the sizes of floral organs, seeds and cotyledons. Analysis of a segregating mapping population suggests that the phenotypes observed for *pla* are inherited as a single recessive locus. To positionally map the *pla* mutation, an F2 mapping population was generated by crossing *pla* (Col-0) to a polymorphic parent, *Landsberg erecta*. Traditional map-based cloning using polymorphic genetic markers as well as next-generation sequencing was used to identify mutations in three genes that are likely candidates for the *pla* phenotype. Complementation studies revealed UBP14, a ubiquitin specific protease, as the causal gene responsible for the *pla* phenotype. Ubiquitin proteases are involved in ubiquitin recycling, and work is currently being carried out to determine if the *pla* phenotype is associated with an accumulation of polyubiquitin chains and a reduction in ubiquitin monomers.

id #27212

Population studies in *Lobesia botrana*

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Accurate species delimitation can have a direct impact on biosecurity preparedness, as it affects various decision making processes such as early detection of intercepted suspect species, and the confirmation of presence/absence of the invasive pest species in the country. Lobesia botrana is a major pest species of the grape and wine industry world wide. L. botrana originated in Europe with periodic successful incursions in various countries, including North and South America, and Africa. In this study we use next generation sequencing (NGS) to characterise the complete mitogenomes of 4 L. botrana individuals from the native range and, to assist with PCR primers design for species delimitation based on the mtCOI barcoding region. We found very little genetic variation at the mtCOI barcoding region across populations of L. botrana sampled from its native range, when compared with individuals sampled from some of the invasive regions. We showed that despite its economically important pest species status, no existing mtCOI gene of L. botrana had been sequenced or available in the public DNA database (e.g., iBoL, GenBank). Its presence in Australia is not confirmed despite the presence of seven unidentified Lobesia spp. sampled from Australia

id #27221

Validation of SNP genetic markers for individualisation in Bigleaf Maple – a forensic tool to combat illegal logging

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Illegal logging is widely recognised as a major global threat to biodiversity and natural heritage. However, despite increased awareness and invocation of laws to prohibit the trade of illegally sourced wood, illegal logging crimes remain largely unprosecuted, principally because there are a lack of appropriate forensic identification tools with which to prove wood illegality.

In this study, we sought to develop and validate genetic markers suitable for forensic individualisation in *Acer macrophyllum*, the Bigleaf Maple, a species prized for its beautiful grain patterns and used to make high-end musical instruments. Bigleaf maple is distributed along the western US states of California, Oregon and Washington, and extends into British Columbia in Canada. The US Forest Service reports multiple thefts throughout its national parks and requires forensic tools to enable wood evidence seized from suspects to be linked back to the stumps of illegally felled trees.

Our projects utilised next generation sequencing (NGS) technologies to identify a suite of potentially informative single nucleotide polymorphisms (SNPs) which were then trialled and assessed for suitability as forensic individualisation markers in the MassARRAY system. A set of >400 reference samples from across the known range of the species was genotyped to produce a robust reference database and a final subset of SNPs selected to form a reliable individualisation test for the species. The resulting test was validated according to the SWGDAM validation guidelines.

Here we presents the specifics of that validation and demonstrate the effectiveness of this new forensic tool. There are currently no published validations of individualisation markers for timber species, representing a significant knowledge gap. Our study takes the first steps to addressing this need in an important US forestry species and should serve a demonstrated framework for future development of forensic timber individualisation resources.

id #27231

Zebrafish models of human disease: the contribution of neural crest to craniofacial and heart disorders in cohesinopathies

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Mutations in subunits or regulators of cohesin cause a spectrum of disorders known as the 'cohesinopathies', the best known example of which is Cornelia de Lange Syndrome (CdLS). CdLS and related cohesinopathies are characterised by broad spectrum, multifactorial developmental anomalies. Mutations in the integral cohesin structural protein RAD21 result in a congenital phenotype that is consistent with a "cohesinopathy". Children with *RAD21* mutations display growth retardation, minor skeletal anomalies and facial features that overlap findings in patients with CdLS. Heart defects occur at high frequency in the cohesinopathies, around 30% in CdLS. The mechanisms by which heart defects occur is enigmatic, but assumed to be developmental in origin. We depleted cohesin subunit Rad21 by 70-80% in zebrafish to create a model of heart pathology in the cohesinopathies. We show that hearts of Rad21-depleted animals were smaller, often failed to loop, and functioned less efficiently than size-matched controls. Functional deficiency was accompanied by valve defects and reduced ejection fraction. Proper cardiac development relies on the correct specification and migration of cardiac neural crest cells. In Rad21-depleted animals, neural crest was specified normally and was competent for migration, but failed to populate the heart. Neural crest cells also exhibited a wandering behavior and failed to condense correctly into pharyngeal arches. Transcriptome analysis revealed that Wnt pathway, chemokine and cadherin genes are dysregulated at the time of cardiac neural crest development. Our results may partially explain the etiology of heart defects in the cohesinopathies, and raise the possibility that mild mutations in cohesin genes, beneath the threshold for syndromic phenotypes, may be causative of a fraction of congenital heart disease in human populations.

id #27400

Rational Therapeutic Approaches in Acute Lymphoblastic Leukaemia Based on New Genetic Lesions Identified using Next Generation Sequencing.

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Acute Lymphoblastic Leukaemia (ALL), a malignant disorder of lymphoid progenitor cells, affects both children and adults, with peak incidence between the ages of 2 and 5 years, and a further peak in older age. While cure rates exceed 80%, relapsed B precursor ALL remains a leading cause of non-traumatic death in children and young adults. The outcome for older patients remains poor. Recent advances in genomic profiling have defined ALL as a heterogeneous disease with multiple subgroups characterized by distinct genetic alterations, with their own prognostic and therapeutic implications. A clinically important finding is the identification of a new subtype of high-risk ALL, termed Ph-like ALL, that exhibits a gene expression profile similar to *BCR-ABL1* ALL (Philadelphia chromosome positive ALL) and harbors a diverse range of genetic alterations activating tyrosine kinases, in addition to other genomic changes. The clinical importance of this finding is that these kinases provide therapeutic targets for which safe and active drugs are already available and in clinical use. We have developed rapid and accurate screening tests to identify these targetable genetic lesions in pre-B ALL patients to enhance the clinical application of rational anti-leukaemic agents in this setting. While there is no doubt that the use of rational therapies will transform outcomes for a large number of patients…there is also no doubt that we will be faced as researchers with new challenges.