71st Anual Meeting of the Genetics Society of AustralAsia

June 30 - July 3 2024

Abstract Book

MACQUARIE University

Meet the local organising committee

As we gather at the scenic Wallumattagal campus of Macquarie University, we extend a warm welcome to all attendees of this year's Genetics Society of AustralAsia (GSA) conference. The event is hosted by the School of Natural Sciences. This event is a product of the tireless efforts of our organizing committee. Their deep commitment to celebrating AustralAsian genetics to bring this conference to life.

Oliver Griffith
Conference convenor

Sally Potter
Conference convenor

Rachael Dudaniec
Local Organising Committee

David Field
Local Organising Committee

Joely Echalar Espinoza
Local Organising Committee

Bridget Campbell
Local Organising Committee

GSA 2024 Scientific Program

Sunday 30th June	
14:00	Registration desk open
15:30	**Conference Open and Welcome to Country** **Location: Mason Theatre**
16:00	**Plenary** **Speaker: Karen Sears** Chair(Oliver Griffith): Location: Mason Theatre
17:00	**Mixer** **UniBar**

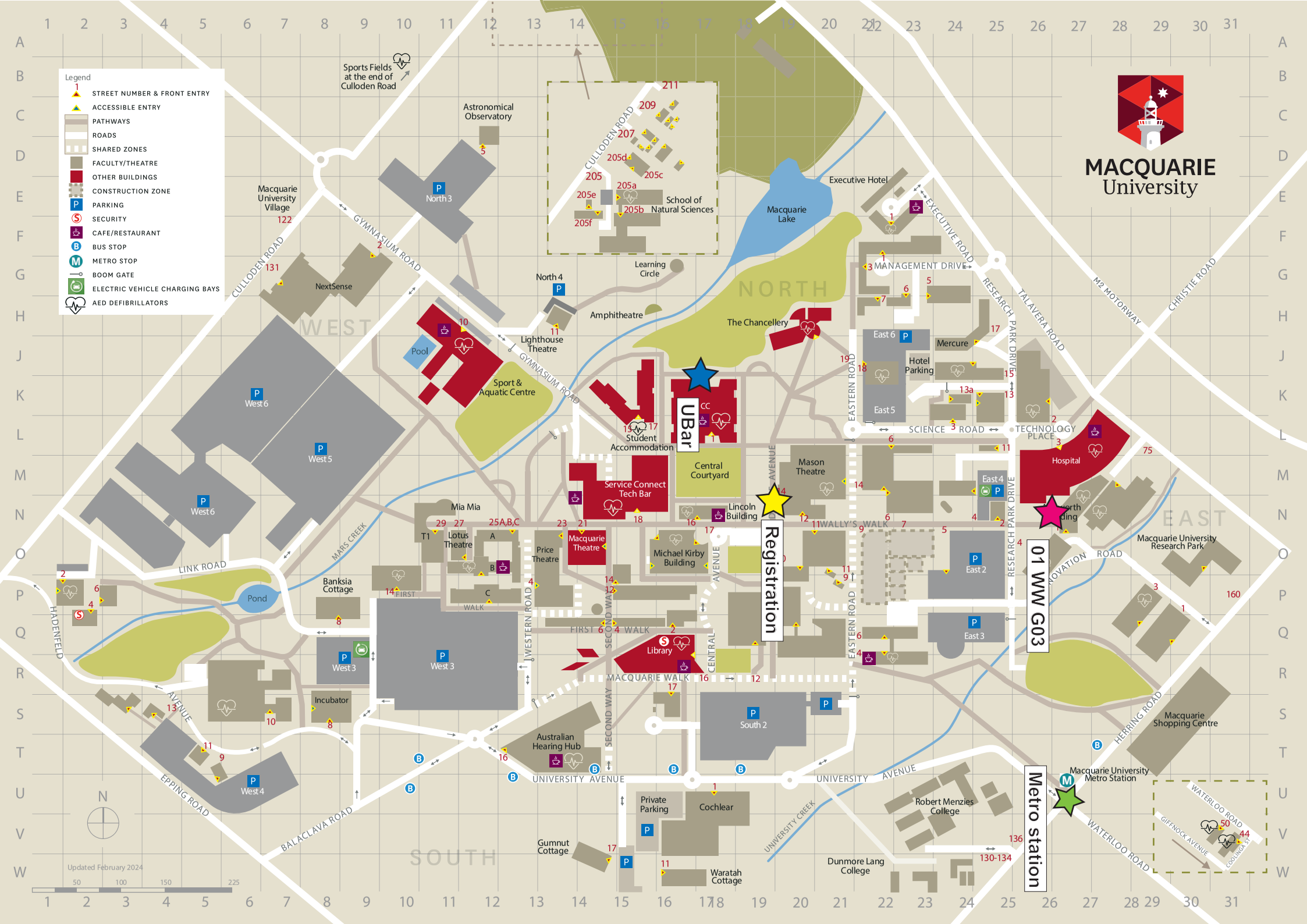
Platinum partner

Silver Partners

	Monday 1st July	
8:00	Registration desk open	
9:00	**Plenary** **MJD White Award: Prof. Melanie Bahlo** **Chair (Anna Santure)** **Location: Mason Theatre**	
10:00	**Morning Tea** Poster Hall	
	Evolutionary Genetics **Chairs (Sally Potter)** **Location: Theatre 3 (T3)**	**Conservation Genetics** **Chairs (John Whale)** **Location: Theatre 4 (T4)**
10:30	Ashley Jones: Eucalyptus genome architecture is driven by structural rearrangements that promote sequence divergence and evolution	Andrea Schraven: Bringing conservation genetics to the forefront of threatened species management: insights from in situ interventions in the face of unmitigated threats.*
10:45	Darren Wong: Three inactivation mutations at a key pigmentation gene underpins flower colour loss in a widespread bee-pollinated orchid	Bridget Campbell: Collaborating with Indigenous Rangers to investigate genetic diversity and connectivity of morphologically cryptic reptile taxa across east Arnhem Land*
11:00	David Field: Genome colliders: using hybrid zones for gene mapping and detecting barriers to gene flow in snapdragons	Holly Nelson: Beyond a reference genome: Downstream applications for management of the critically endangered Bellinger River Turtle*
11:15	Timothy Ghaly: Sowing the seeds of evolution: Agriculture alters protein evolution of soil nutrient cycling genes globally	Katherine Farquharson: Extreme loss of genomic and immunogenetic diversity in the Critically Endangered orange-bellied parrot
11:30	Emma John: Phylotranscriptomics and the evolution of floral colour and specialized pollinator shifts in the most diverse terrestrial orchid genus of Australia.*	Gila Kahila Bar-Gal: Wildlife conservation efforts hinge upon taxonomic status: Gazelles as a case study
11:45	3x Lightning: James Damayo*, Jian Cui, Ash Porter	3x Lightning: Mikaeylah Davidson*, Adi Nugroho*, Melissa Hernández Poveda*
12:00	Lunch Served in the Poster Hall	
12:15	GSA AGM Mason Theatre Grab lunch in the poster hall and bring it into the AGM	
	GSA award symposium **Chair (Anna Santure)** **Location: Mason Theatre**	
13:00	Ross Crozier Medal Speaker: Steven Zuryn	
13:30	Alan Wilton Award Speaker: Emily Roycroft	
14:00	D.G. Catcheside Prize Speaker: Monica Fahey	
14:20	Smith-White Travel Award Speaker: Ana Parra Nunez	
14:35	Batterham Conference Prize Speaker: Maddison Howie	
14:50	Afternoon Tea Poster Hall	
	Evolutionary Genetics **Chairs (Timothy Ghaly)** **Location: T3**	**Conservation Genetics** **Chairs (Bridget Campbell)** **Location: T4**
15:30	Megan Higgie: Novel genomic tools for the Green-eyed Treefrog species complex reveal a likely conserved sex determination pathway	Bernd Gruber: Inferring demographic history of non-model species in a conservation context
15:45	Emma Peel: The marsupial antimicrobial peptide toolkit	Anthony Waddle: Using a reference genome for synthetic biology conservation
16:00	Estefany Karen Lopez Estrada: Disentangling molecular, morphological and diversification rates across the tree of life	Elspeth McLennan: Where are we with genetic monitoring of conservation introductions?
16:15	Felipe Floreste: Genetic insights into spatial variability of immune traits in invasive cane toads across Australia*	Eilish McMaster: On the edge: conservation genomics of the critically endangered dwarf mountain pine Pherosphaera fitzgeraldii*
16:30	Katarina Stuart: Structural variants and transposable elements as hidden components of whole genome variation	Lucy Ockert: Population Structure and Connectivity of the Australian Little Penguin (Eudyptula minor novaehollandiae)*
16:45	Levi Brown: A tale of two vines: convergent evolutionary impacts of two invasive soapberry vines on a native Australian insect	John Black: Genetic consequences of recent population mixing in an endangered marsupial, the Eastern barred bandicoot (Perameles gunnii)*
17:00	Nathan Lo: Pervasive relaxed selection in termite genomes	Patra Petrohilos: Amped Up Immunity: 418 Whole Genomes Reveal Intraspecific Diversity of Koala Antimicrobial Peptides*
17:30	ECR Social Location: Meet at registration to walk to The Ranch Hotel	Students: A career in genetics with Dick Frankham Location: Rm 163 Active Learning Space

	Tuesday 2nd July		
8:00	Registration desk open		
9:00	**Plenary** **Speaker: Prof. Carla Sgro** **Chair (Rachael Dudaniec)** **Location: Mason Theatre**		
10:00	**Morning Tea** **Poster Hall**		
	Evolutionary Genetics **Chairs (Katarina Stuart)** **Location: T3**	**Ecological Genetics** **Chairs (Rachael Dudaniec)** **Location: T4**	**Development and Epigenetics** **Chairs (Oliver Griffith)** **Location: T5**
10:30	Zirui Zhang: Genome biology of pollinator adaptation in the orchid Chiloglottis trapeziformis*	Karina Guo: Would you Mela-look-at that! Genomic prediction of disease resistance in a Paperbark foundation species*	Gabrielle Smith: Genomic underpinnings of species- and sex-specific variation in complex cardiomyocyte morphology*
10:45	Paul Waters: Skink sex chromosomes	Kelton Cheung : What contributes to cane toads' invasion – novel adaptation or allele sorting? *	Nathan Hart: Asymmetrical illumination of embryonic chicken eyes modulates the expression of genes responsible for brain development
11:00	Simon Ho: Genomic insights into evolutionary rates and timescales in birds	Sabrina Haque: Landscape-wide metabarcoding of the invasive bumblebee (Bombus terrestris) shows interactions among gut microbiome and pollenbiome*	Hamutal Mazrier: Establishing clinical and parallel supercomputing pipelines to unfasten hereditary midline closure defects in dogs
11:15	Avneet Kaur: Genetic redundancy underlies the polygenic architecture of adaptation in natural populations	Sanjay Kumar Pradhan: Sigmavirus transmission mode and CO2-triggered paralysis in Queensland fruit fly, Bactrocera tryoni*	Jinglin Wen: Maternal-fetal communication during pregnancy in Australian skinks*
11:30	Anna Santure: Parallel signatures of diet adaptation in the invasive common myna genome	Ryan Nevatte: Of clams and clades: genetic connectivity and diversity of small giant clams (Tridacna maxima) in the southern Pacific Ocean	Carlotta Wills: Investigating the roles of putative granule-associated genes in C. elegans germline epigenetics
11:45	3x Lightning: Ankush*, Maxim Adams, Dineth Pathirana*	3x Lightning Speaker: Christine Chivas, James Weppner* , Teremoana (Tere) Porter-Rawiri*	Jennifer Morrow: Immune response of Bactrocera tryoni to covert viruses in laboratory populations
12:00	**Lunch/Poster Session** **Poster Hall**		
14:00	**Plenary** **Speaker: Prof. Rachel O'Neill** **Chair (Jenny Graves)** **Room Location: 01WW G03**		
15:00	**Afternoon Tea** **Poster Hall**		
	Evolutionary Genetics **Chairs (Paul Waters)** **Location: T3**	**Synthetic Biology** **Chairs (Anthony Waddle)** **Location: T4**	**Development and Epigenetics** **Chairs (Nathan Hart)** **Location: T5**
15:30	Soleille Miller: The Rise of Asexuality: Investigating Early Transitions from Sexual to Parthenogenetic Reproduction in the Peppermint Stick Insect	Kate Tepper: Engineering animals for mercury bioremediation	Boris Yagound: Epigenetics and rapid evolution in cane toads
15:45	Toby Kovacs: Koala mutation rates uncover historical population declines	Michael Clark: Allele Sails: A Novel Method to Alter Wild Populations	Tsering Chan: Effects of parasite infection on microRNA expression of a co-evolved host, the cane toad Rhinella marina*
16:00	Zhuzhi Zhang: Genomic traces of parallel evolution: Insights from Australian wood-feeding and soil-burrowing cockroaches	Samuel Beach: Toxic masculinity – Heterologous venom expression in pest insect seminal fluid reduces mated female lifespan*	Clare Holleley: Epigenetic time travel: mapping historical responses to rapid environmental change
16:15	Steve Cooper: Molecular evolution of Circadian Rhythm genes in subterranean diving beetles from the dark biosphere	Soumitra Bhide: Inverting Invasion: Strategies for Species Control through Inversions*	Jessica Hawes: Investigating the activity of a multidomain histone-lysine methyltransferase involved in establishing epigenetic inheritance in C. elegans*
16:30	Dinithi Rajapaksha: Investigating Germ Layer Evolution in Animals*	Simon Baxter: Advancing insect pest control using genetics	Richard Burke: Signalling pathways control cell growth, apoptosis and remodelling of the extracellular matrix during replacement of the Drosophila larval epidermal cells by histoblast-derived adult epidermal cells.
16:45	Simon Griffith: Mitonuclear interactions impact aerobic metabolism in hybrids and may explain mitonuclear discordance in young, naturally hybridizing bird lineages	1x Lightning: Carly Retief*	2x Lightning Speaker: Benjamin Hanrahan*: Lei Xiong
17:00	2x Lightning: Maggs X, Rhiannon Schembri*	Free	FREE
18:00	**Conference dinner** **UBar**		

	Wednesday 3rd July	
8:00	Registration desk open	
9:00	**Plenary** **Speaker: Prof. Ian Paulson** **Chair (Sally Potter)** **Room Location Mason Theatre**	
10:00	**Morning Tea** **Poster Hall**	
	Insights and outcomes from biodiversity and agricultural genomics (BPA sponsored) **Chairs (Sarah Richmond)** **Room: Mason Theatre**	
10:30	Craig Moritz: Reflections on the Oz Mammal Genomes (OMG) initiative	
10:50	Arthur Georges: The AusARG Genomes Initiative	
11:10	Ary Hoffmann: Empowering pest management and biocontrol with genomics	
11:30	Carolyn Hogg: Threatened Species Initiative: integrating genetics into conservation management	
11:50	David Cantrill: Building a genomic resources for the plant biodiversity sector	
12:10	**Lunch** **Poster Hall**	
13:00	**Plenary** **Speaker: Prof. Masato Nikaido** **Chair(Lee Ann Rollins):** **Room Location Mason Theatre**	
	Insights and outcomes from biodiversity and agricultural genomics **Chairs (Arthur Georges)** **Location: T3**	**Bioinformatics and genomics** **Chairs (David Field)** **Location: T4**
14:00	Sarin "Putter" Tiatragul: AusARG – Phylogenomics of Australian Squamate Reptiles	Hui Zhen Tan: Genomic imputation on a natural population of the hihi (*Notiomystis cincta*)*
14:15	Kirat Alreja: A genome assembly and annotation for the Australian alpine skink *Bassiana duperreyi* with emphasis on the sex chromosomes	Carla Finn: Generating genomics resources to support indigenous aquaculture of haku/Yellowtail kingfish (*Seriola lalandi*) in New Zealand*
14:30	Olly Berry: Genomic platforms transforming environmental research and management	Luke Silver: Empowering Bioinformaticians and Conservation Managers: Generating Workflow Solutions in Galaxy
14:45	Jack Scanlan: Development of sensitive, flexible and generic metabarcoding analysis and database curation pipelines for agricultural biosurveillance	3x Lightning: Carina Paola Cornejo Paramo*, Shyamsundar Ravishankar, Zahra Chew*
15:00	**Afternoon Tea** **Poster Hall**	
	Insights and outcomes from biodiversity and agricultural genomics **Chairs (Carolyn Hogg)** **Location: T3**	**Evolutionary Genetics** **Chairs (Emma Peel)** **Location: T4**
15:30	Margaret Byrne: GAP conservation genomics - resolving taxonomy challenges in species complexes	John Sved: Inbreeding depression and the GRIM (Genome-wide Recessive Infinitesimal Mutation) hypothesis.
15:45	Rod Peakall: Unlocking the secrets of adaptation and evolution in Australian orchids through question-driven transcriptomic and genomic analysis	Mark Tanaka: Why is facultative parthenogenesis uncommon?
16:00	Kym Ottewell: Genomic data empowering conservation of Australia's endangered species	Tom Schmidt: Global, asynchronous partial sweeps at multiple insecticide resistance genes in *Aedes aegypt* i mosquitoes
16:15	Bruce Deagle: Genomics and environmental DNA informs translocations of an endangered Australian freshwater fish	Vaheesan Rajabal: Exploring the role of mobile genetic elements in shaping plant–bacterial interactions for sustainable agriculture and ecosystem health
16:30	2x Lightning: Harrison Eyck, Daniel Powell	Khandaker Asif Ahmed: Chromosome level genome of Blue-tongue virus vector, *Culicoides brevitarsis*
16:45		
17:00	**GSA Awards & Conference Close** **Mason Theatre**	


MACQUARIE University
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ROADS
SHARED ZONES
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OTHER BUILDINGS
CONSTRUCTION ZONE
PARKING
SECURITY
CAFE/RESTAURANT
BUS STOP
METRO STOP
BOOM GATE
ELECTRIC VEHICLE CHARGING BAYS
AED DEFIBRILLATORS
Sports Fields at the end of Culloden Road
Astronomical Observatory
Macquarie University Village
North 3
Culloden Road
Gymnasium Road
NextSense
WEST
West 6
West 5
West 4
West 3
Pool
Sport & Aquatic Centre
North 4
Lighthouse Theatre
Amphitheatre
Learning Circle
Macquarie Lake
NORTH
The Chancellery
School of Natural Sciences
Executive Hotel
Executive Road
Management Drive
Research Park Drive
Talavera Road
M2 Motorway
Christie Road
East 6
Hotel Parking
Mercure
East 5
Eastern Road
Science Road
Technology Place
Hospital
East 4
East 2
East 3
Student Accommodation
UBar
CC
Central Courtyard
Service Connect Tech Bar
Mason Theatre
Lincoln Building
Registration
Wally's Walk
Mia Mia
Lotus Theatre
Macquarie Theatre
Price Theatre
Michael Kirby Building
Library
Banksia Cottage
Incubator
Pond
Mars Creek
Link Road
Hadenfeld Avenue
Western Road
First Walk
Second Way
Central Avenue
Macquarie Walk
University Avenue
Australian Hearing Hub
South 2
Private Parking
Cochlear
Gumnut Cottage
Waratah Cottage
SOUTH
Epping Road
Balaclava Road
University Creek
Robert Menzies College
Dunmore Lang College
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Day 1 – Sunday 30th June

Plenary

Development and aging in mammal

Karen Sears

Institute of the Environment & Sustainability, University of California, Los Angeles

The universality of aging across mammals has engendered much speculation on its causes. We generated genome-wide DNA methylation profiles for 58 tissue types and 185 mammalian species and used the data to develop pan-tissue aging clocks. These clocks' ability to accurately estimate age across mammals suggests that aging might result from defined mechanisms that are largely shared across tissues and species, rather than the accrual of random damage to cells. The clocks also provided clues as to the epigenetic mechanisms that might broadly drive biological aging across mammals; we found that the cytosines that comprise them, i.e., those whose DNAm levels increase with age (i.e., hypermethylation) in tissues from all examined mammals, save one we will discuss shortly, are located near genes enriched in developmental processes, PRC2 binding, and H3K27me3 marks. The tandem enrichment of these factors is likely not coincidental; PRC2 is an essential regulator that maintains hundreds of genes in a repressed state during development and in adults by marking chromatin with H3K27me3. We found only one exception – the opossum (*Monodelphis domestica*). The DNA methylation profiles of aging opossums are not enriched in PRC2 binding, developmental processes, or H3K27me3 marks, the first of which we confirmed with immunohistochemistry. Instead, they tend to be enriched in genes related to RNA processing, cell cycling, and replicative senescence. Furthermore, chromatin state analysis reveals that opossum CpG islands do not show an increase of methylation with age, unlike those of all other examined mammals, and instead exhibit changes in CpGs located in exons and 5'UTRs. Opossum's unique CpG island and PRC2 patterns during later aging might be linked and the result of the unique nature of the opossum genome; it has relatively low CpG content, on the order of half or less that of many other amniotes, and a correspondingly low density of CpG islands, and PRC2 binding primarily overlaps with CpG islands. However, this needs further testing. Based on our adult results, we hypothesized that postnatal development and aging are generally coupled across most mammalian tissues and species because of their shared reliance on PRC2 activity, except for opossums, the "exception that could prove the rule." To begin to test this hypothesis, we next generated genome-wide methylation data for tissues from the first six weeks of post-natal development in opossum and mouse (*Mus musculus*). As in adult mice and most other mammals, we found that age-related CpGs in post-natal mice and, intriguingly, opossums are located near genes associated with developmental processes and PRC2 binding, among other processes. These findings suggest that the epigenetic

processes shaping postnatal development are at least partially conserved in opossums and mice and perhaps most mammals, given these species' deep evolutionary divergence. However, comparisons also discovered some potentially biologically relevant differences in these species' postnatal development, including a strong association of age and CpGs near immune-related genes in opossums but not mice. While further investigation is needed, our findings are consistent with a general connection between the epigenetic processes governing development and aging across most mammals

Day 2 – Monday 1st July

Evolutionary Genetics

Eucalyptus genome architecture is driven by structural rearrangements that promote sequence divergence and evolution

Zixiong Zhuang[1], Scott Ferguson[1], Justin Borevitz[1], and Ashley Jones[1]

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

Eucalyptus trees are widespread across Australia, providing habitat to a rich biodiversity of marsupials, birds and insects, being key foundation species in natural ecosystems. Using long-read sequencing, we investigated how Eucalyptus genome architecture has changed over time to unveil their adaption to the environment. Firstly we assembled the genomes of 33 diverse *Eucalyptus* species, which span millions of years of evolution. We observed a dramatic increase in genome structural variations (SVs) as species diverged, suggesting these play a key role in early architectural divergence. Further divergence led to mutations obscuring rearrangements and a loss of syntenic regions (gene order conservation). Insertions, deletions, duplications, inversions and translocations were observed as major contributors to genome structural divergence. Secondly, we investigated a key species further, *E. viminalis*, which is experiencing high mortality and dieback in NSW. Long-read sequencing of 50 wild *E. viminalis* trees across three populations identified thousands of SVs segregating within the populations, potentially influencing adaptation. These SVs had influenced genes in multiple ways, including deleting gene exons, disrupting gene order, translocation of genes, and complete deletion of genes. This included phenotypically important genes such as the terpene synthase gene family. Our pioneering research on SVs in Eucalyptus provides insights into understanding their role in genome evolution and adaptive traits for this ecologically important genus.

Three inactivation mutations at a key pigmentation gene underpins flower colour loss in a widespread bee-pollinated orchid

Darren C. J. Wong[1], Zemin Wang[2], James Perkins[1], Xin Jin[2,3], Grace Emma Marsh[1], Emma Grace John[1], Rod Peakall[1]

[1]Research School of Biology, The Australian National University, Canberra, ACT, 2600 Australia, [2]State Key Laboratory of Aridland Crop Science, Gansu Agricultural University, Lanzhou, China [3]College of Life Science and Technology, Gansu Agricultural University, Lanzhou, China

Visual cues are of critical importance for the attraction of animal pollinators, however, little is known about the molecular mechanisms underpinning intraspecific floral colour variation. Here, we combined comparative spectral analysis, targeted metabolite profiling, multi-tissue transcriptomics, differential gene expression, sequence analysis and functional analysis to investigate a bee-pollinated orchid species, *Glossodia major* with common purple- and infrequent white-flowered morphs. We found uncommon and previously unreported delphinidin-based anthocyanins responsible for the conspicuous and pollinator-perceivable colour of the purple morph whilst three genetic changes underpinned the loss of colour in the white morph – (1) a loss-of-function (LOF; frameshift) mutation affecting dihydroflavonol 4-reductase (DFR1) coding sequence due to a unique 4-bp insertion, (2) specific downregulation of functional DFR1 expression, and (3) the unexpected discovery of chimeric gypsy transposable element-gene transcripts with potential consequences to the genomic stability and post-transcriptional/ epigenetic regulation of DFR. This is one of few known cases where regulatory changes and LOF mutation in an anthocyanin structural gene, rather than transcription factors, drive intraspecific floral colour variation. Our findings also raise the possibility that there is a strong interplay between environmental stress-driven TE evolution and pollinator-mediated maintenance of adaptive colour variation in nature that has been largely overlooked.

Genome colliders: using hybrid zones for gene mapping and detecting barriers to gene flow in snapdragons

David L. Field[1], Sean Stankowski[2], Arka Pal[3], Parvathy Surendranadh[3], Desmond Bradley[4], Enrico Coen[4], Nick Barton[3]

[1]Macquarie University, [2]University of Sussex, [3]Institute of Science and Technology Austria, [4]John Innes Centre

A fundamental question in evolutionary biology is how phenotypes and their underlying genes interact to generate reproductive barriers in nature. Hybrid zones provide natural laboratories to make these connections and allow for integrated studies of past and contemporary selection and where reproductive barriers operate in the genome. I will present some of the work we've been doing to identify barrier genes and their phenotypic effects by exploiting a natural hybrid zone between subspecies of *Antirrhinum majus* (snapdragons) with a well-defined genotype-phenotype map. A key focus will be on our multi-generational study, where-by genotyping and phenotyping over 30,000 plants over 14 years, we've obtained a pedigree-based estimate of the fitness landscape separating

these recently diverged subspecies. At deeper evolutionary time scales, using whole-genomes along geographic clines we find only a small proportion of the genome resisting introgression around flower colour genes. We will also demonstrate how GWAS and whole genome cline analyses can detect new genes contributing to reproductive isolation. By linking recent ecological time-scales of fitness variation in nature to patterns of deeper genomic divergence, our goal is to provide a general model for how reproductive barriers arise during the early stages of divergence.

Sowing the seeds of evolution: Agriculture alters protein evolution of soil nutrient cycling genes globally

Timothy M. Ghaly[1], Bhumika S. Shah[1,2], Liam D. H. Elbourne[1,2], Johannes J. Le Roux[1], Michael R. Gillings[1], Ian T. Paulsen[1,2], Sasha G. Tetu[1,2]

[1]School of Natural Sciences, Macquarie University, Sydney, NSW, Australia [2]ARC Centre of Excellence in Synthetic Biology, Australia

Addressing escalating food demand whilst safeguarding Earth system functions represents a critical global challenge. A crucial aspect of this endeavour lies in the agricultural use of nitrogen and phosphorous fertilisers. However, the potential for intensive fertiliser application to drive evolutionary change on soil nutrient cycling genes has been overlooked. In this talk, I will present recent work where, leveraging a global dataset of >2,500 soil metagenomes, we identify agriculture-specific signals of selection on important nutrient cycling genes, suggesting convergent evolution in response to agricultural practices globally. Protein structural analyses suggest that agriculture is driving altered enzyme-substrate interactions across different enzyme complexes, particularly those acting upon nitrate and organic phosphorus. This work provides evidence of anthropogenic selection influencing the evolution of microbial biogeochemistry at a global scale, and contributes to a deeper understanding of how human activities shape Earth's nutrient cycles. The overlooked evolutionary consequence of agriculture on microbial nutrient cycling genes may have widespread consequences relevant to food production, ecosystem health, and terrestrial restoration

Phylotranscriptomics and the evolution of floral colour and specialized pollinator shifts in the most diverse terrestrial orchid genus of Australia

Emma Grace John[1], Darren Wong[1], Rod Peakall[1]

[1]Research School of Biology, The Australian National University, Canberra, Australia.

Australian *Caladenia* orchids and allies are characterised by diverse floral colours and multiple pollination strategies. These include ancestral blue-coloured food deceptive bee-pollinated species preceding a large radiation of several hundred green and maroon-coloured sexual deceptive wasp-pollinated species. We investigated the molecular mechanisms underpinning this evolutionary shift by leveraging phylotranscriptomics coupled with floral tissue-specific differential expression across 13 species. Multiple inactivating mutations were found at key anthocyanin pathway genes. At a flavonoid 3’5’-hydroxylase gene, a change in a single amino acid at substrate recognition site 6 (SRS6) is observed, corresponding to a loss of the 5’-hydroxylation activity required for delphinidin

production. Likewise, a single amino acid change is observed in the substrate binding site of dihydroflavonol 4-reductase, decreasing the number of hydrogen bonds formed with the delphinidin substrate, dihydromyricetin. These mutations are predicted to affect the enzyme-substrate specificity linked to the shift from delphinidin (blue)- to cyanidin (maroon)-based anthocyanin chemistry which underpins the key evolutionary change in floral colour. Our findings further suggest that the irreversible loss of delphinidin-based systems may have been a critical adaptation which enabled the runaway evolution of sexual deception in *Caladenia*.

Gene knockdown of RNAi pathways in the honey bee parasite, *Varroa destructor*

James Damayo[1], Rebecca McKee[1], Alyson Ashe[1] and Emily Remnant[1]

[1] *School of Life and Environmental Sciences, University of Sydney*

The ectoparasitic mite *Varroa destructor* is the leading threat to the health of *Apis mellifera* (the Western honey bee), largely due to its role as a vector for many viruses. *V. destructor* employs RNA interference (RNAi) as the main antiviral immune mechanism. Within the RNAi pathway, small interfering RNA (siRNA) are produced in response to a viral infection. *V. destructor* produces an siRNA fragment size profile that contains mostly 24 nucleotide antisense siRNA, which differs from typical arthropod profiles that are 22 nt with near equal sense and antisense fragments. To better understand the molecular mechanisms underpinning *V. destructor*'s unique siRNA response, we conducted double stranded (dsRNA) knockdowns of key RNAi genes. Specifically, we successfully knocked down the expression of five RNAi genes (Ago-1, Ago-2, Dcr-1, and two Dcr-2 genes) and an RNA-directed RNA-polymerase (RdRp), a gene which has an unknown function in *V. destructor*'s RNAi pathway. We will then conduct small RNA sequencing to identify the impact of successful dsRNA knockdowns of RNAi genes on viral siRNA fragment size profiles. An understanding of the function of *V. destructor*'s antiviral immune genes can help mitigate the impact that it has on honey bee health and aid in developing novel treatments.

Toll-like receptors diversity in the koala

Jian Cui[1], Kimberley Batley[1], Carolyn Hogg[1], and Katherine Belov[1]

[1] *School of Life and Environmental Sciences, The University of Sydney, Sydney, Australia*

The koala population has declined due to its vulnerability and susceptibility to various threats. Diseases, particularly chlamydia and retrovirus infections, have been identified as major disease threats for this species. Toll-like receptors (TLRs) play a crucial role in innate immunity, recognising and responding to various pathogens. Variations in TLR genes can influence an individual's susceptibility or resistance to infectious diseases. This study surveyed genetic diversity at TLRs in 413 wild koalas from Queensland, New South Wales and Victoria. We showed that one gene, TLR1/6like is monomorphic, while the other nine TLR genes have between 3 and 15 alleles. Our results show that koalas have five distinct genetic clusters in their TLR genes that are consistent with

the diversity of the Koala MHC. The bioinformatic approach presented here has broad applicability to other threatened species with existing genomic resources in Australia.

Novel genomic tools for the Green-eyed Treefrog species complex reveal a likely conserved sex determination pathway

Lorenzo V. Bertola[1], Conrad J. Hoskin[1], and Megan Higgie[1]

[1] College of Science & Engineering, James Cook University, Townsville, Australia

The Green-eyed Treefrog species complex is a set of closely-related Australasian Treefrog species (Family: Pelodryadidae) in north Queensland. Three genetic lineages meet at two contact zones and provide an excellent opportunity to study the recent speciation of the Kuranda Treefrog, which was one of the first systems to demonstrate speciation via reinforcement. We have developed a set of genomic tools to understand both the genetic basis of this recent speciation event and the genetic basis of sex determination in Australasian Treefrogs. The highly variable nature of frog sex determination systems is important to further our understanding of genetic sex determination and its evolution. However, candidate sex determining genes have only been identified in a few frog families worldwide. Here, using high-density linkage maps, a chromosome-level genome assembly, and sexed adult individuals, we investigate genome-wide synteny and sex determination mechanisms in the Green-eyed Treefrog species complex and identify the sex determining region in the southern *Litoria serrata* lineage. Applying these findings to conservation, strongly or completely sex-linked loci within this region may be valuable for early identification of sex in immature individuals in captive breeding programs of threatened Australasian Treefrog species.

The marsupial antimicrobial peptide toolkit

Emma Peel[1,2], Carolyn Hogg[1,2], and Kathy Belov[1,2]

[1]School of Life and Environmental Sciences, The University of Sydney, Sydney, New South Wales, Australia
[2]Australian Research Council Centre of Excellence for Innovations in Peptide & Protein Science, The University of Sydney, Sydney, NSW, Australia

Marsupials give birth to altricial and immunologically naïve young yet develop within a pouch rich in microorganisms. Antimicrobial peptides (AMPs) may provide protection, as they directly kill microorganisms and modulate the immune response. However, little is known of AMP families cathelicidins and defensins in marsupials. We characterise the marsupial AMP toolkit using bioinformatics and 'omics data, identifying 128 cathelicidins and 321 defensins amongst 14 species from 12 families. Cathelicidins and alpha defensins have undergone large gene expansions in all marsupials compared to eutherians, while beta defensins were similar. AMP gene clusters were encoded in syntenic regions of the genome across all mammals. Marsupial cathelicidins also displayed synteny with chicken and may represent an evolutionary step between birds and eutherians. AMPs were expressed in the mammary gland, milk, and pouch, had antimicrobial activity or were orthologs of human AMPs with known antimicrobial functions, hence may protect pouch young. Antimicrobial screens identified 32 cathelicidins with activity, including predicted ancestral

cathelicidins with rapid and prolonged activity at low concentrations that have therapeutic potential against bacteria and fungi, including drug-resistant strains. Our study highlights the value of 'omics resources in understanding species biology, gene family evolution, and discovery of potential novel therapeutics.

Disentangling molecular, morphological and diversification rates across the tree of life

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The evolution of Earth's vast genetic and morphological diversity has been explained by two contrasting models, phyletic gradualism and punctuated equilibrium. Under a phyletic gradualism framework, species are steadily transformed into new forms over time, via the accumulation of small, incremental changes. In contrast, under punctuated equilibrium, evolutionary change tends to occur in bursts at speciation events. Previous studies of morphological and molecular data have found varying levels of support for the two evolutionary models. We examined these models using comprehensive morphological and molecular data sets from 40 clades across the Tree of Life. Testing for associations between species richness and the amount of evolutionary change in sister clades, we find little evidence supporting the punctuated equilibrium model. Our results suggest that both morphological and molecular evolution occur gradually through time. Highlighting that phyletic gradualism could be the dominant mode of macroevolution across the Tree of Life.

Genetic insights into spatial variability of immune traits in invasive cane toads across Australia

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Cane toads (*Rhinella marina*) have posed significant threats to Australian ecosystems since their introduction to the east coast in the 1930s. Since then, toads have expanded westward carrying along the co-introduced lungworm *Rhabdias pseudosphaerocephala*. Cane toads now range from Queensland (QLD; range-core) to the Northern Territory (NT) and Western Australia (WA; range-edge) and differ with respect to their physiology and immunity to parasites. Range-edge toads have lower metabolic rates during an immune challenge and higher susceptibility to lungworm infection than their range-core counterparts, yet the underlying genetic and evolutionary mechanisms remain unclear. We assessed innate

immunity parameters (neutrophil:lymphocyte ratio – NLR, bacteria killing ability – BKA) and mRNA expression of naïve common-garden bred offspring derived from the range-edge and range-core families in response to an induced parasite infection. Our preliminary results show that experimental parasite infection upregulated gene expression of IL-6, IL-10, and IL-25 cytokines in the spleen of infected toads compared to controls. Interestingly, QLD toads show overall lower immune parameters and gene expression compared to NT and WA toads. We suggest that the harsh environment at the front of the invasion range selects for higher constitutive immune traits to compensate for the novel challenges of long-distance dispersal and colonizing new environments.

Structural variants and transposable elements as hidden components of whole genome variation

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Quantifying genetic variation is needed in order to study population structure, patterns of demography and dispersal, and identify regions of putative adaptation within and across populations and species. Evolutionary and conservation genomics has historically focused on genetic variation identified through SNPs, but now new technologies are facilitating the exploration of the distribution and impacts of complex, non-SNP variants. In this study, we explore the dynamic landscape of non-SNP variants present in the globally invasive *Acridotheres tristis*, a bird that has gone through multiple and sequential bottlenecks within its invasive ranges. We compare patterns in SNPs, as well as two major groups of non-SNP variants: structural variants (SVs) and transposable elements (TEs). We assess how the fundamental properties of these three different types of variants shape their nature and distribution, both throughout the genome and across separate populations of this globally spread invasive species. We also examine how these different types of variants have different functional impacts and responses to demographic processes. Our results place particular emphasis on the need to distinguish between different types of non-SNP variants explicitly, as we find that SVs and TEs have very different characteristics when examined within a population genomics context.

A tale of two vines: convergent evolutionary impacts of two invasive soapberry vines on a native Australian insect

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The levels of eco-evolutionary experience shared between native and invasive species influence how they respond evolutionarily to each other. Soapberry bugs in the genus Leptocoris are obligate seed predators of soapberries in the family Sapindaceae and have colonised numerous invasive species of the family. Two such invaders, commonly known as balloon vines, *Cardiospermum halicacabum* and *C. grandiflorum*, have been colonised by the Australian soapberry bug, *L. tagalicus*. The bug has evolved longer proboscides to feed on these large-fruited invasive plants. Here we demonstrate the genetic implications of the invasion of these two invasive balloon vine species for *L. tagalicus*. We found that bugs utilising *C. halicacabum* in the Northern Territory are distinct from those found on *C. grandiflorum* in Queensland and New South Wales and those on the native *Alectryon tomentosus*. We also show that, while the east coast bugs appear to be a panmictic population, there is a strong latitudinal association with levels of genetic admixture, likely driven by the merging of two independent *C. grandiflorum* invasion fronts. Our study highlights how the introduction of invasive balloon vines into Australia may simultaneously be driving differentiation, as well as the genetic breakdown of distinct populations, of Australian soapberry bugs.

Pervasive relaxed selection in termite genomes

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The genetic changes that enabled the evolution of eusociality have long captivated biologists. In recent years, attention has focussed on the consequences of eusociality on genome evolution. Studies have reported higher molecular evolutionary rates in eusocial hymenopteran insects compared with their solitary relatives. To investigate the genomic consequences of eusociality in termites, we sequenced genomes from three of their non-eusocial cockroach relatives. Using a phylogenomic approach, we found that termite genomes experienced lower rates of synonymous mutations than those of cockroaches, possibly as a result of longer generation times. We identified higher rates of nonsynonymous mutations in termite genomes than in cockroach genomes, and identified pervasive relaxed selection in the former (24–31% of the genes analysed) compared with the latter (2–4%). We infer that this is due to a reduction in effective population size, rather than gene-specific effects (e.g., indirect selection of caste-biased genes). We found no obvious signature of increased genetic load in termites, and postulate efficient purging at the colony level. Our results provide insights into the evolution of termites and the genomic consequences of eusociality more broadly.

Conservation Genetics

Bringing conservation genetics to the forefront of threatened species management: insights from in situ interventions in the face of unmitigated threats.

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Supplementations (or population reinforcements) provide an in situ management strategy for threatened species, involving the release of conspecifics into existing populations to alleviate small population pressures. My PhD research delves into the long-term genetic consequences of management interventions for the endangered marsupial species, the Tasmanian devil (*Sarcophilus harrisii*). Firstly, I identified current gene flow patterns and source-sink dynamics among twenty-one wild devil sites across Tasmania to recommend locations for future interventions. Substantial bi-directional gene flow was observed within central Tasmania while coastal sites were relatively isolated. Next, I quantified changes in genetic differentiation among eight devil sites before and after supplementation and assessed this action's impact on genetic diversity and relatedness over time, within each site. Using nine years of sampling across eight wild, diseased sites, four supplemented and four not supplemented, genetic differentiation among supplemented sites significantly declined, compared to among not supplemented sites. Changes in genetic diversity and relatedness were substantially variable among all sites over time. Finally, long-term empirical data has been used to parameterise individual-based models to project demogenetic trajectories of wild sites under various management options. This research highlights how genetic assessments can support species-level conservation management programs.

Collaborating with Indigenous Rangers to investigate genetic diversity and connectivity of morphologically cryptic reptile taxa across east Arnhem Land

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Across northern Australia, high levels of species richness, localised endemism and cryptic diversity have been found for many reptilian taxa, including *Carlia* and *Ctenotus* skinks and *Diporiphora* dragons. However, in some remote regions, such as Arnhem Land, scientific data has historically been scarce, leaving gaps in the understanding of the squamate diversity. Additionally, these genera contain morphologically cryptic species complexes and are very challenging to identify in the field using morphological keys. This study showcases the findings of from eight years of collaborative cross-cultural fauna surveying with Indigenous Ranger groups across east Arnhem Land in which *Carlia*, *Ctenotus* and *Diporiphora* were sampled. Phylogenetic and genetic structure analyses using

DArTseq SNP data (~5,000-12,000 SNPs) and previously analysed reference samples were conducted to identify species and lineages across the three genera. For four comonly found species, genetic structure and estimated effective migration surfaces (EEMs) analyses were conducted to better understand regional population structure and gene flow. Our results clarified the regional *Carlia*, *Ctenotus* and *Diporiphora* species richness and distribution, across 13 species and 16 lineages. The genetic structure results showed a clear pattern in gene flow in relation to landscape features such as floodplains and ridges, demonstrating the importance of conservation corridors. Achieving these results was only possible through collaborative cross-cultural work that valued and supported both local Indigenous and Western scientific priorities and knowledge systems. The surveys used to sample the study species included cultural knowledge recordings and locally preferred modifications of the standard Northern Territory Government fauna survey design with high multigenerational Indigenous involvement. Researchers aspired to follow best practice guidelines for working with Indigenous research partners throughout this project and undertook significant time to explore mutual benefits, co-design elements with local people, and communicate the results and implications for local Indigenous land management.

Beyond a reference genome: Downstream applications for management of the critically endangered Bellinger River Turtle

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Although reference genomes alone do nothing for conservation, the downstream applications based on a high-quality genome are broad. Here we present the first high-quality (HiFi + HiC) scaffolded reference genome with associated transcriptomes for a side-necked turtle, the Bellinger River turtle. This species experienced significant population declines in 2015 due to a novel disease outbreak. Working with our conservation partners, our applied genetic tools have allowed us to make management recommendations for the conservation breeding and recovery program. Using reference aligned DARTseq data, we investigated temporal and contemporary genetic diversity showing that the current wild and captive populations have low yet consistent levels of diversity. Using MitoHiFi, we bioinformatically extracted the mitochondrial genome and compared this to the existing published mitogenome, answering the question, is a bioinformatically extracted mitogenome suitable? We also developed eDNA primers for non-invasive field detection of the species. Finally, we manually characterised the MHC regions, sequencing 37 whole genomes to investigate immunogenetic diversity and gain insights into the species' disease susceptibility. This project showcases the value and importance of both conservation-academic partnerships and high-quality referential material for informing conservation management strategies and the broad applicability of reference genomes for aiding management efforts of threatened species.

Extreme loss of genomic and immunogenetic diversity in the Critically Endangered orange-bellied parrot

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The orange-bellied parrot (*Neophema chrysogaster*) is a Critically Endangered migratory species with an estimated 87% likelihood of extinction by 2038. Numerous diseases (including those caused by viral, bacterial, and fungal pathogens) threaten the single remaining wild population and captive breeding program. The species has undergone multiple population crashes, reaching a low of two breeding females and 12 males in the wild in 2016. We assembled and annotated a high-quality long-read reference genome and resequenced 19 contemporary and 16 historic genomes dating back to 1829 to examine temporal patterns in genomic and immunogenetic diversity. Extreme losses of genomic diversity (62%) were observed between historic (mean autosomal heterozygosity = 0.00149 ± 0.000699 SD) and contemporary (0.00057 ± 0.0000263) parrots. Immunogenetic diversity was characterised by manually annotating immune genes with transcriptome data. Interestingly, we observed unusually restricted immunogenetic diversity. Our work demonstrates the value of long-read genomic sequencing and museum collections in investigating temporal patterns despite the challenges associated with limited standing variation in contemporary birds. We discuss the implications of our findings on disease susceptibility and efforts to manage the species in the wild.

Wildlife conservation efforts hinge upon taxonomic status: Gazelles as a case study

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The rapid expansion of the human population in the southern Levant over the last 70 years has resulted in significant anthropogenic activities, posing a serious threat to biodiversity. Habitat modification and poaching are the principal drivers pushing wildlife populations, particularly gazelles, towards extinction. Presently, three species of Gazelles are extant in the southern Levant: the endangered mountain gazelle (*Gazella gazelle*), the vulnerable Dorcas gazelle (*G. dorcas*), and the critically endangered endemic Acacia gazelle (presumed *G. arabica* or *G. gazella* subspecies, *G. arabica acaciae* / *G. gazella acaciae*). These flagship species play a pivotal role in ecosystem conservation. However, their taxonomic status remains unresolved, impacting conservation

management programs. This study aimed to clarify the taxonomic and phylogenetic status of these three species to bolster conservation efforts. Mitochondrial genomic analysis of modern an museum samples revealed that (I) the mountain gazelle is indeed a species exclusively distributed in the southern Levant. (II) The Acacia gazelle represents a "Ring species" of *G. arabica*, trapped in its evolutionary process due to allopatric speciation. The rapid decline of these unique gazelle populations underscores the urgent need for conservation actions to avert their extinction.

Increasing chytrid resistance in Southern Corroboree Frogs through Selective Breeding

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Functionally extinct in the wild, the Southern Corroboree frog is dependent on captive management and breeding to survive. Their main threat to survival is the deadly amphibian chytrid fungus. With no effective management strategies to control chytrid in wild frogs, this project aims to enhance their resistance through selective breeding. While this method has been successfully achieved in domesticated animals, its application to wildlife remains unexplored. Given that the entire extent of the genetic diversity of Corroboree frogs is held in captivity and a successful breeding program is already established, we are uniquely positioned to evaluate the efficacy of this approach in this species. Traits correlated with chytrid resistance are required for targeted genetic improvements. To identify these, we have conducted a 1,000 animal chytrid exposure experiment to collect chytrid susceptibility data, and designed and manufactured the first custom SNP array for amphibians to genotype the frogs in our experiment. We will use the collected phenotypic and genetic information to conduct a GWAS for chytrid resistance/susceptibility. The overarching goal of our research is to implement a breeding strategy across the captive colonies to increase beneficial alleles, and decrease deleterious alleles, thereby increasing the tolerance of Corroboree frogs to chytrid infection.

Evolutionary and population dynamics of introduced rusa deer (*Cervus timorensis*)

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Rusa deer (*Cervus timorensis*), native to Indonesia, have been introduced and are established across Oceania. These populations were formed from independent and serial introductions, as well as admixture events, making rusa deer an excellent model for studying how introduction patterns and differing environments affect evolutionary trajectories. This project utilises sample from the native range, and invasive samples from New Caledonia, New Zealand, and Australia to characterise genetic diversity and population structure, and to investigate evidence of adaptation following introduction to new environments. We will place our invasive range samples within existing phylogenies produced using mitochondrial DNA from the native range to clarify the origin of founding individuals of these invasive populations. To further investigate invasive range population structure and selection, we are using SNP panels generated from DArTseq data. This project will advance our understanding of the molecular dynamics of invasion, and will address the potential for invasive rusa deer to act as genetic reservoirs for conserved native populations. We also aim to provide insight for population managers regarding dispersal patterns across invasions and the impacts of landscape features on movement patterns of rusa deer, both which are key to devising effective control strategies for these growing populations.

Evaluating chytridiomycosis susceptibility in Northern and Southern Corroboree Frogs

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Southern Corroboree Frogs (*Pseudophryne corroboree*), endemic to Australia, have become functionally extinct in the wild. Meanwhile, populations of Northern Corroboree Frogs (*P. pengilleyi*) still exist, but they are in a rapid decline, pushing them towards the brink of extinction. This concerning situation is primarily attributed to the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (Bd). Given the urgency of the situation, we want to understand the factors influencing resistance to chytridiomycosis in these frogs. Our research focuses on susceptibility, gene expression, individual and population variations, and the age-related effects on infection outcomes. By investigating gene expression's role in infection outcomes, we will collect toe-clips and record signs development during the infection process. This approach aims to identify the most vulnerable age

groups to chytridiomycosis, and the difference between populations and species providing crucial insights for protective measures.

Inferring demographic history of nonmodel species in a conservation context

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The ongoing and widespread decline of species and biodiversity loss due to drivers such as urbanisation, species introductions, and climate change, has triggered research interest in developing methods to study historic population trends using easily attainable genetic data. Through understanding historic population trends, we can identify drivers and trigger suitable management actions for the conservation of species. We ran an extensive simulation exercise (>200k computer hours on NCI), by simulating population trends and tested three popular model free methods (GONE, Epos and Stairways2), which use genetic data based on SNPs (single nucleotide polymorphisms) to reconstruct population histories. The simulations were setup to mimic variations of four historic scenarios (decline, increase, stable and bottleneck), on time scales which are most important in a conservation context (within the last 200 years) and under realistic conditions in terms of available sample sizes and number of loci within a conservation context. Overall, we found that methods were able to identify the correct population scenario once certain thresholds in terms of sample sizes and number of loci were achieved. Interestingly, there was no clear winner and therefore we give specific recommendations, about the circumstances under which produces reliable reconstructions of the four historic population trends.

Using a reference genome for synthetic biology conservation

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Biodiversity conservation faces many contemporary challenges but new technologies in molecular biology may offer creative solutions. Affordable genome sequencing and a new generation of molecular tools (e.g. CRISPR) have the potential to change the way we study and combat intractable threats such as climate change and infectious diseases. The pandemic fungal disease chytridiomycosis has caused devastating declines of amphibians, but using modern tools we may unravel the host mechanisms that determine disease outcomes and greatly diversify our options for

conservation. For example, if disease resistance is governed by one or a few genes, this information would be useful for efforts to increase host resistance via selective breeding or synthetic biology. We propose a conceptual framework using modern methods to study and promote amphibian resilience to chytridiomycosis. This includes generating a high-quality reference genome, optimising methods to produce amphibian embryos, and developing and using molecular tools to produce genome-edited animals. We show this conceptual model in practice, illuminating the feasibility and potential for synthetic biology conservation research.

Where are we with genetic monitoring of conservation introductions?

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Conservation introductions, moving species beyond their native ranges, are becoming increasingly necessary. We performed a systematic review to quantify conservation introductions globally and determine whether genetic monitoring is occurring. Despite an abundance of empirical evidence to guide them, conservation introductions are still rare. Of the 167 conservation introductions we identified, most were performed in North America, Australia and China with megadiverse developing nations underrepresented. Plants were disproportionately represented in the literature, with climate change the most common motivator of all conservation introductions. Only ten works reported genetic monitoring, with only two of these measuring changes over time. Both works showed a worrying trend of rapid genetic diversity loss detectable within a single generation. Early detection of genetic diversity loss is paramount to implementing corrective measures such as reinforcement before adaptive potential is eroded. We urge conservation practitioners to perform conservation introductions for a more diverse range of taxa, across more megadiverse countries, and to perform genetic monitoring on existing and future populations. Only through more rigorous applications of conservation introductions will we learn how to establish self-sustaining populations into an uncertain future.

On the edge: conservation genomics of the critically endangered dwarf mountain pine *Pherosphaera fitzgeraldii*

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Pherosphaera fitzgeraldii, or Dwarf Mountain Pine, is a critically endangered conifer found exclusively in the Greater Blue Mountains Area of New South Wales, Australia. Threats such as pollution and invasive weeds have led to population decline and limited recruitment. We used conservation genomics to assess its population health and propose management strategies. Genomic analysis revealed two distinct genetic groups aligned with prevailing wind currents, suggesting limited pollen or seed exchange between subpopulations. Despite geographic proximity, gene flow was minimal, increasing the risk of genetic differentiation. All subpopulations showed signs of inbreeding and genetic depletion due to historical isolation and small population sizes. We propose genomic-based recommendations for prioritizing conservation sites, enhancing genetic diversity in ex situ collections, and guiding future investigations for conservation practitioners. Additionally, further genomic studies to uncover the sex determination mechanism of *P. fitzgeraldii* could assist in maintaining balanced sex ratios in ex situ collections and facilitate future genetic rescue efforts. This study emphasizes the urgent need for conservation efforts and demonstrates how genomics can inform protection and recovery strategies for *P. fitzgeraldi*i.

Population Structure and Connectivity of the Australian Little Penguin (*Eudyptula minor novaehollandiae*)

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The Australian little penguin (*Eudyptula minor novaehollandiae*) is the only penguin species (Family: Spheniscidae) to breed on mainland Australia. Despite being classified as “least concern" by the IUCN Red List due to their widespread presence along the southern coast of Australia, localised declines are evident. Colonies without active conservation measures are particularly vulnerable to human disturbances. Using reduced-representation sequencing, this project aims to examine population structure of this species across Australia and identify populations with poor connectivity that may be of conservation significance. We will present estimates of differentiation between colonies and insight into the connectivity of little penguins along the coast of Australia based on a subset of 94 individuals from 17 sites. We will discuss how these data inform future directions of the wider project covering the full range of this species. We also plan to interrogate these data in conjunction with environmental data for evidence of selection to determine whether environmental threats such as rising sea surface and ambient air temperatures may be impacting the evolutionary trajectory of this species.

DnaDot - Fixing Ecology and Evolution’s Blind Spot, Population size

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To understand and manage any ecosystem, one of the most critical indicators is census population sizes ('NcNc'). This is why the IUCN needs to know census size to an accuracy of ±10% for each managed, endangered or exploited population. Knowledge of population size is also critical for: general ecological studies; biodiversity measures with low error margins; and assessing potential for evolutionary adaptation. Unfortunately, all existing methods for estimating NcNc have many difficulties, especially requiring independent knowledge or assumptions about demography, including: immigration, emigration, family size and its variance. This article introduces 'DnaDot', a strong new addition to our armory of census population size estimates, being accurate, with few assumptions. DnaDot is based on mark-release-recapture, but instead of marking, uses pre-existing polymorphisms to divide the population into separate groups. The method uses one sample, minimizing effort, uses no demographic assumptions or data, and does not require genotyping to be accurate enough to identify individuals and kin, which is problematic for other genetic methods especially when degraded field samples are used. DnaDot outperformed close competitors on minimal assumptions, and good detectability of marks. Also, in simulations of a wide range of scenarios, DnaDot had superior accuracy and precision to competing methods.

Genetic consequences of recent population mixing in an endangered marsupial, the Eastern barred bandicoot (*Perameles gunnii*)

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Population mixing aims to assist conservation of isolated or captive populations by increasing genetic variation. This genetic variation will be reduced by drift in the generations after mixing, but the extent of reduction is not well studied and may be species specific. Here, I examine the genetic outcomes of population mixing in a large, captive population of a specialist marsupial – the Eastern barred bandicoot (*Perameles gunnii*). This population was established using animals from Victoria, Australia, shortly before the species became locally extinct. Declining genetic diversity in this population led to the implementation of assisted gene flow from a Tasmanian population, a divergence of ~10,000 generations. First, I present work on genetic structure and diversity in the Tasmanian source populations, finding widespread population structure over several hierarchical levels. Next, I reevaluate estimates of genetic diversity in the Tasmanian and Victorian populations using an improved pipeline for assessing autosomal heterozygosity. Finally, I compare changes in genetic diversity and morphometrics of the captive population over approximately eight generations of hybrid offspring, and I further contextualise results with comparison to another captive site that only contains Victorian animals.

Amped Up Immunity: 418 Whole Genomes Reveal Intraspecific Diversity of Koala Antimicrobial Peptides

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Characterising functional diversity is a vital element of conservation genomics yet most immunogenetic studies in non-model organisms have been focused on the MHC and TLR gene families. Antimicrobial peptides (AMPs) are an ancient component of the eukaryotic immune system. The two major groups of mammalian AMPs are cathelicidins and defensins, with the former having undergone species-specific expansions in marsupials. Here, we utilised data from the recently published 418 whole koala genomes to undertake the first comprehensive analysis of AMP diversity across a species' range. Allelic diversity was lower than other immune gene families such as MHC, suggesting that AMPs are more constrained. Some SNPs are predicted to change AMP function through stop gains, change in structure and increase in peptide charge. Copy number variants (CNVs) were observed in five AMPs. Interestingly, the most common CNV was the duplication of PhciCATH5, a cathelicidin with activity against chlamydia, which was more common in the southern part of the species range than the north. AMP copy number is correlated with expression levels, so we hypothesise that there is a selective pressure resulting in this widespread CNV. Future studies should use phenotypic metadata to assess the functional impacts of this CNV.

Day 3 – Tuesday 2nd July

Evolutionary Genetics

Genome biology of pollinator adaptation in the orchid *Chiloglottis trapeziformis*

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Orchids represent the second largest family of flowering plants, with approximately 28,000 species. Genomic studies within this diverse group are limited. Fewer than 20 orchids have a reference genome when compared to thousands of other plant families containing model and agriculturally important crop species. This gap in knowledge is especially pronounced for Australian orchids that lack any genomic resources. This is despite the fact that Australia has a rich terrestrial orchid flora of some 2000 species and the highest diversity of sexually deceptive orchid species globally. We have utilized a combination of long-read sequencing technologies (PacBio HiFi and Oxford Nanopore) and Hi-C data, to achieve the first Australian orchid chromosome-level de novo genome assembly for the *Chiloglottis trapeziformis*. The resulting genome assembly has a BUSCO score that is among the highest reported for any orchid genome. We are now employing transcriptome-guided approaches to predict and functionally annotate genes. With a fully annotated genome, we will then explore the role of gene duplication in the adaptation of pollination strategies. This work will establish a crucial genomic resource and lay the foundation for future studies on the evolutionary genomics of plant-pollinator interactions in the diverse Australian orchid flora.

Skink sex chromosomes

J King Chang[1], Kirat Alreja[2], Benjamin J. Hanrahan[1], Duminda S. B. Dissanayake[3], Andre L. M. Reis[4,5], Nicholas C. Lister[1], Terry Bertozzi[6,7], Oliver W. Griffith[8], Camilla M. Whittington[9], Hardip R. Patel[10], Ira W. Deveson[4,5,11], Arthur Georges[3], Paul D. Waters[1]

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Skinks, a diverse group of lizards within the family Scincidae, typically have homomorphic XY sex chromosome systems. The Australian Amphibian and Reptile Genomic Initiative (AusARG) recently generated chromosome-level genome assemblies for five Australian skink species, which we annotated to compare genome wide synteny to uncover insights into their sex chromosome structure. Using genome sequence read depth in both sexes and Y-enriched kmers, we successfully identified X and Y scaffolds in four of the five skink genomes. However, in Lampropholis delicata, we did not find clear X or Y scaffolds, hinting at a turnover in the sex chromosome system. Our comparative

analysis revealed that most skink species possess poorly differentiated X and Y chromosomes, with the exception of two species that had differentiated X and Y chromosomes.

Genomic insights into evolutionary rates and timescales in birds

Simon Y. W. Ho[1], Al-Aabid Chowdhury[1], Jacqueline M. T. Nguyen[2,3], and David Duchêne[4]

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Genomic data provide valuable opportunities for studying evolutionary rates and timescales on an unprecedented scale. These analyses can be performed using phylogenomic methods in combination with molecular clocks, allowing the patterns and drivers of rate variation to be characterised in detail. Based on analysis of 363 genomes sequenced by the Bird 10,000 Genomes consortium, we have reconstructed the evolutionary timescale of modern birds and identified the key biological correlates of their genomic evolutionary rates. We have found that the Neoaves, a group that includes 95% of living bird species, underwent an explosive diversification shortly after the end-Cretaceous extinction event. This diversification was associated with rapid changes in microchromosomes. More generally, genome-wide mutation rates in birds show a close relationship with several life-history characteristics and morphological traits associated with ecology. Our study illustrates the evolutionary insights that can be gained from applying phylogenetic and molecular-clock approaches to whole genomes.

Genetic redundancy underlies the polygenic architecture of adaptation in natural populations.

Avneet Kaur[1,2], Maddie E. James[1,2], Hyungtaek Jung[1], Candice Bywater[1,2], Jan Englestädter[1,2], Melanie J. Wilkinson[1,2], and Daniel Ortiz-Barrientos[1,2].

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The challenge of defining the genetic complexity of natural adaptation remains fundamental to understanding Earth's diversity. This study focuses on the polygenic composition of adaptation and the often-overlooked role of genetic redundancy in forming phenotypic diversity. In this study, we examined the genetic structure of adaptive traits derived from two populations of the ecologically diverse *Senecio lautus* species complex using the Multiparent Advanced Generation Intercross (MAGIC) Population. Our genome-wide association studies (GWASs) reveal the genetic base of polygenic adaptation, characterised by many small-effect alleles throughout the genome. We explored the contribution of genetic redundancy to trait differences, finding that different populations have different allele combinations and still produce similar phenotypic adaptations. In

subsequent population genomic analyses, we proved the adaptive significance of some of the GWAS-identified locations and showed consistent selection signatures in independent headland and dune populations. Many of the GWAS-identified sites are concentrated in some regions, suggesting that the interaction between recombination and selection facilitated the evolution of gravitational motion in the system. Our findings reveal that the adaptation landscape is both polygenic and redundant, suggesting that early models underestimated the complexity and redundancy observed today. The research has improved our understanding of polygenetic adaptation and emphasises the need for evolutionary models to adapt to the multifaceted and resilient nature of the genetic architecture underpinning adaptation.

Parallel signatures of diet adaptation in the invasive common myna genome

Kamolphat Atsawawaranunt[1], Katarina Stuart[1,2], Annabel Whibley[1,3], Kyle M Ewart[4,5], Richard E Major[5], Rebecca N Johnson[5,6], Anna W Santure[1]

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Invasive species offer uniquely replicated model systems to study rapid adaptation. The common myna (*Acridotheres tristis*) has been introduced to over a dozen countries and is classified as one of the most invasive birds in the world. Their multiple invasions provide an opportunity to identify parallel adaptation, as invasive populations originate from multiple source populations. We compared whole-genome resequencing data from 82 individuals from four native and seven invasive populations, representing two independent introduction pathways. Results from three selection scan methods were combined and identified a strongly selected region on chromosome 8 that spans two copies of AMY2A, part of the alpha-amylase gene family, and a structural variant (SV). Outlier SNPs and the SV are polymorphic in native populations, but fixed or close-to-fixed in the two invasive pathways, providing evidence for independent parallel evolution. Intriguingly, the second copy of AMY2A has a missense mutation, and the SV contains a transposable element, suggesting adaptation via changes to protein function or expression. Genes in this family have been associated with human commensalism in house sparrows and linked to adaptation to high-starch diets in humans and dogs. This study illustrates the value of replicated analyses within and across species to predict rapid adaptation.

Tracking the evolution of wildlife viruses using historical specimens

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Understanding the evolution and emergence of wildlife viruses has proven to be more important than ever for preparing for future zoonotic outbreaks impacting human and animal health, such as SARS-CoV-2, Ebolavirus, and avian influenza. However, there are many gaps in our knowledge of the current and past viruses circulating in wildlife, or the "virome". In this project, we aim to understand the viral diversity in Australian wildlife over the past century, using the priceless specimens held in the Australian National Wildlife Collection. Novel methodology has enabled the previously genetically inaccessible formalin-fixed specimens, allowing this project to explore a range of important species, such as microbats, rodents, and reptiles, from over 60 years ago. Using the collections vast metadata, we will model environmental changes alongside genetic changes, and provide insight into how major ecological shifts have impacted viral diversity in Australian wildlife (e.g., loss of environments, rising temperatures). We will utilise co-phylogenetic and phylodynamic modelling to determine the host-viral evolutionary patterns, and to determine viral movement between species. This project will provide insights into the diversity of Australian wildlife viruses, how the viral landscape has shifted over time, and how to prepare for future viral outbreaks.

Is Sycon capricorn one species or more?

Ankush, Rodger Yan, Thomas McKinlay, Marcin Adamski and Maja Adamska

Research School of Biology, the Australian National University, ACT, Australia

Sponges (Porifera) are important research models because of their phylogenetic position, spectacular regenerative capacities and complex symbiotic relationships with diverse microbes. This research project investigates the population structure of the *Sycon capricorn* complex, a taxonomically challenging group of calcareous sponges found in the Capricorn region of the Great Barrier Reef and along the South-Eastern coastline in the Dharawal country. By analyzing nuclear SNP data and constructing phylogenies using both nuclear and mitochondrial genes, we aim to gain insight into population structure within this sponge species or species complex. Our current results indicate existence of *Sycon capricorn* species complex including two morphologically distinct forms: branching and radially symmetrical. In parallel, using long read sequencing, we are also investigating highly derived mitochondrial genome organization characterized by presence of multiple linear chromosomes, each containing a single gene flanked by terminal inverted repeats.

Testing the inbreeding hypothesis of multiple sex chromosome evolution

Maxim W.D. Adams[1], Aaron R. Jex[2], Emily J. Remnant[1], Kenji Matsuura[3] and Nathan Lo[1]

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Chromosomes are a fundamental unit of inheritance, and their size, structure and number vary dramatically across the Tree of Life. While sex is often determined by a single pair of chromosomes, an increasing number of species have been discovered with multiple sex chromosomes (MSCs), which fuse into multivalent chains during male meiosis. The cytological mechanisms behind MSC systems are well characterised, yet little is known about the evolutionary pressures that drive their emergence within a species. Here we investigate a leading – but untested – hypothesis, which suggests that MSCs evolve to fix heterozygosity and preserve genetic diversity in response to inbreeding. We will perform reduced-representation genome sequencing and chrosomome-scale long-read genome sequencing of the termite species *Glyptotermes nakajimai* and *Neotermes insularis*, both of which have multiple populations displaying multivalent chromosome chains of varying lengths. By characterising intraspecific patterns of inbreeding, genetic load, heterozygosity and chromosome structure, we aim to provide the first evidence of an association between inbreeding and the occurrence of MSCs. This talk will outline the methodology and background to the study, and present any preliminary findings regarding population structure and inbreeding in the focal species.

The Rise of Asexuality: Investigating Early Transitions from Sexual to Parthenogenetic Reproduction in the Peppermint Stick Insect

Soleille M. Miller[1], Katarina C. Stuart[2], Daniela Wilner[1], Lee A Rollins[1], and Russell Bonduriansky[1]

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Transitions from sexual to asexual reproduction have occurred in numerous lineages across the tree of life, but it remains unclear why asexual populations rarely persist. Transitions to parthenogenesis can occur through various mechanisms, leading to diverse methods of reproduction among parthenogens. Here, we use a facultative parthenogenetic stick insect, *Megacrania batesii*, to investigate the origin and initial stages of transitions from sexual to parthenogenetic reproduction in a species that is capable of both. Using insects from a previous crossing experiment, we follow lineages of *M. batesii* across multiple generations. Each generation, we manipulated reproductive mode and quantified heterozygosity and within-family genetic variation in order to determine the mechanism of parthenogenesis in this species. We identified apparent variation in the mechanism of parthenogenesis being employed between lineages, and even within the same individual. Our findings suggest that the origin and immediate consequences following transitions to parthenogenesis could have large implications for the evolutionary trajectories of parthenogenetic lineages and could influence which parthenogenetic lineages persist in nature.

Mutation rate estimates uncover the timing of severe historical population declines in koalas

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Australia has the worst mammal extinction rate in the world, with many remaining species now restricted to small, isolated populations. Previous demographic estimates for Australian marsupials have suggested historical population sizes significantly declined in the late Pleistocene, coinciding with the arrival of indigenous Australians on the continent. This indicates that human arrival may have negatively impacted extant marsupial species, akin to assertions made about the extinct Australian megafauna. However, these demographic estimates may be unreliable due to a lack of accurate species' specific mutation rate estimates, a key scaling parameter in these analyses, casting uncertainty on the size, timing and hence drivers of these declines. To address this, we resequenced genomes of parent-offspring koala trios, a marsupial of high conservation concern and low genetic diversity. Using this new mutation rate estimate, we model historical population sizes in koalas over the last million years. Our results suggest that koalas experienced large population declines before the arrival of humans, and that other factors such as climate are more likely to be responsible. This highlights the impact of historical climate change on past population health, which raises concern for already struggling marsupial populations under a currently changing climate.

Genomic traces of parallel evolution: Insights from Australian woodfeeding and soil-burrowing cockroaches

Zhuzhi Zhang[1] and Nathan Lo[1]

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Incidences of parallel evolution provide a powerful framework to elucidate the predictability of evolution. Australian soil-burrowing cockroaches (Geoscapheinae) provide an ideal study system in this context. These cockroaches, which create burrows up to a metre deep in the soil and feed on dried leaf litter, have evolved independently multiple times from their Panesthia wood-feeding ancestors. To investigate genomic changes associated with the evolution of soil-burrowing, we are sequencing the genomes of five soil-burrowing cockroaches and their wood-feeding sister species. We aim to understand the genomic architecture that underpins this complex trait, and potential differences between the mechanisms governing this trait. Further, significant life history changes may leave traces in the genome. How the major lifestyle changes affect natural selection from a genomic perspective is still not well understood. Here we report patterns of selection across more

than 500 conserved insect genes to understand the genome-wide evolutionary changes as a consequence of transitioning to a soil-burrowing lifestyle.

Molecular evolution of Circadian Rhythm genes in blind water beetles from the dark biosphere

Steven J. B. Cooper[1,2], Tessa M. Bradford[1,2], Adrián Villastrigo[3], Barbara L. Langille[2], Simon M. Tierney[4], Eliza R. Casey[2], Terry Bertozzi[1,2], Andrew D. Austin[2], Michael Balke[3], and William F. Humphreys[5,6]

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Independently evolved blind water beetle (Dytiscidae) species from island-like subterranean aquifers in Western Australia provide a powerful system to explore changes to the genome that accompany evolution in the dark. Using next-generation sequence data, we investigated the molecular evolution of Circadian Rhythm (CR) genes, to determine whether subterranean species have maintained the genetic pathways associated with clock-like gene expression. We hypothesised that genes specifically associated with the light entrainment of CRs in blind beetle species would evolve under neutral evolution and accumulate deleterious mutations. Using data from transcriptomes, exon capture, and newly generated Illumina and Nanopore data from 14 surface and 48 subterranean species of the genera *Paroster* and *Limbodessus*, we identified 16 genes, including three cryptochromes and one non-visual opsin gene putatively associated with light entrainment of CRs. Comparative sequence analyses revealed that two cryptochrome genes and the opsin gene showed evidence of pseudogenisation (insertions or deletions resulting in frameshift mutations or mutations introducing stop codons) in multiple subterranean species, in contrast to surface species. The remaining 13 genes retained open reading frames in all beetle species and are likely to be functional. Overall, these analyses suggest that subterranean beetles have maintained conserved CR genetic architecture, but have lost the function of light entrainment. Given the temperature stability of their groundwater environment, it is unclear what other features of their environment might entrain CRs in subterranean species or whether they have arrhythmic biological clocks.

Investigating Germ Layer Evolution in Animals

Dinithi Rajapaksha, Di Pan, Cuneyt Caglar, Rachel Rathjen , Marcin Adamski, Maja Adamska

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The origin of complex animals remains poorly understood. A critical aspect of animal evolution concerns the origin of germ layers. Haeckel's hypothesis suggested a germ layer homology between sponges and corals, and thus all eumetazoans. According to this hypothesis, sponge choanoderm (composed of choanocytes) and sponge pinacoderm (the outer epithelium) would be homologous to eumetazoan endoderm (from which the digestive system originates) and the ectoderm (giving rise to the epidermis), respectively. We addressed this hypothesis comparing tissue-specific transcriptomes derived from single-cell transcriptome datasets of sponges and cnidarians. The sponges studied represent the two major, distinct lineages in this phylum—the calcareous and siliceous sponges. We have sequenced single cell transcriptomes of Australian calcareous sponge, *Sycon capricorn*, and identified its cell types. Single-cell transcriptome datasets for the remaining species were extracted from recent literature. Homology was assessed using the SAMap algorithm. Our results are fully consistent with Haeckel's hypothesis, supporting homology between the innermost layers of sponges and cnidarians as well as the outermost layers of sponges and cnidarians. Thus, sponge body plan appears to represent an intermediate step between single cell protists (choanoflagellates) and complex animals, rather than being independent experiment in animal multicellularity as suggested by alternative hypotheses.

Mitonuclear interactions impact aerobic metabolism in hybrids and may explain mitonuclear discordance in young, naturally hybridizing bird lineages

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Understanding genetic incompatibilities and genetic introgression between incipient species are major goals in evolutionary biology. Mitochondrial genes evolve rapidly and exist in dense gene networks with coevolved nuclear genes, suggesting that mitochondrial respiration may be particularly susceptible to disruption in hybrid organisms. Mitonuclear interactions have been demonstrated to contribute to hybrid dysfunction between deeply divergent taxa crossed in the laboratory, but there are few empirical examples of mitonuclear interactions between younger lineages that naturally hybridise. Here we use controlled hybrid crosses and high resolution respirometry to provide the first experimental evidence in a bird that inter-lineage mitonuclear interactions impact mitochondrial aerobic metabolism. Specifically, respiration capacity of the two mitodiscordant backcrosses (with mismatched mito-nuclear combinations) differ from one another, although they do not differ to the parental groups or mitoconcordant backcrosses as we would expect of mitonuclear disruptions. In the wild hybrid zone between these subspecies the mitochondrial cline centre is shifted west of the nuclear cline centre, which is consistent with the direction of our experimental results. Our results therefore demonstrate asymmetric mitonuclear interactions that

impact the capacity of cellular mitochondrial respiration and may help to explain the geographic discordance between mitochondrial and nuclear genomes observed in the wild.

Cracking the case on reversals from viviparity to oviparity: A phylogenetic analysis of *Saiphos equalis*

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Although viviparity (live birth) has evolved over 150 times in vertebrates, 'reversals' back to oviparity (egg-laying) are considered to be rare due to difficulties in regaining complex physiological processes needed for oviparity. The lizard *Saiphos equalis* contains populations of viviparous, oviparous, and a unique, intermediate, 'transitional' form across its geographic range. This transitional form is hypothesised to be a result of a rare reversal 'back' from viviparity. Viviparity evolved recently in this species, meaning the mechanisms required for egg-laying may not have been lost, making *S. equalis* an excellent model to test for potential reversals in reproductive strategy, an issue my project addresses. I carried out Maximum-Likelihood phylogenetic analysis on mitochondrial sequences across 28 populations of *S. equalis* to test for reversals from viviparity back to the transitional phenotype or oviparity. I found strong support for the basal positioning of viviparous animals in two clades containing transitional populations, suggesting at least two reversals may have taken place. However, the paraphyletic grouping of a viviparous population suggests incomplete lineage sorting or introgression between populations, requiring further investigation. My results indicate reproductive mode evolution may be more complex than previously thought, and that reversals may play an important role in evolutionary processes.

Impact of structural variants on the evolution of parity modes using a pangenome of *Lerista bougainvillii*, a reproductively bimodal skink lizard

Maggs X[1,2], Dineth Pathirana[1], Daren Card[3], Scott Edwards[3], Catherine Grueber[1], Camilla Whittington[1]

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Viviparity (live birth) has arisen 115 times independently in squamates (snakes and lizards). The genomic changes required to transition from oviparity (egg-laying) to viviparity remain unknown. Prior research on reproductively bimodal species, those with both oviparous and viviparous

populations, has associated gene expression profiles and single nucleotide polymorphisms with parity mode. Until recently, technological limitations prevented researchers from investigating the impact of structural variants (SVs), those ≧ 50bp, on the evolution of parity modes. An emerging approach with great promise for robustly profiling SVs is pangenome graphs, alignment of whole genomes from multiple individuals from the same or closely related species. Through the construction of a pangenome with assemblies from oviparous, viviparous, and transitional populations of *Lerista bougainvillii*, a reproductively bimodal skink endemic to Australia, our study aims to identify SVs associated with parity mode evolution. Here, we report current progress on individual genome assemblies to be included in the pangenome, the first for any lizard. We discuss how we'll apply this new, cutting-edge resource to investigate the impact of SVs on gene expression in the uterus and embryonic tissues of oviparous and viviparous *L. bougainvillii* during and outside of pregnancy.

Ecological Genetics

Would you Mela-look-at that! Genomic prediction of disease resistance in a Paperbark foundation species

Karina Guo[1,2], Collin Ahrens[1,3], Stephanie Chen[1,3], Karanjeet Sandhu[2], Maurizio Rossetto[1], Ashley Jones[4], Chloe Tan[4], Justin Borevitz[4], Richard Edwards[3], Jason Bragg[1]

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Melaleuca quinquenervia (Broad-leaved paperbark) is a foundation species across large land areas and diverse vegetation types, from internationally important wetlands to forested grasslands. In many locations, *M. quinquenervia* are dominant canopy species, contributing substantially to local ecological form and function. However, *M. quinquenervia* populations are being impacted by Myrtle rust, a disease that arrived in Australia recently, and attacks hundreds of Myrtaceae species globally. This disease actively kills new leaves, affecting tree form and reproduction. Among *M. quinquenervia* individuals, variation exists in resistance to this disease. Opportunistically, we initiated an experiment to study the genetic basis of this resistance. Individuals of different maternal lines across NSW were grown and artificially inoculated with Myrtle rust. We then conducted a genome wide association study (~2,000,000 SNPs), linking the resistance phenotype to seedling genotypes, and identifying alleles that putatively conferred resistance. Using these, we estimated a genomic prediction model that predicts resistance accurately based on a modest number of SNPs (~600). This model potentially enables the identification of genetically resistant individuals using high-throughput genotyping, at relatively low cost. This talk will discuss methods, and how these

approaches could be deployed in restoring areas that have been impacted by this devastating disease.

What contributes to cane toads' invasion – novel adaptation or allele sorting?

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Knowledge about a species' native range is important for understanding the underlying evolutionary processes that occur following their introduction to a novel environment. Cane toads (*Rhinella marina*) were introduced to Australia in 1935 and are still expanding their range across the Kimberley region of Western Australia. Despite low genetic diversity, cane toads appear to have adapted to novel, arid environments as they travelled westward across Australia, and differ to their eastern Australian conspecifics in morphology, physiology and behaviour. The recent discovery of two ecomorphs in the native range (rainforest and coastal areas) raises the question of which was introduced to Australia, or whether hybrids of the two ecomorphs were introduced. If genetic variants from both ecomorphs exist in Australia, it is possible that what appears to be novel adaptation may be sorting of native range ecomorph alleles into the environments to which they are pre-adapted. We leveraged whole-genome and reduced representation sequence data to 1) investigate if native range ecomorphs are genetically distinct, 2) evaluate the genetic composition of toads across the invasion trajectory and 3) detect signals of selection in native and invasive populations. This study will provide insights into rapid evolution and resources for future management of this species.

Landscape-wide metabarcoding of the invasive bumblebee (*Bombus terrestris*) shows interactions among the gut microbiome and pollenbiome

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Many social insects introduced to regions beyond their native ranges have become highly invasive. The ~30-year-old introduction of the eusocial European buff-tailed bumblebee, *Bombus terrestris*, into the island of Tasmania raises concerns due to ecological impacts and the risk of transmitting pathogens to native bees or commercially important honeybees. The health of *B. terrestris* is intricately connected to its gut microbiome and diet, however, environmental variables may also interact with this, particularly during invasion into novel environments. Using landscape-wide sampling and metabarcoding approach to characterize the gut bacteria (16S rRNA) and diet composition from foraged pollen (ITS2: floristic diversity of pollen baskets), this study investigates how the *B. terrestris* gut microbiome is affected by nutritional (pollenbiome) diversity and environmental variation in *B. terrestris* across Tasmania. Analysis of 92 *B. terrestris* workers from 16 sites revealed that the composition and diversity of their gut microbiome were significantly predicted by site annual precipitation and pasture. Pollen analysis from 18 sites indicated a dominance from introduced plants, and interaction between pollen diversity X wind velocity significantly predicted gut microbial diversity. These insights help to unravel how pollinator-environment interactions influence bee gut health and invasion success into a new environment with novel nutritional resources.

Sigmavirus transmission mode and CO2-triggered paralysis in Queensland fruit fly, *Bactrocera tryoni*

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Populations of Queensland fruit fly (*Bactrocera tryoni*), Australia's most significant horticultural pest, are associated with RNA viruses that are closely related to other insect-specific viruses (ISVs). Common ISVs in B. tryoni are cripavirus (Dicistroviridae) and iflavirus (Iflaviridae), both of the Picornavirales, and sigmavirus (Rhabdoviridae) of the Mononegavirales. Transmission mode and efficiency of sigmavirus in *B. tryoni* are unknown, and so are its host effects. Through a set of comprehensive mating and cohabitation experiments with infected and uninfected flies we investigated sigmavirus transmission parameters in laboratory populations of *B. tryoni*. We also tested their response to CO2 because it had previously been found that sigmavirus causes paralysis in CO2-anaesthetised Drosophila flies. We found that sigmavirus is transmitted biparentally. Maternal transmission is more effective than paternal transmission and results in high viral loads in the offspring. Horizontal transmission was also observed, however, did not result in high viral loads and no vertical transmission to the next generation. Sigmavirus-infected flies experienced CO2-

triggered paralysis and mortality when exposed to CO2 at 12 °C for ten minutes. Insights gained from our research provide important new information about sigmavirus transmission and host effects that is of relevance to the biology and management of *B. tryoni*.

Of clams and clades: genetic connectivity and diversity of small giant clams (*Tridacna maxima*) in the southern Pacific Ocean

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Giant clams are large marine bivalves that make important contributions to reef environments in the Indian and Pacific Oceans. However, populations of these bivalves are currently threatened by harvesting for their meat and shells and environmental changes. The small giant clam (*Tridacna maxima*) has a wide distribution across the Indo-Pacific, but many areas of this distribution have received little to no assessment of genetic connectivity and diversity. Here, we assess genetic connectivity and diversity of *T. maxima* in the Coral Sea and Cook Islands using the mitochondrial CO1 gene. Our results show that the Coral Sea comprises a single population with high gene flow between reefs, whereas the Cook Islands shows evidence of a structured population within the archipelago. Both regions also display very high haplotype diversities, indicating these regions are important repositories of genetic diversity for *T. maxima*. Additional CO1 data from across the Indo-Pacific revealed that the Cook Islands clams belong to a unique *T. maxima* clade found exclusively in the Central Pacific Ocean, whilst the Coral Sea clams belong to a South-Western Pacific clade. This information will assist with the development of effective management plans for the species.

The application of mosquito iDNA for monitoring of mammals and birds in Kakadu

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Globally a pronounced pattern of accelerating biodiversity decline is occurring. This pattern extends to Australia, with prominent declines in Australia's unique mammals. Since European settlement this has amounted to the extinction of 39 mammals and the listing of a further 107 as at risk of extinction.

The first crucial step in protecting at risk species lies in gaining in-depth knowledge into their occurrence and distribution. A task traditionally facilitated through physical capture, visual observation or collection of traces such as fur or scat, techniques that are commonly skewed towards the detection of large and/or common species. Further to this multiple techniques are commonly required when information is needed for multiple vertebrate groups, further increasing the resource related cost. This highlights the importance of developing novel approaches that can provide biodiversity information about multiple groups in a sensitive and resourceful manner. Recently invertebrate derived (iDNA) has gained interest as a novel approach that allows for the simultaneous detection of multiple vertebrate groups with varying ecologies and body size with a single technique. By harnessing hematophagous invertebrates ability to gather vertebrate DNA through their feeding behaviours. This presentation seeks to provide an overview of how mosquito derived iDNA has been applied for monitoring of birds and mammals in Kakadu, demonstrating the ability of this approach to detect a broad range of mammalian and avian fauna.

Landscape genetics of invasive rusa deer: A geospatial perspective for improving predictions

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Landscape genetics is used extensively in conservation management to map gene flow across a landscape, highlighting barriers to and corridors of animal movement. Despite its utility, landscape genetic approaches are much less commonly used in invasive species management. Although existing landscape genetic studies often identify factors affecting animal movement, the identification and visualisation of the specific pathways utilised are less well-characterised. Knowledge of these pathways is key to implementing effective population management strategies. This project uses the rusa deer population located in the Illawarra region of New South Wales to demonstrate the power of applying landscape genetic approaches incorporating circuit theory to invasive species management. In addition to modeling how landscape features affect deer movement, we are using faecal pellet counts to validate our predictions. We will also characterise the landscape genetics of a second rusa deer population to determine whether the identified landscape features generally affect movement in this species or if they are site-specific. The outcomes of this study will contribute directly to the literature on invasion species applications of landscape genetics, highlighting its strengths outside of conservation management.

Exploring Fungal Diversity: The Puāwaitanga of Restored Wairarapa Wetlands?

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Wairarapa Wetlands in Aotearoa New Zealand, like many worldwide, face degradation, prompting restoration efforts. Despite their vital role in ecosystem health, wetlands and fungi are often overlooked, as insufficient data hinders our understanding of fungi's ecological importance. While Māori (Indigenous peoples of Aotearoa) connections to wetlands are well-documented, the relationship between Māori with fungi remains limited to traditional uses. In this era of nurturing the evolution of mātauranga Māori (Māori knowledge), there's a chance to expand our understanding of ecological restoration and diverse fungi. By aligning mātauranga Māori with scientific methods, I hope to gain a holistic understanding of how fungal communities in Wairarapa wetland forests respond to restoration efforts, and to identify fungal communities as indicators of restored processes, framed in the context of te ao Māori (a Māori worldview). I will interview Māori who are connected to Wairarapa to explore Indigenous ties to place and how fungi fits in with their environmental aspirations for wetlands. I will also sequence and analyse soil samples from different ecosystem states within Wairarapa wetland sites using environmental DNA techniques. Ultimately, this work seeks to contribute to improved wetland restoration outcomes that align with te ao Māori.

Development and Epigenetics

Genomic underpinnings of species- and sex-specific variation in complex cardiomyocyte morphology

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Zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*) are two bony fishes utilised as complementary comparative models to understand vertebrate evolution and cardiovascular biology. However, insights into the cellular and molecular profiles of fish hearts, particularly of cardiomyocytes, have

been hindered by limited methods to dissociate heart cells. Accounting for fishes' lower physiological temperature, we developed a new enzymatic dissociation protocol for fish cardiac tissue to isolate high yields of live single cells. Using our protocol, with confocal microscopy and imaging flow cytometry, we have identified species- and sex- specific cardiomyocyte morphologies in zebrafish and medaka ventricles. Quantitative morphological analysis reveals that female cardiomyocytes are significantly larger and elongated compared to male cardiomyocytes in both species. We also observe diverse cardiomyocyte shapes in zebrafish, while medaka cardiomyocytes are more morphologically consistent and resemble mammalian "brick-like" cardiomyocytes. Based on these findings, we are investigating the species- and sex-specific gene expression and histone modification profiles of FACS-purified cardiomyocytes and endothelial cells to identify potential underpinnings of morphological divergence. By doing so, we aim to build our fundamental understanding of the relationships between genomics, epigenomics, and cellular morphology, and characterise cellular and molecular diversity within highly conserved organs such as the vertebrate heart.

Asymmetrical illumination of embryonic chicken eyes modulates the expression of genes responsible for brain development

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In many vertebrates, the left and right sides of the brain are specialised for different cognitive functions, a phenomenon known as brain lateralisation. In birds, the optic nerve of each eye projects exclusively to the contralateral hemisphere of the brain and, accordingly, brain lateralisation is manifested as preferential eye use for certain visual tasks, such as feeding. Moreover, some aspects of visual lateralisation in birds are influenced by asymmetrical illumination of the eyes during late development, which arises from the orientation of the embryo in the egg. However, the molecular mechanisms underlying light-dependent lateralisation and the associated changes in brain connectivity have yet to be established. In this study, we used different techniques (differential gene expression, differential gene co-expression and gene co-expression network analysis) to trace patterns of gene expression in the left and right sides of the brain of the domestic chicken (*Gallus gallus*) exposed to different lighting regimes during incubation. Our results indicate that differential light exposure of the eyes during development impacts a suite of genes related to neuronal development and function in the brain, providing new avenues for discovery regarding the mechanisms involved.

Establishing clinical and parallel supercomputing pipelines to unfasten hereditary midline closure defects in dogs

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Our recently developed clinical and bioinformatics pipeline allows us to characterize and investigate rare canine inherited developmental anomalies. To date, the pipeline has been used to investigate two syndromic midline-closure-defects (MCD): a dorsal MCD in Shetland sheepdogs (Neural tube defect; NTD); and a ventral MCD in papillon dogs (Pentalogy of Cantrell; PoC). Advanced imaging (computed tomography), pathology investigation, and pedigree analyses were performed on affected stillborn and neonatal death cases and their families. The underlying MCDs were diagnosed, and evidence for familial inheritance of these rare developmental anomalies in the investigated breeds was provided. Whole genome sequencing (WGS) was performed on affected cases (n=2 PoC; n=2 NTD) and analysed alongside 106 previously generated WGS samples from various breeds. The high-throughput bioinformatics pipeline utilising NCI Gadi supercomputer (National Computational Infrastructure) aligned WGS data to the CanFam4 reference genome. Base quality score recalibrated BAMs were joint genotyped with GATK Best Practices workflow using publicly available canine VCF for recalibration. Exploration of the joint-genotyped VCF file of 110 dogs (n=4 affected; n=106 controls) is currently underway to identify variants unique to the affected dogs after accounting for breed-associated variants. Promising variants from this analysis will be taken forward for comparison against Dog-10K-data.

Maternal-fetal Communication during pregnancy in Australian Skinks

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During the transition from oviparity (egg laying) to viviparity (live birth), placentas arise to support the embryo during development. Placentation has evolved more than 100 times independently in reptiles and relatively recently in some lineages, making reptiles a great system to study this evolutionary transition. To understand the evolution of maternal-fetal communication, we compared placental gene expression patterns of oviparous and viviparous skinks, and cultured uterus with and without fetal membranes (specifically the chorioallantoic membrane or CAM) in vitro. Gene expression analysis identified that many signalling genes of viviparous skink placenta are involved in embryo development. We found 28 genes upregulated and 15 down-regulated in the uterus by the

presence of the CAM. Gene ontology analysis showed that genes involved in extracellular organization and tissue development were over-represented in our upregulated gene list. To validate the gene-expression date we performed immunohistochemistry on one of the up-regulated genes, the calcium transport protein calbindin 1 (CALB1). CALB1 localised to luminal and glandular epithelial cell in the uterus co-cultured with the CAM, consistent with where it is expected to function. Our results show that fetal tissue can influence gene expression of maternal tissue to support embryo development during pregnancy.

Investigating the roles of putative granule-associated genes in *C. elegans* germline epigenetics

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As our understanding of epigenetic regulation grows, it is becoming clear that such pathways are involved in a myriad of complex cellular processes that have far-reaching impacts on organisms and populations. One area of interest is the involvement of granules – biomolecular condensates of RNA and protein which are strongly implicated in germline epigenetic processes. We have identified two genes of interest in *Caenorhabditis elegans*, lotr-2 and tdrd-3, that we predict are involved in epigenetic regulation. To characterise these unexplored genes, we are investigating how knockouts affect animals at the epigenomic, transcriptomic and phenotypic levels. LOTR-2 contains a LOTUS domain, a domain found in germ granule-related proteins. We have imaged endogenously tagged germ granules in strains containing a lotr-2 mutation and have found granule formation and organisation to be perturbed in various parts of the germline when compared to wild-type. In line with a hypothesised role in cytoplasmic stress granules, we have found tdrd-3 mutants to respond poorer to heat stress than their wild-type counterparts. We have also performed total RNAseq on lotr-2 and tdrd-3 single mutants and found downregulation of genes in related to development and fertility respectively and will share our results to date on investigating these findings further.

Immune response of Bactrocera tryoni to covert viruses in laboratory populations

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Insect-specific RNA viruses (ISVs) are common and can affect host fitness. In Queensland fruit fly (Qfly; *Bactrocera tryoni*), Australia's most significant horticultural pest, cripavirus and iflavirus (both (+)ssRNA viruses), and sigmavirus ((-)ssRNA), are persistent in laboratory populations. RNAi is a component of insect immunity that is detectable through small RNA sequencing of virus-derived siRNAs, piRNAs and miRNAs. Our study investigated the virome and small RNA response to viruses

in Qfly laboratory lines. Total RNA was isolated from eight Qfly samples with a priori known differences in cripavirus, iflavirus and sigmavirus infections. Pools of whole flies were used except for two Qfly populations from which pools of ovaries and head/thorax were isolated separately. The sequenced transcriptomes revealed new viruses within the samples: a (+)ssRNA picorna-like virus; dsRNA orbivirus and toti-like viruses; and a new genus of (-)ssRNA Xinmoviridae. The small RNA sequencing highlighted a siRNA response to most viruses and also a piRNA response to sigmavirus and the Xinmovirid. Viruses of pest insects may present novel biological control agents, but may also impact pest management strategies such as the sterile insect technique (SIT) which requires the mass-rearing of flies whose fitness and performance may be affected by these viruses.

Epigenetics and rapid evolution in cane toads

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Released in coastal North Queensland 89 years ago, cane toads (*Rhinella marina*) have spread westerly at ever-increasing speeds and are now approaching the outskirts of Broome. 'Western toads' from newly-established populations differ widely in their morphology, physiology and behaviour compared to 'Eastern toads' from long-colonised areas. The ecological data documenting the dispersal of toads across Australia is close to unmatched, yet the molecular underpinnings of their rapid evolution remain mostly unresolved. Genetic data indicates reduced diversity compared to the native range, and overall high homogeneity across the introduced range. By contrast, the role of epigenetics as a potential driver of rapid adaptation compensating for low genetic diversity is still an open question. Here we focused on DNA methylation, a key epigenetic mechanism regulating gene activity and that can be influenced by environmental factors. Contrasting Western and Eastern toads at an early developmental stage revealed distinct DNA methylation profiles, particularly in genic regions putatively involved in dispersal-related traits. We discuss the links between epigenetic variation and genetic polymorphisms, the influence of transposable elements and their epigenetic regulation on adaptation, the potential for epigenetic inheritance, and the overall role of epigenetics in rapid evolution in this system.

Effects of parasite infection on microRNA expression of a co-evolved host, the cane toad *Rhinella marina*

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MicroRNAs (miRNAs) are a crucial regulatory mechanism that mediates important biological functions, including the immune response. They are a potentially critical interface in host-parasite associations. Our study investigated the effects of infection by a parasite lungworm (*Rhabdias pseudosphaerocephala*) on the miRNA expression of their co-evolved host, the cane toad (*Rhinella marina*). These species were co-introduced to Australia and the toad's ongoing range expansion has generated substantial divergence in host-parasite dynamics. We experimentally infected two groups of naïve, common-garden bred, hosts either once or twice with lungworms. We sequenced whole transcriptomes of infected and uninfected hosts' spleens to investigate miRNA expression. Overall, we identified 103 known miRNAs and 134 potential novel miRNAs in cane toads. We found all differentially expressed miRNAs to be downregulated in infected hosts compared to non-infected hosts, with stronger downregulation in hosts that received a second infection compared to single-infected individuals. Interestingly, downregulated miRNAs included mir-137, a well-known miRNA linked to innate immune function, which could indicate host manipulation by the lungworm parasite. Our study suggests that miRNAs play a key role in mediating interactions between lungworms and cane toads.

Epigenetic time travel reveals rapid evolutionary responses in vertebrates

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The physical environment plays a large role in the manifestation of an organism's phenotype, fitness and survival. Gene expression plasticity enables organisms to respond to changes in their environment. The degree to which such plasticity contributes to species' resilience or vulnerability to change is currently unknown. New technology to characterise century old chromatin architecture and historical RNA (including RNA virus detection) can combine to transform formalin-fixed biological collections into an accurate, comprehensive, and global record of environmental impact on gene expression and phenotype. I will discuss how a temporal understanding of gene expression trends advances our evolutionary understanding of species resilience and how this new technology has practical applications in threatened species management and environmental monitoring.

Investigating the activity of a multidomain histone-lysine methyltransferase involved in establishing epigenetic inheritance in *C. elegans*

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SET-25 is a H3K9 methyltransferase necessary for the establishment of transgenerational epigenetic inheritance in *C. elegans*. In a SET-25 null strain, animals exposed to an RNAi stimulus are unable to pass silencing on to their progeny to the same degree as wild-type animals. Interestingly, some SET-25 null progeny can still inherit silencing, and these animals are then able to pass silencing on to their offspring at a level similar to wild-type. With only an annotated SET domain, structural predictions and homology searches using SET-25 have identified a putative chromodomain, hypothesised to be involved in protein-protein or protein-nucleic acid interactions, and an n-terminal intrinsically disordered region. In this study, we generated transgenic strains with mutations in each of these domains and used them to investigate their individual contribution to H3K9me3 deposition with CUT&RUN, and their role in localisation using fluorescence microscopy. We found that, though both the chromodomain and SET domain are important for the deposition of H3K9me3, mutations to the SET domain did not significantly affect protein localisation. In contrast, mutations to the chromodomain appeared to decrease localisation specificity and foci formation, suggesting it is necessary for the targeting of specific loci for silencing.

Signalling pathways control cell growth, apoptosis and remodelling of the extracellular matrix during replacement of the *Drosophila* larval epidermal cells by histoblast-derived adult epidermal cells.

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Drosophila larval epidermal cells (LECs) are large, endo-replicating, polyploid cells that form an epithelial monolayer between the larval cuticle and basal lamina. During pupariation / metamorphosis, the LECs are gradually displaced and removed by apoptosis and by the lateral expansion of histoblasts that differentiate to form the adult epidermal cells (AECs). We have found that by manipulating the AKT and Hippo signalling pathways to increase LEC growth or block LEC apoptosis, some LECs can survive metamorphosis and contribute to the adult abdomen, resulting in a dorsal split in the abdomen. In combination, increased growth and blocked apoptosis leads to tumour-like midline outgrowths of LECs. Using a pan-LEC Gal4 driver, we are able to induce an adult abdomen where the ventral side consists almost exclusively of perduring LECs that partially take on the characteristics of AECs. We also find that dysregulation of the E3 Ubiquitin ligase Nedd4 disrupts key signalling pathways regulating normal LEC development. The process of LEC replacement elegantly illustrates the spatial and temporal coordination of signalling within and between tissues that is required for the transition from larval to adult structures that occurs during metamorphosis.

Sex chromosome dosage compensation in a skink with sex reversal

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Sex chromosome dosage compensation is an evolutionary response to correct imbalanced gene dosage from the X (or Z) chromosome with the autosomes, and between the sexes. It is thought that gene expression of the single X (or Z) chromosome is upregulated in the heterogametic sex. Studies of dosage compensation have historically focused on eutherian mammals which have a conserved XY sex determination system. In contrast, lizards have varying sex determination systems between even closely related species, including both XY and ZW systems, temperature-dependent sex determination, and some cases where temperature can override genetic sex determination. The eastern three-lined skink (*Bassiana duperreyi*) has an XY sex determination system, in which low temperature incubation during development can cause female to male sex reversal (i.e. XX males). To investigate how biological and genetic sex affects dosage compensation we generated transcriptomes from brain tissue of normal XY males and XX females, along with XX males. With this, we aimed to observe the gene dosage of the X chromosome in normal males compared to normal XX females and to identify if X chromosome dosage in sex reversed XX male skinks is distinct compared to the canonical sexes.

Gonadal transcriptome sequencing reveals the temperature response of gene expression at the chromosomal level in *Mauremys reevesii*

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Mauremys reevesii is a model organism with temperature-dependent sex determination (TSD), with a male-producing temperature (MPT) of 26°C and a female-producing temperature (FPT) of 32°C. Gonadal tissues were collected at stages 15, 18, and 21 for embryos incubated at the MPT and FPT respectively. These stages occur during the thermosensitive period for sex determination. Significant differential expression was observed. CYP19A1 and FOXL2 were upregulated at FPT, whereas DMRT1, SOX9, and AMH were upregulated at MPT. Expression of DNA methyltransferase genes and histone demethylase genes were assessed using qPCR. The expression of NO66 and KDM6B

significantly decreased with increasing temperature, and the expression of NO66 and KDM6B were each highly positively correlated with the expression of DMRT1, SOX9, and AMH. These results suggest that KDM6B and NO66 may promote male gonad development. Moreover, a comparative analysis of expression of genes residing on different chromosomes, drawing upon the assembled whole genome of M. reevesii, revealed heightened sensitivity to lower temperatures on chromosomes 12, 14, and 17. This result suggests that the response to temperature in TSD species involves not only individual genes or a few genes but potentially larger-scale regions at the chromosomal level, a complex interplay of epigenetic and genetic mechanisms.

Synthetic Biology

Prospects for a Y-linked editor to control Dipteran pests

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Y-linked editors (YLEs) are constructs inserted into the Y chromosome that cleave chromosomes required for female reproduction and survival. These edits cause dominant lethality or sterility in the progeny; therefore, the fitness of female progeny is reduced. We have designed a CRISPR/Cas9-based approach to bias sex ratios by targeting the haplolethal wings up A (wupA) gene on the X-chromosome of *Drosophila melanogaster*. Most haplolethal genes require two copies for viability; however, dosage compensation mechanisms allow haplolethal genes to occur on the X chromosome of *D. melanogaster*, where males are XY. Creating mutations in one copy of the gene kills females. Three sgRNA sequences targeting wupA were inserted into a fly line, successfully disrupting wupA during spermatogenesis under a nosCas9 germline promoter. Our fly crosses resulted in 14% female F1 progeny, a significant deviation from Mendelian ratios. We screened surviving females for allelic resistance, evaluated the Cas9 and sgRNAs' efficiency, and assessed the fitness of wupA-poisoning males. YLEs that hinder the functionality of haplolethal genes in pests may ultimately lead to the decline of the target population through female elimination. Our ultimate aim is to transfer this sex-biasing system into the agricultural pest *Drosophila suzukii*, whose Y-chromosome we have successfully assembled.

Engineering animals for mercury bioremediation

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Mercury is one of the most toxic environmental pollutants and impacts neural, reproductive, and immune health. Methylmercury is highly bioavailable and readily accumulates in food webs, where it has been inaccessible to remediation technologies. We demonstrate that invertebrate (*Drosophila melanogaster*) and vertebrate (*Danio rerio*) genetic animal models can be engineered to express the microbial enzymes, organomercurial lyase (merB) and mercuric reductase (merA), to bioremediate methylmercury. The engineered animals could catalyse the removal of methylmercury in their biomass as gaseous elemental mercury (Hg0). They accumulated >50% less total mercury relative to their wild-type counterparts, and the majority of the mercury in their biomass was in the less bioavailable form, inorganic mercury (Hg2+). Furthermore, the engineered animals could withstand higher exposures to methylmercury. This research could be applied to remediate mercury in contaminated hotspots for species conservation, as well as for recreational and subsistence fishing. Insects that can compost organic wastes could bioremediate and restore value to organic wastes typically contaminated with mercury.

Allele Sails: A Novel Method to Alter Wild Populations

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Both beneficial and deleterious alleles can be spread through a population with genome editing approaches. Synthetic gene drives can rapidly increase the frequency of an allele. However, the gene drive transgene may persist indefinitely or spread into non-target populations. Here, we propose a new system to alter wild populations – an Allele Sail. An Allele Sail uses a genome editor (the Wind) to create DNA edits (the Sail), which permit the development of viable and fertile offspring. This creates a unique dynamic where the editor is transmitted at a standard Mendelian ratio, while the frequency of the edits increases at a super-Mendelian rate. Using agent-based modelling we show that edits can reach very high frequencies with a single, low frequency release. We also show that the spread of edits that redirect sexual development towards males can suppress a pest population. As a proof of concept, we genetically modified zebrafish to express a CRISPR/Cas9 system targeting fancl, a gene required for female sex determination. The sex-ratios of zebrafish carrying the Cas9 transgene were heavily skewed towards males. This successful proof of concept should promote the development of Allele Sails to alter wild populations.

Toxic masculinity – Heterologous venom expression in pest insect seminal fluid reduces mated female lifespan

Samuel J. Beach[1] and Maciej Maselko[1]

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The growing prevalence of insecticide resistance has increased the need for alternative pest management strategies. Current alternatives such as mating-based genetic biocontrol technologies are limited in their potential to rapidly suppress existing pest populations, as they function by reducing the reproductive capacity of mated females by affecting offspring viability or sex-ratio. However, if the lifespan of mated females were reduced rather than their reproductive capacity, it would be possible to achieve intragenerational pest management through genetic biocontrol. We have demonstrated the first proof of concept of such a technology, the Toxic Male Technique (TMT). Heterologous expression of venom proteins within the reproductive tract of *Drosophila melanogaster* males resulted in 37 - 59% reduction in the median lifespan of mated wild type females compared to females mated to wild type males. Agent-based models of *Aedes aegypti* mosquito genetic biocontrol release programs found that compared to current state of the art techniques, TMT may be capable of reducing the incidence of potentially disease-transmissible blood feeding by a further 40 ~ 60%. Our results demonstrate the feasibility of intragenerational genetic biocontrol, which presents a new strategy for combatting outbreaks of agricultural pests and disease vectors.

Inverting Invasion: Strategies for Species Control through Inversions

Soumitra Bhide[1], Isabelle Lohery[1], Ben Phillips[2] Charles Robin[1]

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Invasive species continue to be a significant factor in the loss of native biodiversity and economic loss. Once introduced, these species form smaller subpopulations on their boundaries, furthering the invasion. Eliminating these small subpopulations is the key to containment, but conventional means have proven ineffective. However, these smaller populations tend to have low genetic diversity and thus are vulnerable to extinction if the variants present in these subpopulations are deleterious. We are exploring whether we can push these subpopulations into extinction by loading the core population with deleterious recessive alleles, thus introducing a genetic Allee effect. To do this, we aim to construct supergenes/haplotypes (?) consisting of deleterious recessive alleles within the *Drosophila melanogaster* model. Our goal is to employ *Drosophila* deficiency lines to mimic deleterious recessives and quantify the genetic Allee effect under various conditions. To spread potential deleterious alleles despite their fitness cost, we seek to construct synthetic inversions that tightly link them, driven by a toxin-antidote gene drive. Such synthetic constructs will provide insight into building a more efficient tool for containing and eliminating invasive species.

Advancing insect pest control using genetics

Amanda Choo[1], Thu Nguyen[2], Elisabeth Fung[3], Anzu Okada[1], Peter Crisp[3] & Simon Baxter[2]

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The Queensland fruit fly, *Bactrocera tryoni*, is a serious horticultural pest that can be controlled using the sterile insect technique (SIT). Sterile factory reared males are released and mate with wild

females that produce inviable embryos. A major challenge of SIT is removal of factory females, which hinder release efficiency. Here we use CRISPR/Cas9 genome editing to develop multiple *B. tryoni* pupal colour mutants, and use one to create a genetic sexing strain to visually identify males. Mutations created in the pigmentation gene ebony resulted in a black puparium and a dark adult body colour, however, fitness costs were relatively high. This phenotype was akin to the black pupae genetic sexing strain used for SIT against the Mexican fruit fly, and genome sequencing subsequently confirmed ebony as the causal gene. Next, a previously described white pupae gene was used to create a *B. tryoni* genetic sexing strain. Low dose X-ray radiation translocated part of chromosome 5, including a wild-type copy of white pupae, to the male Y-chromosome. This produced a strain where male pupae are brown, and females pale grey. This research can potentially improve SIT efficiency through mechanical sorting and removal of female pupae.

Heterologous expression of phytases in an insect host

Carly Retief, Kate Tepper, Sheemal Kumar and Maciej Maselko

Macquarie University

Phytic acid is the main source of phosphate in grains and legumes. Phytic acid constitutes around 80% of total seed phosphorus and 1-3% of plant seed weight. This rich source of phosphorus is not bioavailable to many agriculturally significant animals such as pigs and chickens. Due to multiple negatively charged phosphate groups, phytic acid has a chelating effect on other essential micronutrients such as iron and zinc. Waste runoff from animals reared on diets rich in phytic acid contaminate nearby waterways, leading to increases of algal blooms and eutrophication. Phytases are microbial/fungal enzymes capable of breaking down phytic acid and are often added to animal feed to boost the bioavailability of phosphorous. Commercially available phytases are produced via microbial fermentation that is dependent on complex infrastructure and highly refined feedstocks. We propose genetically engineering insects such as *Hermetia illucens* (black soldier fly) to heterologous produce phytases as an animal feed supplement. Insects are increasingly used as feed ingredients and can be reared on waste that is not suitable for microbial fermentation with comparably small infrastructure needs. Producing feed additives will help to generate more value from organic waste. I will present data demonstrating that the model insect, *Drosophila melanogaster*, can be engineered to produce an active microbial phytase. These proof of concept results demonstrate the potential for insect biomanufacturing of animal feed enzymes.

Day 4 – Wednesday 3rd July

Plenary

Comparative Genomics of Cichlid Fishes in East African Great Lakes

“Evolution” and “Conservation”

Masato NikaidoDay

Evolutionary Biology at School of Life Science and Technology, Tokyo Institute of Technology

One of the most interesting phenomena describing the evolution of the cichlids (fresh water fish) inhabiting the East African Great Lakes (Tanganyika, Malawi and Victoria) is the morphological parallelisms within the context of adaptive radiation. Indeed, many researchers have focused their attention on the cichlids to elucidate the genetic mechanism underlying adaptive radiation as well as parallel evolution. In contrast, the cichlids of Lake Victoria are also regarded as a target species for conservation study because of their drastic population decline caused by the introduction of Nile perch, a commercial fish. In this talk, I introduce our recent studies on evolution and conservation of the East African cichlids by focusing on 1. the parallel evolution in lip hypertrophy and 2. genetic signature of bottleneck both revealed by comparative genomics. 1. Our group has focused on “lip hypertrophy” as a representative example of parallelism observed in cichlids the East African Great Lakes. Comparative genomics, RNA-seq analyses revealed that the hypertrophied lips were characterized by the higher expression of extracellular matrix proteins such as versican and proteoglycans. In addition, cross-breeding, QTL mapping analyses revealed the tight link between the loss of MAGP4 genes and lip hypertrophy. These phenomena were common in all examples of lip hypertrophy in East African Great Lakes, providing important insight into the genetic mechanism of parallel evolution in cichlids. 2. In the 1980s, the upsurge of Nile perch, a carnivorous fish introduced to the lake, drove the extinction of more than 200 endemic cichlids. Based on our comprehensive comparative genomics and population genetic analyses, discovered genomic signatures of severe bottleneck in a paedophage, egg-eating cichlid species, which began during the 1980s, and population size rebounded during the 1990s-2000s. The timing of the bottleneck corresponded to the historical records of disappearance and later resurgence, which is likely associated with the introduction of Nile perch. The elucidation of population dynamics at the genomic level would be an important benchmark for designing future conservation strategies in Lake Victoria.

Insights and outcomes from biodiversity and agricultural genomics

AusARG – Phylogenomics of Australian Squamate Reptiles

Sarin Tiatragul[1], Ian G. Brennan,[1,2] Damien Esquerre[1,3], Elizabeth S. Broady[1], Craig Moritz[1], and Scott Keogh[1]

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The Australian Amphibian and Reptile Genomics Initiative (AusARG) is a national collaborative project to facilitate genomic research on Australia's remarkable native species. Phylogenomics of Australian squamate reptiles are one of three major themes of the initiative. The overarching goal is to produce species-level dated phylogenomic hypotheses for every squamate radiation in Australia and their relatives from other regions. We aim to include representatives of all recognised taxa, as well as many known undescribed cryptic lineages. Through generating a consistent set of molecular data, we can compare across Australian groups and integrate with other global phylogenomic initiatives. Through a huge effort we have already collected data for more than 1,000 (?) samples of Australian lizards and snakes. These data complement existing phylogenomic data generated for all Australian frogs, pythons, and goannas, thus completing all Australian of squamate reptiles and amphibians. These data sets will be important for a wide variety of purposes including deciphering macroevolutionary patterns, biogeographic history, resolving complex taxonomic issues, and patterns of molecular evolution. In this talk we will give an overview of the project, talk about progress to date and plans for the future.

A genome assembly and annotation for the Australian alpine skink Bassiana duperreyi with emphasis on the sex chromosomes

Benjamin J. Hanrahan[1^], Kirat Alreja[2^], Andre L. M. Reis[3,4,5], J King Chang[1], Duminda S.B Dissanayake[6], Richard J. Edwards[7], Terry Bertozzi[8,9], Jillian M. Hammond[3,4], Denis O'Meally[10], Ira W. Deveson[3,4,5], Arthur Georges[6,11+], Paul Waters[1+] and Hardip Patel[2*+]

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The Australian three-lined skink (*Bassiana duperreyi*) inhabits the Australian high country in the south west of the continent including Tasmania. It is distinctive because it undergoes sex reversal (XX genotype to male phenotype) at low incubation temperatures. We present a genome assembly

from an individual *Bassiana duperreyi* assembled, scaffolded and annotated using a combination of HiFi, ONT and HiC reads. The genome sequence is 1.59 Gb with a scaffold N50 of 211 Gb. Most of the assembly is scaffolded into chromosomal elements, confirmed by novel identification of centromeric and telomeric elements, that correspond in number to the 6 macro-chromosome pairs, 8 micro-chromosome pairs and one pair of sex chromosomes in this species. With the exception of kmer completeness, the assembly exceeded the standard recommended by the Earth Biogenome Project. We identify 187 bp and 199 bp alpha satellite repeat elements predicted to be centromeric repeat elements. The *B. duperreyi* genome assembly has one of the highest completeness levels for a skink, and will provide a resource for research focussed on sex determination and thermolabile sex reversal, and as an oviparous foundation species for studies of the evolution of viviparity, and genomics of the Scincidae more generally.

Genomic platforms transforming environmental research and management

Oliver Berry[1], Mark Wallace[1], Andrew Young[2]

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A casual observer of medicine and agriculture will know that those fields have been radically transformed by the molecular biology revolution along with parallel advances in materials, engineering, and data science. In 2016, we started thinking about what next for genomics and environmental science and took the view that a similar revolution was possible and needed. We were presented with a unique opportunity to execute our plan through a partnership with Bioplatforms Australia and CSIRO's Future Science Platform program. In addition to an emphasis on national-benefit, distinct attributes of our brief included: a focus on building "platforms" – generalisable new ways of employing genomics to address natural resource management; license to fail (i.e. an elevated technical risk profile); a medium-term funding envelope; and encouragement to build national capability in environmental genomics through training and other mechanisms. This unusual, and we believe, enlightened brief has yielded failures, but they are vastly outnumbered by successes. In this presentation we will introduce some of these scientific successes plus the emerging partnerships and communities of practice that are shifting the dial on how genomics can contribute to more effective fisheries, biosecurity and biodiversity management.

Development of sensitive, flexible and generic metabarcoding analysis and database curation pipelines for agricultural biosurveillance

Jack L. Scanlan[1], Alexander M. Piper[1], Lea Rako[1], Francesco Martoni[1], Brendan C. Rodoni[1,2], Mark J. Blacket[1]

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Metabarcoding, the use of high-throughput DNA barcode sequencing to identify numerous individual taxa in complex mixed communities, has revolutionised the study of biodiversity. However, its adoption in agricultural biosecurity and biosurveillance, to detect exotic and endemic pests and pathogens, has been comparatively slow, due to these fields' requirements for highly sensitive detection, rapid turnaround times, and broadly interpretable and actionable reports. To address these challenges, we are developing a suite of next-generation metabarcoding analysis and reference database curation pipelines, alongside refined sample-processing and sequencing methodologies. Our pipelines, written in the Nextflow scientific workflow language, support the entire metabarcoding workflow, from deriving high-quality barcode sequences from public repositories to underpin identifications, through sequencing data analysis, to reporting to different stakeholders. They are designed to be taxonomically generic, flexible to different sequencing platforms, and accessible to users with varying bioinformatic experience and computational resources. Additionally, non-destructive DNA extraction methods for arthropods allow us to re-examine specimens to confirm detections of priority species, providing a triaging system for large numbers of samples. We expect our tools and methodologies to further support the diagnostic use of metabarcoding in Australian agricultural biosurveillance and biosecurity, while simultaneously producing valuable community-level datasets for biodiversity research.

GAP conservation genomics - resolving taxonomy challenges in species complexes

Margaret Byrne[1], Rachel M. Binks[1], Benjamin M. Anderson[2], Kelly A. Shepherd[2], Andrew D. Crawford[1], Barbara L. Rye[2], Michael Hislop[2], Robert Davis[2], Terry D. MacFarlane[2]

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Genomics for Australian Plants is a national collaborative BioPlatforms initiative that has developed genomic resources for understanding of the evolution and conservation of the diverse Australian flora. Conservation genomics is one of its three components, applying an integrated approach to taxonomic resolution of species complexes where there are likely taxa of conservation concern so they can be defined and considered in conservation listing and implementation of management actions. Genomic approaches assist in this challenge through identification of relationships at the species-population interface providing an evolutionary basis for identifying taxon boundaries, including where ongoing hybridisation confuses morphological relationships. Species relationships have been clarified in three species complexes in south-western Australia, an area with high species diversity and endemism and complex evolutionary history, using ddRAD genomic data in combination with morphological assessment. The relative outcomes of ddRAD and the angiosperm 365 bait kit were also tested for one of these species complexes. These analyses have led to the identification of species of conservation concern, and clarification of situations where morphological variation does not reflective taxonomic separation, or where hybridisation obscures morphological

relationships. The outcomes demonstrate the benefits of using genomic methods in taxonomic resolution of species complexes for effective conservation action.

Unlocking the secrets of adaptation and evolution in Australian orchids through question-driven transcriptomic and genomic analysis

Darren Wong[1], Emma John[1], Zirui Zhang[1], Ashley Jones[1], Ryan O'Donnell[1], Benjamin Schwessinger[1], Celeste Linde[1], Rod Peakall[1]

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While transcriptomic and genomic knowledge is valuable alone, the most powerful insights emerge from comparative analyses. In this question-driven approach, which is often overlooked when generating genomic resources, prior biological knowledge is used to inform strategic sampling. This tactic is employed in our studies of Australian terrestrial orchids and their repeated evolution of pollination by sexual deception, where specific male pollinators are sexually attracted to dull-coloured flowers by chemical and visual mimicry. Capitalising on carefully chosen contrasts between study species and tissue types, transcriptomic analysis has enabled us to elucidate the volatile and colour biosynthetic pathways underpinning pollinator attraction. As we finalise the first Australian orchid genome, gene duplication is a logical subject for deeper investigation following the discovery of its key role in the evolution of this pollination adaptation. Drawing on our extensive multi-tissue transcriptomes atlas, our multi-tiered sequence capture dataset covering the phylogeny, and the growing number of publicly-available datasets (facilitated by Bioplatforms Australia), we have uncovered novel phylogenomic insights into the intertwined evolution of Australian terrestrial orchids, their pollinators, and their mycorrhizal symbionts. As we expand our genome sequencing effort, we aim to identify the genomic changes contributing to the astonishing diversity of Australian orchids.

Genomic data empowering conservation of Australia's endangered species

Kym Ottewell[1] and Margaret Byrne[1]

[1] *Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions, Kensington, Western Australia*

Conservation management agencies are increasingly using genetic data to inform conservation and management actions for threatened species. The Department of Biodiversity, Conservation and Attractions in Western Australia is no exception, with a long history of uptake of genetic data and genetic monitoring tools into conservation operations, underpinning threatened species population management and conservation actions such as translocations. Through engagement with BioPlatforms Australia Oz Mammal Genomes and Threatened Species Initiative, and collaborations spanning university researchers, government and non-government conservation organizations, we have generated genomic data sets for more than 10 threatened fauna species, including some of

Australia's most critically endangered. Population genomic datasets have been invaluable in understanding the consequences of population decline and past management of species such as the boodie (*Bettongia lesueur*) and mala (*Lagorchestes hirsutus*), and transformed current conservation practices. In many cases, genomic data have been translated into management tools to facilitate ongoing monitoring of species, for example, in developing molecular sexing markers and SNP arrays for the ghost bat (*Macroderma gigas*), enabling monitoring of populations across Australia from non-invasive samples. With these examples and others, we will highlight how genomic data has empowered conservation outcomes for threatened species managed by our organization.

Genomics and environmental DNA informs translocations of an endangered Australian freshwater fish

Bruce Deagle[1], Rob Freeman[2], Charlotte Jense[3], Floriaan Devloo-Delva[1], and Christopher Burridge[3]

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Conservation genetics is a discipline with a rich theoretical and applied knowledge base; however, genetics is often still overlooked in conservation actions. There have been recent debates about how to change this, and about what the true value of genetic data is to conservation managers. In this talk we illustrate practical genetic applications in the conservation of Swan galaxias (*Galaxias fontanus*). This endangered Tasmanian freshwater fish has a fragmented distribution in headwater streams of two watersheds and population connectivity is restricted by the downstream presence of introduced brown trout. Swan galaxias were translocated to several fish-free streams 30 years ago and further translocations are ongoing to secure the few remaining populations. As part of the Bioplatforms Australia Threatened Species Initiative we carried out full genome sequencing and RAD-seq of natural and previously translocated populations. Our data show unexpected patterns of natural genetic diversity within the Swan River watershed, retention of genetic diversity in previous translocations, and provide information on priority source populations for new translocations. We also used environmental DNA methods to help identify translocation sites that were trout-free, for the detection of *G. fontanus* in population surveys and for non-invasive collection of population genetic data.

Assessing the Potential of Airborne eDNA for Targeted Weed Detection

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Environmental DNA (eDNA) sampled from the air could transform the way we monitor terrestrial weeds. While recent advances have proven plant DNA is readily detectable from air samples, crucial questions remain. Foremost among these is determining how sensitive these methods are at detecting rare weeds in natural environments and establishing optimal protocols for capturing air DNA. We sought to examine these questions by conducting two experiments with soybean (*Glycine max*) as a substitute for a weed. First, we used a field experiment to test detection sensitivity in a realistic environment. Here, we set up an outdoor transect line with soybean in the centre to evaluate the effects of distance from source, and plant density on detection. Second, we manipulated environmental variables in a controlled context within a glasshouse with soybean at a fixed distance. We manipulated airflow, sampler height, sampling method, and plant density. This experiment was designed to determine how controlled covariates impact detection of eDNA sampled from air. We sought to demonstrate whether a targeted approach with air eDNA is effective at detecting a rare plant, and the impact of environmental variables. These results could help inform future efforts to detect weeds before they become established.

Genome of the eastern water dragon (*Intellagama lesueurii*), an agamid model for urban adaptation

Daniel Powell[1,2*], Nicola Jackson[1], Parwinder Kaur[3], Olga Dudchenko[4,5], Erez Lieberman Aiden[4,5,6], Arthur Georges[7] and Céline, H. Frère[1]

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Squamate reptiles are a highly diverse and intriguing group of tetrapods, offering valuable insights into the evolution of amniotes. The eastern water dragon (*Intellagama lesueurii*) is a large, semi-aquatic member of the Agamidae family of iguanian lizards that inhabits riparian landscapes along the east coast of Australia. The eastern water dragon exhibits temperature-dependent sex determination (TSD) and is renowned for its urban adaptability and complex social systems. We report a chromosome-length genome assembly with accompanying gene annotations and a first look at a comparative analysis with other squamate genomes highlighting genomic features with implications for immune function and energetic homeostasis. We also provide lessons learnt from producing the genomic data together with highlights from our initial studies and how this reference genome is serving as a valuable resource for our studies of evolution and environmental resilience in this species.

Bioinformatics and genomics

Genomic imputation on a natural population of the hihi (*Notiomystis cincta*)

Hui Zhen Tan[1] and Anna W. Santure[1]

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Sampling strategies in genomic studies often present a trade-off between number of samples sequenced and sequencing depth. Genomic imputation is a useful tool for elevating low coverage sequences to whole-genome level by utilising information from a reference panel of individuals selected to capture species-wide diversity and sequenced at higher coverage. While genomic imputation is routinely applied in model organisms, it has had limited applications to natural populations despite its potential in reducing genotyping costs for large sample sizes. In this study, we apply genomic imputation on the hihi, an endangered passerine bird endemic to Aotearoa New Zealand. A comprehensive set of genomic resources has been generated for the hihi including reference genomes, linkage maps, and whole genome re-sequences of hundreds of individuals. We introduced missingness among reference panel individuals prior to imputation to allow fine-tuning of parameters and to assess imputation accuracy, with and without a high-density linkage map. We illustrate that setting an appropriate effective population size parameter is crucial to improving imputation accuracy, achieving up to 96% concordance with known genotypes. Surprisingly, application of a genetic linkage map alone provided little improvement. These results provide one of the first benchmarks for imputation on a small, inbred, natural population.

Generating genomics resources to support indigenous aquaculture of haku/Yellowtail kingfish (*Seriola lalandi*) in New Zealand

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New Zealand's aquaculture industry relies heavily on the farming of three species, which includes only one finfish species (the non-indigenous king salmon *Oncorhynchus tshawytscha*). To enhance food security and better support local communities, particularly Māori (indigenous) communities, The Smart Māori Aquaculture initiative has identified *Seriola lalandi* (yellowtail kingfish) as a high-potential indigenous finfish for aquaculture. Utilising genomic information in aquaculture settings is still novel when compared to terrestrial farming; genetic information for wild *S. lalandi* populations surrounding New Zealand is limited. This community-led project uses bioinformatics applied to combination of long- and short- read data, and Hi-C data, to produce the first high-quality genome assembly for a New Zealand -based *S. lalandi*, revealing insights into its unique genetic diversity. This

project will also investigate the population structure and genetics of wild New Zealand *S. lalandi*, informing stock structure and broodstock selection for local breeding programmes.

Empowering Bioinformaticians and Conservation Managers: Generating Workflow Solutions in Galaxy

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The importance of using genetic data to inform conservation decisions has been well documented, however, there are still barriers limiting the uptake of genetic data into conservation management programs. A recently identified barrier is the required bioinformatic knowledge and access to large-scale compute to undertake analysis of genetic data. As the cost of obtaining genetic sequence data decreases, the volume of data in exponentially increasing. The Galaxy Australia platform offers a user-friendly, 'point and click' interface with an array of analysis tools to reduce some of the barriers for conservation managers. In conjunction with the Galaxy Australia team, the Australian BioCommons and the Threatened Species Initiative has generated a series of bioinformatic workflows to enable bioinformaticians and conservation managers to assemble and annotate reference genomes for threatened vertebrate species. These workflows will be available to users via the newly developed customised interface called the Genome Lab. Bioinformaticians and conservation managers can effortlessly navigate, customize, and execute these workflows, enabling them to conduct sophisticated analyses without extensive computational knowledge. By harnessing the power of Galaxy's user-friendly interface and collaborative environment, we aim to empower bioinformaticians and conservation managers alike, facilitating impactful life sciences research and informed conservation decisions.

A Bag-Of-Motif Model Captures Context-Specific Distal Regulatory Elements

Paola Cornejo-Páramo[1,2], Xuan Zhang[1], Lithin Louis[1], Yi-Hua Yang[1], Zelun Li[1], David Humphreys[1], Emily S. Wong[1,2]

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Enhancers are cis-regulatory regions that orchestrate the spatiotemporal gene expression by mediating transcription factor (TF) interactions. Deciphering their sequence properties is challenging due to rapid evolution, low conservation, and binding complexity (1, 2). We developed

"Bag-of-Motifs" (BOM), a tree-ensemble–based machine-learning framework designed to identify cell type-specific enhancers using TF binding motif composition. BOM is an adaptable framework for dissecting the key TF binding motifs within regulatory sequences between specific cell types/states, and conditions. We use Shapley values, a concept rooted in game theory, to elucidate the contribution of binding motifs to the classification tasks (3). BOM excels in classifying candidate enhancers from multiple cell types across various species and developmental stages and outperforms deep-learning models in the same classification task (auROC=0.98). In conclusion, BOM not only excels in classifying candidate enhancers across diverse biological contexts but also sheds light on the critical motifs governing enhancer function and gene regulation.

Filtering Out the Noise: A Pre-Alignment Metagenomic Classifier Approach to Reduce Contamination in Ancient DNA Datasets

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Contamination with exogenous DNA presents a significant challenge in ancient DNA (aDNA) research. Failure to address contamination from microbes, reagents and present-day sources can lead to false conclusions. Although significant strides have been made in mitigating exogenous contamination through improvements in field and laboratory protocols, there is still a need to accurately distinguish between endogenous and exogenous data computationally. Here we propose a novel workflow to minimise contamination in aDNA datasets. Unlike current protocols that rely exclusively on the specificity of the mapping to a single reference genome to remove contaminating reads, our approach uses Kraken2-based, a metagenomic classifier, filtering before mapping to the reference genome. Using both simulated (human and dog) and empirical shotgun aDNA datasets we show that this workflow offers a simple and efficient method that can be run on a wide range of computational environments—including personal machines—while enabling the removal of contaminating sequences with limited loss of authentic endogenous data. Compared to mapping alone, filtering and mapping drastically reduces the overall computational resources required. Based on simulated data we identify that the database used to filter shotgun data is crucial for the success of this workflow, hence, we benchmark two strategies: filtering by identifying endogenous reads and filtering by identifying contaminants. We show both strategies remove more contaminating sequences better than mapping alone and the choice depends on the available computational resources and prior information about the dataset. The cumulative time of filtering and mapping reads using our workflow in the empirical data reduced mapping time by 27.24% to 93.97%, with the most significant impacts being observed in low endogenous samples. Furthermore, we identify potential contaminants that mapped to the reference but were filtered out using our strategy. We also confirm pre-mapping filtering has minimal impacts on downstream population genetics analysis.

Our method provides a straightforward and effective solution to mitigate exogenous contamination in aDNA datasets, while significantly decreasing computing time.

The CFTR Locus in Ancestrally Diverse Populations

Zahra Chew[1], Hardip Patel[1], and Shafagh Waters[2]

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Cystic fibrosis is an autosomal recessive disorder caused by mutations in the CFTR gene, which encodes an epithelial transmembrane chloride and bicarbonate channel. There are hundreds of causative variants across CFTR with different mutations linked to ancestrally diverse populations. There remains bias in favour of European ancestral groups in testing and targeted treatment. This project explores the genetic and protein structural landscape of the CFTR locus in the ancestrally diverse populations represented in publicly available datasets. At a haploblock level the vastly different structures present in continental populations of 1000 Genomes (ranging between 5 and 34 haploblocks) are reflective of corresponding population histories. CFTR contains between 2675 and 5202 total SNPs and between 7 and 32 pathogenic SNPs across the corresponding populations in the GnomAD database. 22.5% of these pathogenic variants are specific to a population and only 7.5% are present in all 5 groups. In addition, non-reference protein haplotypes exist in high frequencies within the global population. Alphafold predictions provide insight into the structural variation present within healthy populations, laying the foundation for future exploration of altered drug responses. Further investigation of CFTR genetic and protein diversity will facilitate more equitable access to diagnosis and treatment of cystic fibrosis.

Evolutionary Genetics

Inbreeding depression and the GRIM (Genome-wide Recessive Infinitesimal Mutation) hypothesis.

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An important finding in recent years has emerged from studies comparing genomes of *Drosophila* species, confirmed by studies comparing primate species. These genomic evolutionary rate profiling (GERP) studies have shown conservation of sequences throughout the genome, including intron, inter-gene, 5' and 3' regions. This implies the deleterious nature of mutations throughout the genome, not just the much more easily identified exon mutations. On a very different level, dating from Darwin's day, inbreeding depression has been shown to be ubiquitous in outbreeding organisms. Are these two very different observations related? This question is addressed by a meta-analysis of studies measuring the fitness of chromosome homozygotes in *D. melanogaster* using the Balancer

Equilibration (BE) test. These experiments show substantial differences between chromosomes but indicate that no chromosome in homozygous condition has anything approaching wild type fitness. A key unknown in deciding whether deleterious mutation can explain this low fitness is the question of dominance (or recessiveness). Deleterious mutation can only explain low homozygous fitness if the mutations are at least partly recessive. Is it possible that low impact mutations are closer to completely recessive than currently believed?

Why is facultative parthenogenesis uncommon?

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Sexual reproduction and recombination confer long-term genetic advantages over asexual reproduction. For some species, like the Australian walking stick, females can reproduce sexually or, if they do not mate, asexually. This strategy of facultative parthenogenesis enjoys the best of both worlds: females can combine their genes with males to produce offspring or they can reproduce without mating and immediately receive the 2-fold advantage of asexual reproduction. Given these advantages, it is puzzling that facultative parthenogenesis is not widespread among diploids. It is possible that developmental constraints limit the rate at which this ability arises. However, it has evolved a number of times across a large number of taxa. Why then has it failed to replace obligatory sexual reproduction? One explanation invokes sexual conflict and selection for "male coercion". Here, we explore a more parsimonious explanation. By developing a population genetic model, we show that the invasion of facultative parthenogenesis is hindered by a combination of dispersal and genetic drift.

Global, asynchronous partial sweeps at multiple insecticide resistance genes in *Aedes aegypti* mosquitoes

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Aedes aegypti (yellow fever mosquito) is a globally invasive pest that confers most of the world's dengue burden. Insecticide-based management has led to the evolution of insecticide resistance in this species, though the genetic architecture and geographical spread of resistance remains incompletely understood. This study investigates partial selective sweeps at resistance genes on two chromosomes and characterises their spread across populations. We identified three sweeps at the voltage-sensitive sodium channel gene (VSSC) on chromosome 3, each corresponding to a single nucleotide substitution including two at the same nucleotide position (F1534C) that have evolved and spread independently. We also identified partial sweeps at a second locus on chromosome 2. This locus contained 15 glutathione S-transferase (GST) epsilon class genes with significant copy number variation among populations and where three distinct genetic backgrounds have spread across the Indo-Pacific region, the Americas, and Australia. Local geographical patterns and linkage

networks indicate VSSC and GST backgrounds probably spread at different times and interact locally with different genes to produce resistance phenotypes. These findings highlight the rapid spread of resistance genes globally and are evidence for the critical importance of GST genes in resistance evolution.

Exploring the role of mobile genetic elements in shaping plant–bacterial interactions for sustainable agriculture and ecosystem health

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Integrons are genetic systems that contribute to bacterial genome evolution via their capacity to capture and express mobile genes cassettes conferring diverse functions. Integrons are extensively studied for their role in spreading antibiotic resistance genes among human pathogens. They have been identified in many bacterial lineages across varied ecosystems but there has been little investigation in plant-associated bacteria. Bacteria and plants have complex, dynamic relationships that influence plant health and productivity. To investigate whether integrons contribute to adaptative processes in plant microbiomes, we examined gene cassette and microbial taxonomic profiles in rhizoplanes of four important crop species grown under controlled glasshouse conditions. We identified 38,546 unique gene cassettes, including elements carrying genes associated with antibiotic resistance, type II toxin–antitoxin systems and genes with putative functions associated with plant growth promotion, along with a larger set encoding genes of unknown functions. We show that rhizoplane microbiomes of different plant species exhibit more similarity in their community composition profiles than in their gene cassette profiles, with complex and distinct suites of gene cassettes associated with each plant species, suggesting that gene cassettes might have a role in establishing and maintaining rhizoplane communities.

Chromosome level genome of Blue-tongue virus vector, *Culicoides brevitarsis*

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Biting midges (*Culicoides* spp) are hematophagous insects, which are proven vectors of several arboviruses of medical and veterinary importance. Recent emergence of several *Culicoides*-transmitted diseases necessitates developing high resolution genomic resources to determine their vector competencies and disease transmission potentials. In response to this problem, as a WOAH reference laboratory for bluetongue virus (BTV), we have assembled the chromosome-level genome of major bluetongue vector of Australia, *Culicoides brevitarsis*. We used state-of-the-art Oxford nanopore based long-read and Illumina based short-read sequencing technologies and scaffolded the genome with Hi-C. Gene models were supported by high-coverage RNAseq data. The genome (2n=6) is 130Mb in size, with scaffold N50 and L50 statistics of 43 Mb and 2 respectively, covering 11,708 genes and >91.5% dipteran BUSCO completeness. Our ortholog-based search has identified several immune and antiviral genes, which may confer BTV competency in other *Culicoides* species. We shall present genome-wide comprehensive analysis by comparing our genome with nearby mosquito, sand-fly and vinegar-fly taxa, to underpin evolution of important gene families within the species. We shall discuss our findings; ongoing vector competency works for new emerging diseases i.e. African Horse Sickness Virus, challenges, and community engagements to work this notorious pest species.

Poster section

Genetics and Medicine

01 - Ancestry and Paternity Testing in the Northernmost Region of South America: Bogotá's Genetic Heterogeneity.

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Over the past decade, the rise in disputed paternities in Colombia has involved at least 65,400 fathers of diverse ethnic backgrounds and ancestries. This study examines the sensitivity of Paternity Index (PI) and Probability of Paternity (W) to the selection of the population's STR genetic database. It was found that while the results of paternity exclusion and inclusion do not differ between populations, numerical values vary significantly, up to three orders of magnitude, depending on the reference population. Furthermore, populations with higher conservatism require adjustments in minimum allele frequencies to obtain accurate PI. The study investigates how variance in PI and W estimators is affected by different reference populations, utilizing 1797 Colombian trio cases and 5 reference populations of indigenous, Afro-descendant, and mixed ancestry. A larger sample size was observed to maintain a constant number of effective alleles, resulting in higher PIs. Differences in PI and W values when employing reference populations with different ethnic origins and sample sizes

allow expressing paternity test results as confidence intervals. Future research is suggested to delve deeper using reference populations with larger sample sizes and consistent genetic markers to assess how the number of markers affects PI and W values.

02 - The Description of Genetic Variability in Blood Group Genes to Assist Evidence-Based Transfusion Medicine

Sarah F. Jackson[1], Hardip Patel[1]

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In Australia, 1 in 3 people will require blood transfusions across their lifetime. For these procedures, matching donor and recipient blood types is essential to prevent adverse reactions which may cause illness or in some cases, death. Severe responses occur due to alloimmunisation, a process in which recipient antibody proteins recognise foreign red blood cell surface antigens from the donor and subsequently destroy them. To prevent this, blood matching generally via serological tests is performed prior to blood transfusions. There are currently 45 blood groups recognised by the International Society of Blood Transfusions (ISBT) broken down into over 300 blood type alleles with new groups, types and subgroups identified every year. Newly discovered or previously uncharacterised variants may not be detectable using existing serological methods, potentially resulting in misidentification. This study aims to characterise genetic variability for all blood group systems using publicly available datasets such as gnomAD and the 1000 Genomes Project. Haplotypes will be developed to assess the number and frequency of blood type variations and will be compared across datasets and population groups. Results of this investigation will provide a platform for the development of more accurate serological tests to improve future blood transfusion therapies.

Ecological Genetics

03 - Investigating the influence of predation cues on SOCS3 expression and DNA methylation in cane toad

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The effects of predation cue exposure on the morphology and behaviour of larval amphibians are well characterised. However, epigenetic responses to these cues are less well understood. Suppressor of Cytokine Signalling 3 (SOCS3) regulates the inflammatory response and DNA methylation levels of this gene have been linked to body mass index in other taxa. SOCS3 displays strong DNA methylation changes in cane toads (*Rhinella marina*) in response to predation cues. Because DNA methylation levels can affect gene expression, this project aims to characterise DNA methylation matched with expression level of SOCS3. Cane toad tadpoles were raised in a common-garden experiment and full-siblings were either exposed to predation cues or a sham treatment (controls). We will quantify DNA methylation of SOCS3 using targeted bisulfite sequencing and expression levels of SOCS3 using RT-qPCR. This research will determine whether previously observed changes in DNA methylation following exposure to predation cues are correlated with expression of SOCS3, providing key insights into the function of DNA methylation, and the interaction of environment and gene activity in amphibians. Moreover, this research will be an important step forward in addressing the lingering question of how invasive species adapt to new environments despite limited genetic diversity.

04 - Bird Brained: Effect of lead pollution on vision and cognition in wild sparrows

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Lead is a systemic heavy metal toxin associated with health deficits and cognitive impairment in humans. However, the sub-lethal impacts of lead on wild animals are poorly understood. Here, we studied house sparrows in the lead mining town of Broken Hill, NSW, where the blood lead levels for sparrows in some areas far exceed World Health Organisation guidelines. House sparrows have small home ranges, so their blood lead level reflects the level of pollution in their immediate environment, allowing birds from high and low lead areas within Broken Hill to be compared. This project assessed the effects of chronic lead exposure on cognition and visual performance. We assessed behaviour using a range of visual based cognitive tests and paired this with differential gene expression in the brain and eye. Results suggest that lead contamination has minimal impacts on cognitive and visual behaviour in these birds, and they may have adaptive mechanisms to cope with high lead levels. This study sheds light on the impacts of lead pollution in urbanised environments, with important considerations for wildlife health and contaminated land remediation.

05 - Identification of olfactory receptor genes in the highly invasive Eastern mosquitofish (*Gambusia holbrooki*)

Louise Tosetto, Nathan Hart

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The Eastern mosquitofish (*Gambusia holbrooki*) is a highly pervasive invasive freshwater fish that competes for food and resources with many native aquatic animals. Invasive mosquitofish may use chemical cues (pheromones) as sex signals, and there is potential to use these olfactory attractants for control. However, there very little is known of the olfactory receptors used in cue detection, particularly in teleost fish. Through the construction of olfactory transcriptomes, we first identified the olfactory genes of this highly invasive species. We then identified differences in olfactory receptors in male and female fish during breeding through differential gene expression analysis. This project provides information on the olfactory receptor genes present in the eastern mosquitofish revealing genes that may play a role in pheromone cue detection. Taking a genetic approach and assessing the molecular mechanisms driving olfactory driven behaviours has not been widely done. These findings lay the foundation for further research into molecular mechanisms for cue detection in *Gambusia holbrooki* and invasive fish more broadly.

06 - Assessing the potential to control the Australian sheep blowfly using *Wolbachia*

Matthew Lyons, Laura Wines and Simon Baxter

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Wolbachia are maternally inherited bacterial endosymbionts found in approximately half of all insect species and can impose several fitness costs on their hosts. Some *Wolbachia* strains increase their transmission and spread through insect populations via cytoplasmic incompatibility, causing a bias in reproductive success. These features can potentially be used to control insect pest populations by intentional release of insects infected with specific *Wolbachia* strains. This project focuses on the potential for *Wolbachia* to control the Australian sheep blowfly, *Lucilia cuprina*, a serious sheep ectoparasite and complex pest to control. 450 blowflies from seventy populations across Australia were screened for *Wolbachia* using diagnostic PCR primers. *Wolbachia* was absent from all samples, indicating the potential to infect and release novel endosymbionts into naïve populations. Next, approximately 3000 embryos were microinjected with the wMel *Wolbachia* isolate from *Drosophila melanogaster*, which was previously shown to cause strong cytoplasmic incompatibility in *Aedes* mosquitoes. Despite infection persisting in injected individuals, females showed poor fecundity and *Wolbachia* failed to transmit between generations. Microinjection of alternative *Wolbachia* strains or different endosymbiotic species could be performed to develop insect strains for area-wide pest management strategies.

07 - Tracking the source population of Simulium blackfly invasion in urban settings in Ghana through population genomics

Millicent Opoku[1], Neha Sirwani[1], Emily N. Hendrickson[1], Himal Shrestha[1,4], Kwadwo K. Frempong[2], Sampson Otoo[2], Franklin Ayisi[3], Millicent S. Afatodzie[2], Abena Nyarko[2],

Sarah M. Dogbe[2], Sellase Pi-Bansa[2], Joseph Harold Nyarko Osei[2], Sindew M. Feleke[1], Warwick Grant[1], Daniel Boakye[2] and Shannon M. Hedtke[1]

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Simulium blackflies of the damnosum species complex exhibit long flight ranges of 20 to 600km when assisted by wind. They are vectors of the parasitic nematode that causes human onchocerciasis (river blindness), and characterised by severe skin lesions, irreversible blindness, and epilepsy. Extensive small-scale mining in Ghana have led to pollution of fast-flowing rivers, natural breeding habitats for Simulium. These blackflies are likely to migrate in search of suitable breeding habitats. Reports of blackflies in parts of the capital city, Accra in June 2023, where blackflies had not previously been found, warranted prompt investigations. We collected 270 female adult blackflies by human landing catch (HLC) from 14 communities in Ghana. Based on principal components analysis (PCA) of 138,128 SNPs from Whole genome sequences, blackflies from the Volta region (Elavayno, n=10; Holuta, n=10) were genetically distinct from those collected from Accra (Paradise Valley, n=10; Teiman Borga Town, n=9), Eastern regions (Asuoyaa, n=14), and elsewhere in Ghana. A PCA and k-means clustering of these distinct groups showed that, blackflies from Accra exhibited greater genetic similarity to those from the Eastern region. This implies a potential origin in southeastern Ghana, warranting further investigation into the corridors for movement and risk of onchocerciasis transmission.

08 - Evolutionary Rescue in Ecotonal Landscapes: Genome-Environment Links under Climate Change in Neotropical Lizards *Ameiva ameiva*

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The impact of climate change on biodiversity is often analyzed under a stable evolutionary perspective, focusing on species' current tolerance to warmer climates. However, species can adapt, especially in low-fragmentation habitats where adaptive genetic variation spreads across populations, increasing the potential for evolutionary rescue. Our study integrates genomic data, niche modeling, and landscape ecology to predict range shifts and evolutionary rescue potential in the Neotropical lizard, *Ameiva ameiva*. We sampled 266 individuals of A. ameiva from the Amazon, Cerrado, and Atlantic Forest biomes, generating a genomic matrix of 40,000 SNPs. Using genome-environment association analyses, we aim to identify loci under environmental selection while accounting for neutral genetic variation. We will model the distribution of genotypes adapted to

different climatic conditions and predict range shifts under future climate scenarios, incorporating dispersal constraints. Our study seeks to elucidate key climatic and environmental drivers of selection, assess genomic vulnerability, and understand the adaptive potential of *A. ameiva*. By combining molecular markers, paleoclimate data, and future bioclimatic variables, this research will support conservation strategies aimed at preserving biodiversity amidst rapid environmental changes. Understanding the potential for evolutionary rescue in *A. ameiva* will provide insights into maintaining ecological resilience and biodiversity.

Conservation Genetics

09 - Conservation genomics of Sharman's rock-wallaby, *Petrogale sharmani* in north-east Queensland

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Sharman's rock-wallaby, *Petrogale sharmani*, is a narrow range endemic species, restricted to a small area of north-east Queensland, adjacent to the Wet Tropics World Heritage area. Due to its small distribution (~200,000 ha) and a series of threats it is currently listed as "Vulnerable" under the federal EPBC Act. Much of its distribution is managed for cattle grazing and its tropical woodland habitat is largely intact. The species is currently known from ~50 sites but the degree of connectivity amongst sites is unknown. Here we report of DaRTseq analysis (DNA SNPs) of ~70 individuals sampled from 12 sites throughout the known distribution of the species. Preliminary analysis indicates limited differentiation across the sampled distribution.

Evolutionary Genetics

10 - Genes under selection due to Batrachochytrium dendrobatidis in the alpine tree frog (*Litoria verreauxii alpina*)

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With an ever-growing number of amphibian species being affected by *Batrachochytrium dendrobatidis* (Bd), research into this parasitic fungus and how it impacts amphibian populations is crucial for the preservation of the taxa. Our understanding of Bd and its interaction with amphibians has increased with much of the focus on the distribution and limitations of the fungus itself. The alpine tree frog (*Litoria verreauxii alpina*) has shown no resistance to Bd, yet populations are able to persist despite an almost 100% infection rate in adults and a low infection rate in juveniles. Therefore, recruitment of new individuals plays an important role in maintaining population levels. With the decrease in generational length and increase in selection for reproduction caused by Bd, this makes them a suitable candidate to observe selection at an accelerated rate. Samples were collected from the field in 2013 and 2018 from populations that were both pre and post Bd introduction, some of which were Bd endemic for >25 years. These samples were then sequenced using high-coverage DArTseq™ in order to locate loci under selection. With this data, we expect to find genes under positive selection due to endemic Bd.

11 - Investigating the embryonic transcriptome of the Pot-belly Seahorse (*Hippocampus abdominalis*)

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The Syngnathidae is a family of fishes comprising the seahorses, seadragons and pipefishes. They are unique in their morphology, behaviour, and most famously, their male pregnancy, an exceptional trait among vertebrates. This unique male pregnancy has made these fish popular models for the study of convergent evolution, comparative biology, and sex role-reversal, as it provides the opportunity to study an independently evolved and physiologically complex pregnancy. To date, all transcriptome studies focused on reproduction in syngnathids have focused on the gestational tissue (brood pouch) of the pregnant father. In this study we aim to analyse the transcriptome of embryonic pot-belly seahorses (*Hippocampus abdominalis*) at three time points in their development, in order to analyse changes in gene expression levels through development. This work will shed light on the physiological relationship between seahorse father and young during gestation.

12 - Mutation rate and selection in the unusual duplicated mitogenomes of Australasian stingless bees (Apidae: Meliponini)

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The overall architecture of the mitochondrial genome (mitogenome) is highly conserved, particularly in animals. However, there are exceptions: the mitogenomes of the Indo-Malay/Australasian (IM/AA) clade of stingless bees (Apidae: Meliponini) were recently discovered to exhibit long inverted repeats. Depending on the species, these mitogenomes contain two identical copies of either all, or some, mitochondrial genes. Here, we investigated the ratio of mitochondrial to nuclear mutation rate and selection pressures experienced by the amphimeric mitogenomes found in the IM/AA clade, in comparison to related stingless bees, in order to investigate links between mutation rate and the amphimeric mitogenome structure. This analysis was conducted in silico using assembled mitochondrial and nuclear genes from species taken from across the three main clades of Meliponini, with the use of several newly sequenced mitogenomes. Given the potential for mitogenome structure and mutation rate to impact co-evolved nuclear genes, we examined the possibility of mito-nuclear co-evolution in the stingless bees and the role it may have played in divergence within the IM/AA clade. Through this comparative examination of stingless bee genomes (both mitochondrial and nuclear), we contribute to an increased understanding of mito-nuclear co-evolution and speciation of the Australasian stingless bees and beyond.

13 - Macroevolutionary insight into the role of introgression analysis in speciation of Australasian rodents

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Our understanding of the evolutionary processes that drive speciation and adaptation continues to grow as more studies cover diverse model systems. Introgression has been recognised to have a role in the dynamics of the divergence history of varied species. Here I explore the role of introgression in the divergence of native rodent murines in Australia and New Guinea. They are an excellent model system to examine processes of divergence due to their rapid speciation dated to the late Miocene (~5-10 Ma), chromosome variation, and widespread distribution across diverse environments. Using exon capture and whole exome data sets, I will assess the discordance between gene and species trees, and then use the D-statistic (ABBA BABA test) to evaluate the past gene flow between species at a macroevolutionary scale. I will also evaluate the trends of speciation related to biome adaptation and relatedness between clades. The outcomes of this study will help to provide new insights into the role of introgression in speciation processes.

14 - Exploring Feeding Habits and Genomic Patterns of Divergence in two subspecies of the Australian sheep blowfly

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The Australian sheep blowfly, *Lucilia cuprina dorsalis*, is a facultative ectoparasites whose larvae feed on the living tissues of domestic sheep in agricultural regions. A closely related subspecies, *Lucilia c. cuprina*, is only common in urban areas and may lack parasitic behaviour. Here we investigate genetic and behavioral aspects underlying the trophic adaptations in the two subspecies and aim to identify genes and genomic regions that contribute to the physiological diversification and feeding habits. Behavioral assays were conducted to explore larval feeding preferences which involved measuring the larval response to different diets (rotten and fresh meat) at two temperatures (33ºC and 25ºC). These assays, along with comparative genomic analysis, will shed light on potential differences in feeding behaviour between the Australian subspecies. These findings will contribute to our understanding of the genetic mechanisms driving trophic specialization and the evolutionary processes involved in the speciation of *Lucilia cuprina*.

15 - Identifying and Exploring Sex Chromosomes in Australian Skinks: Comparative Analysis and Synteny Mapping

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Skinks are a diverse group of lizards belonging to the family scincidae. They are known for their sleek and typically elongated body with short limbs. While their external appearance varies widely, skinks generally have a poorly differentiated XX/XY sex chromosome system where the X and Y are cytogenetically indistinguishable. The Australian Amphibian and Reptile Genomic Initiative (AusARG) recently made genomic resources, including chromosome-level genome assemblies and gene annotations, available for 5 Australian skink species. This has provided us with a unique opportunity to identify sex chromosome in the genome assemblies of these species, allowing identification of sex chromosomes synteny. We successfully identified X and Y chromosome scaffolds in four out of the five new skink genomes using genomic sequencing read depth and Y-

enriched kmers. In *Lampropholis delicata*, no obvious X or Y were found in the assembly, suggesting a sex chromosome system turnover. From our comparative analysis, we found that majority of the skink species in our study have poorly differentiated XY chromosomes, except for *Bassiana duperreyi* and *Pseudemoia entrecasteauxii*, which have differentiated X and Y chromosomes.

Genetics of quantitative traits

16 - Investigating the genetic and biochemical basis of *Anigozanthos* flower colour

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This study aims to characterize biochemical and genetic basis of flower colour in the *Anigozanthos* (kangaroo paw) genus, including hybrids important to the horticulture industry. Flower colour is a key phenotype shown to be an adaptive trait in many species and is considered relatively simple for genetic and biochemical analysis. Colour is also a very important trait for the horticulture industry. The recent development of a blue-flowered kangaroo paw by Kings Park and Botanic Gardens has led to increased interest in understanding the genetic architecture of these uniquely coloured flowers. To understand the biochemical variability of floral pigments in *Anigozanthos*, we collected over 200 flower samples representing each of the species and subspecies from across WA. Both wild and cultivated samples are undergoing quantitative phenotypic and biochemical analysis (HPLC-MS) to get a clear picture of the floral pigments present in the genus. The genes contributing to the novel blue colour will be mapped using reduced-representation genome sequencing of an F2 population similar to the one which produced the first blue-flowered individual. The results of this project will aid future breeding and conservation efforts of kangaroo paw and may provide a road map to conserving other native WA species.

Bioinformatics and Genomics

17 - Insights into platypus venom composition

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Amongst the thousands of venomous taxa, there are only 15 venomous mammalian species worldwide, including Australia's own platypus. Despite this, little is known about platypus venom composition, and previous research over a decade ago was limited by genome quality. Utilising the new high-quality platypus genome and a suite of publicly available RNA-seq datasets comprising of nine tissue types and over 50 samples, this study capitalises on 15 years of 'omics resources to provide a comprehensive characterisation of the platypus crural (venom) gland to identify genes associated with venom in this iconic species. Using transcriptomics, we have identified over 5000 upregulated genes in the crural gland, with over 150 having expression highly specific to the crural gland. Amongst the highly expressed crural gland-specific genes are a hyaluronidase, a tissue factor pathway inhibitor, and kallikrein-like serine proteases which are also present in other venomous mammals. 20% of the identified genes are previously uncharacterised and not similar to any known protein in curated databases such as UniProt. This study highlights the value of improved genome assemblies for venom discovery and has leveraged a high-quality genome and large RNA-seq library to improve our understanding of platypus venom composition and their venom system more broadly.

18 - Establishing Genomics Infrastructure for Australian Researchers

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Genomics is increasingly employed across research and industry. However, keeping pace with the management, analysis and publication of genomic data is a global challenge. Since 2020, Australian BioCommons has developed community-scale digital capacity, training, and bioinformatics infrastructure to support Australia's life scientists. By engaging with the national community of practitioners, we have distilled their roadblocks and needs, and created Infrastructure Roadmaps that describe a blueprint to resolve these challenges. As a result, we have established a number of computational systems and services, including: The Australian Reference Genome Atlas (ARGA, arga.org.au/), a data discovery platform that indexes genomic data from Australian-relevant species. The Galaxy Australia Genome Lab (genome.usegalaxy.org.au/), a user-friendly web-based, data analysis platform with access to pre-installed tools, workflows and training. Fully tested workflows sourced from global initiatives, such as the Vertebrate Genomes Project, which are made available with How-to Guides for use on Genome Lab. The Australian Apollo Service, (apollo-portal.genome.edu.au/), a fully supported manual curation, visualisation, and collaborative genome annotation service. All BioCommons services cater to a variety of expertise levels with dedicated user support, are delivered collaboratively through partnerships with infrastructure and informatics providers and are fully subsidised for Australian-based researchers.

Genetic and Epigenetic Regulation

19 - Sex-specific epigenetic profiling of a monotreme

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Monotremes are egg-laying mammals native to Australia and New Guinea. Extant members of this group include four species of echidna, and the platypus *Ornithorhynchus anatinus*. Monotremes are the most distantly related mammals to eutherians, and the least understood mammalian clade. When contrasted with eutherians and marsupials, monotreme epigenetics remain somewhat unexplored. To address this, we used high-throughput chromosome conformation capture (Hi-C) and chromatin immunoprecipitation followed by sequencing (ChIP-seq) to investigate both genome structure and epigenetic features in male and female platypus. Hi-C data allowed us to compare intra- and inter-chromosomal interactions between the sexes. Using ChIP-seq we quantified the distribution and abundance of several histone modifications, both at transcription start sites (TSSs) and on a whole chromosome level. We uncovered notable differences between male and female platypus, suggesting that genome structure and histone modification patterns are sex-dependent in monotremes. To date, this is a phenomenon not observed in other mammals. These sex-specific disparities may relate to mechanisms of monotreme dosage compensation, which are yet to be thoroughly characterised.

20 - Incomplete transcriptional dosage compensation of chicken and platypus sex chromosomes is balanced by post-transcriptional compensation

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Heteromorphic sex chromosomes (XY or ZW) present problems of gene dosage imbalance between sexes and with autosomes. A need for dosage compensation was long thought to be critical in vertebrates. However, this was questioned by mRNA abundance measurements in mammals and birds. Here, we demonstrate unbalanced mRNA levels of X genes in platypus males and females that

correlate with differential loading of histone modifications. However, protein abundance ratios were 1:1 between the sexes, indicating a post-transcriptional layer of dosage compensation. We conclude that sex chromosome output is maintained in platypus via a combination of transcriptional and post-transcriptional control, consistent with the importance for sex chromosome dosage compensation.

21 - Analysis of miRNAs in the honey bee parasite, *Varroa destructor*

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The honey bee mite, *Varroa destructor*, is a major parasite of the western honey bee, *Apis mellifera*, causing colony losses and devastating the beekeeping and pollination industries after establishment. Current treatment methods are costly and inefficient, with growing resistance to chemicals reducing treatment efficacy. RNA interference (RNAi) is a promising area for novel control methods that could provide specific targeting of *V. destructor*. The endogenous small RNAs of *V. destructor* have only recently been examined, revealing both conserved and Varroa-specific miRNAs. While the function of conserved miRNAs may be assumed, based on the functions of their homologues, the function of *Varroa*-specific miRNAs and their potential as *Varroa* control targets remains unknown. This research aims to determine the function of these Varroa-specific miRNAs by treating mites with mimics and inhibitors to manipulate their expression. Sequencing and analysis of differentially expressed genes coupled with prediction tools to identify miRNA:mRNA binding sites will determine which mRNAs are regulated by *Varroa*-specific miRNAs. The expression of these miRNAs throughout mite development will also be investigated, as currently small RNA sequencing has only been done on adult mites. This research will reveal the function of Varroa-specific miRNAs and their potential as targets for Varroa treatment.

22 - The Role of Small RNAs in Honey Bee Immune Regulation

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The Western honey bee, *Apis mellifera*, suffers from a range of viral infections that can be exacerbated by infection with the global bee parasite *Varroa destructor*. Australian bees have historically been free of *Varroa* and the damaging viruses that it spreads, such as Deformed wing virus (DWV). *Varroa* invaded NSW on 22nd June 2022, providing a final opportunity to investigate the genetic impact of bee viruses in isolation in the remaining *Varroa* naïve honey bee populations. This project explores the role of small RNAs (siRNAs and miRNAs) in honey bee immune regulation during experimental viral infections. We will perform small RNA and mRNA transcriptome sequencing to determine whether honey bee pupae injected with DWV and a local virus, Black queen cell virus (BQCV) possess differentially expressed miRNAs and whether these miRNAs regulate the expression of honey bee genes involved in immunity. Additionally, this project will investigate the antiviral siRNA response within honey bees, specifically the relationship between viral load and siRNA viral degradation within the pupae. The outcomes from this project will increase our scientific

understanding of honey bee immunity during DWV-A, DWV-B and BQCV infections in *Varroa* naïve honey bees, which is critical for understanding bee biology.

23 - Short Direct Repeats-mediated splicing of the candidate master sex determining gene nr5a1 in the dragon lizard *Pogona vitticeps*

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The Australian central bearded dragon *Pogona vitticeps* has ZZ male: ZW female sex chromosomes while high temperature can override the genetic influence, causing male-to-female sex reversal. The mechanisms of interchange of genetic and temperature dependent sex determination remains unknown. Nr5a1, a putative master sex determining gene for the dragon resides on both Z and W chromosomes, produces three splicing isoforms in ZZ male gonad, and sixteen splicing isoforms in ZW female gonad, most of which would translate into truncated polypeptides with altered function or could act as a competitive inhibitor to the intact protein. These noncanonical isoforms contain pairs of short direct repeats (SDR) of 2-5 nucleotides in both the 5' and 3'excision sites of individual isoforms and only one copy of the repeats remains in the junction after excision and jointing. The splicing sites occurred within C- and G-rich microsatellite tandem repeats (STR region), which is predicted to form stem and loop structure, bridging the splicing sites into approximately. In addition, the SDR sites overlap or border to binding motifs for RNA splicing factors, which express differentially in two sexes. These results suggest interactions of pre-mRNA structure and RNA splicing factors are implicated in the SDR-mediated splicing of nr5a1.

Synthetic Biology

24 - Promoting Toxic Masculinity

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Vector borne illnesses make up 17% of infectious disease globally, with the *Aedes aegypti* mosquito being responsible for the spread of zika virus, chikungunya, and dengue fever — with an excess of 90,000,000 annual cases. Population control strategies such as sterile insect technique (SIT) and female-specific insects carrying dominant lethal (fsRIDL), function intergenerationally — suppressing future mosquito generations once released — thus allowing for the initial population to continue to spread disease before any population decline is observed. A new methodology, toxic male technique (TMT), utilizes tissue-specific expression of ion-channel venom peptides within insect seminal fluid, transferred from males to females via mating, thereby acting intragenerationally.

Modelling indicates a more rapid population suppression is attained with TMT compared to current techniques, and crucially, biting rates are quickly curtailed. Designing a TMT system within *Ae. aegypti* requires the characterization of promoters specific to the adult male accessory glands (MAG, organs of the mosquito reproductive tract responsible for the production and secretion of seminal fluid proteins), for venom expression, and subsequent transfer from male to female through mating. In this poster presentation I will discuss the characterization of several MAG specific promoters within Ae. aegypti.

Insights and outcomes from biodiversity and agricultural genomics

25 - Metabolic effect of raspberry ketone (RK) ingestion in *Bactrocera tryoni*: A comprehensive approach through tissue-specific transcriptomics and proteomics

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Mature Queensland fruit fly (Qfly) males are pollinators of plants producing raspberry ketone (RK), and actively feed on RK to boost their sexual performance. This study explores the metabolic effects of RK ingestion on Qfly males, focusing on molecular changes. In insects, reproductive investment often trades off with immune defense; RK-fed flies show increased reproductive activity potentially at the expense of their immune system. The research has two objectives 1)To analyze the metabolic impact of force-feeding RK to sexually immature Qfly males by conducting transcriptomic analysis of reproductive organs (accessory glands, testes, apodeme, rectal gland, and carcasses) on days 3, 6, 9, and 12. 2)To examine the metabolic effects of RK ingestion in mature Qfly males, particularly how it interacts with mating and immune responses. This involves a) Transcriptomic and proteomic analysis of reproductive organs, fat body, and carcasses after RK ingestion and mating. b) Transcriptomic and proteomic analysis of the fat body and carcasses following PBS injection. c) Proteomics analysis of hemolymph at intervals (6, 15, 17, 20, 23 days) after an immune challenge with *Serratia marcescens*. Studying tissues-specific multi-omics will provide a comprehensive understanding of RK's metabolic impacts and elucidate the molecular basis of potential trade-offs between reproductive and immune functions.

26 - Transgenics for control of stored grains pests

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This research project aims to elucidate the genetic mechanisms by which Coleopteran pest *Tribolium castaneum* has become resistant to the fumigant phosphine. This species infests stored grain of all types, endangering food security and cutting into farmer's profits on a global scale. Understanding and confirming which genes significantly impact phosphine resistance phenotype will allow agriculturalists to build better integrated pest management strategies for the control of *T. castaneum*. Here we report CRISPR mutagenesis of the rph1 gene and some P450 genes that have previously been associated with phosphine resistance. Additionally, this project seeks to improve the genetic tractability of this species by honing techniques for genetic manipulation and eventually lead to the implementation of genetic pest control methods (for example, gene drives). The implementation of genetic control methods requires a base understanding of the mechanisms shaping standing genetic variation and gene flow in a target population. Therefore, we also report our preliminary experiments and plans for future genomic analyses of beetles collected from Australian silos.