Genetics Society of AustralAsia 2010

Annual Conference Canberra 4-8 July

Institute for Applied Ecology

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Sunday 4th July	Monday 5th July			Tuesday 6th July			Wednesday 7th July			Thursday 8th July	
	Time	Discovery	PI Lecture Theatre	Time	Discovery	PI Lecture Theatre	Time	Discovery	PI Lecture Theatre		Discovery
AM	8.00-9.00	Registration		8.00-9.00	Registration		8.00-9.00	Registration			
	9.00-9.15	Welcome: Jeremy Burdon									
	9.15-10.15	Keynote Speaker John Thompson		9.00-10.00	Keynote Speaker John Pannell		9.00-10.00	Keynote Speaker Rachel O'Neill		9.30-10.00	Coffee from 9.30am
	Track	Evolutionary genetics: insects Chair: Rowell	Comparative genomics: reptiles Chair: Deakin	Track	Population genetics Chair: Miller	Human Genetics and Genomics Chair: Williams	Track	Mammalian Genetics I Chair: Ezaz	Disperal and Invasion Chair: Coates	Track	Epigenetics Chair: Williams
	10.15-10.30	Gilchrist	Ezaz	10.00-10.15	Rymer (30 min)	Paz-Filho	10.00-10.15	Deakin	Tooman	10.00-10.15	Huttley
	10.30-10.45	Brownlie	O'Meally	10.15-10.30		Ofi	10.15-10.30	Murtagh	McGraw	10.15-10.30	Ying
	10.45-11.15	Morning Tea		10.30-10.45	Peakall	Ballouz	10.30-10.45	Al Nadaf	Chong	10.30-10.45	Wafers
	11.15-11.30	Lee	Young	10.45-11.15	Morning Tea		10.45-11.15	Morning Tea		10.45-11.00	Searle
	11.30-11.45	Kemp	Georges	11.15-11.30	Byrne	Das	11.15-11.30	Livernois	Hopley	11.00-12.00	MJD White: Michael Hynes
	11.45-12.00	Newcomb	Murphy	11.30-11.45	Padovan	Ho	11.30-11.45	Miller, R	Houliston	12.00-12.30	Prize giving
	12.00-12.15	Robin	Sarre	11.45-12.00	Nemri	Feng	11.45-12.00	Wong	Elliott	12.30	Conference Close
PM				12.00-12.15	Ravensdale	White	12.00-12.15	Cheng			
	12.15-1.15	Lunch		12.15-1.15	Lunch - Committee meeting		12.15-1.15	Lunch - AGM			
	1.15-2.15	Keynote Speaker Richard Frankham		1.15-2.15	Keynote Speaker John Buckleton		1.15-2.15	Keynote Speaker Claire Wade			
	Track	Conservation genetics: general Chair: Peakall	Gene expression Chair: Easteal	Track	Emerging Technologies Chair: Huttley	Systematics and phylogeography Chair: Byrne	Track	Ross Crozier Symposium Chair: Johnson/Oldroyd	Mammalian Genetics II Chair: Belov		
	2.15-2.30	Bill Sherwin (30 min)	French	2.15-2.30	Mills	Bissett	2.15-2.30	Painter	Dobbie (30 min)		
	2.30-2.45		Vassilieva	2.30-2.45	Gardner	Gonzalez	2.30-2.45	Bromham			
	2.45-3.00	Lister	Remnant	2.45-3.00	Henderson	Krosch	2.45-3.00	Oldroyd	Lillie		
	3.00-3.15	Sinclair, J	Trevaskis	3.00-3.30	Afternoon Tea		3.00-3.15	Goudie	Blyton		
	3.15-3.45	Afternoon Tea		3.30-3.45	Begum	Bryant	3.15-3.30	Oxley	Baldwin		
	3.45-4.00	Bradman	Balasubramaniam	3.45-4.00	Hudson	Miller, J	3.30-4.00	Afternoon Tea	Afternoon Tea		
	4.00-4.15	Coates	Delaney	4.00-4.15	Gillings	Boomer	4.00-4.15	Johnson	Attard		
	4.15-4.30	Sinclair, E	Grant	*Special session: Teaching*			4.15-4.30	Dowton	Kirby		
	4.30-4.45	Whitehead	Warr	4.15-4.30	Behm		4.30-4.45	Newman	Wilton		
	4.45-5.00	Hoehn	Prodhan	4.30-4.45	Grant		4.45-5.00	Tay	MacDonald		
5-7 pm Mixer - Discovery Foyer				5.00-7.00	Poster Session		5.00-5.15	Smith			
							7.30-10.30	Conference Dinner - The Lobby			

Keynote: Monday morning

T1: Relentless Coevolutionary Dynamics

John N. Thompson
Department of Ecology and Evolutionary Biology, University of California, Santa Cruz CA, USA

Coevolution among species is a pervasive process. All complex organisms require coevolved interactions with other species to survive and reproduce, and many, possibly most, microbial species are involved in coevolving parasitic or mutualistic interactions. In recent years we have learned that coevolving interactions are more genetically and ecologically variable and dynamic than we previously suspected, creating constantly changing geographic mosaics of adaptation and counter–adaptation. These coevolutionary mosaics sometimes occur over surprisingly small geographic scales and arise over surprisingly short periods of time. As we alter the composition of species in all major ecosystems worldwide, we are also altering coevolutionary dynamics in ways that we are only beginning to understand.

Evolutionary genetics: insects

T2: A model system for the study of species differences: Hubert Jarvis fruit fly.

A. S. Gilchrist
Fruit Fly Research Laboratory, University of Sydney

The divergence of morphological characters in different species is the stuff of evolution. However, the fact that most species pairs wont hybridise complicates the genetic analysis of naturally evolved differences. Consequently, evo–devo studies tend to rely on comparative studies or genetic transformation. Where different species do hybridise, the genetics of the divergent trait is usually unclear, hindering the identification of loci underlying QTLs. This presentation introduces a new system for studying the genetics of species differences that overcomes these problems. The system consists of two distinct native fruit fly species that unexpectedly hybridise, allowing QTL analysis of the divergent traits. Significantly, the genetics of one divergent character (a major bristle) has been dissected in detail by developmental biologists and shown to be highly conserved. The advantages of this system will be discussed along with some initial results regarding physiological (heat tolerance) as well as morphological (bristle) divergence. This system promises to allow more efficient analysis of the genetics of naturally evolved species differences than other current model systems.

T3: Symbionts in conflict: Wolbachia and pathogen resistance in insects

J. C. Brownlie[1], *G. D. D. Hurst*[2], *A. Fenton*[2], *K. N. Johnson*[3]
[1]*School of Biomolecular and Physical Sciences, Griffith University, Brisbane, Queensland,* [2]*School of Biological Sciences, University of Liverpool, Liverpool, U.K,* [3]*School of Biological Sciences, The University of Queensland, Brisbane, Queensland*

Wolbachia fitness is intimately associated to the productivity (*i.e.* the number of offspring produced) of their host. By contrast horizontally transmitted pathogens, such as viruses or fungi, re–direct host resources away from reproduction to complete their own life cycles. This sets up a classic inter–symbiont conflict between maternally inherited symbionts such as *Wolbachia* and horizontally transmitted pathogens. Recently we showed that *Wolbachia* infected *Drosophila melanogaster* are less susceptible to viral infection when compared to *Wolbachia* free flies. This unexpected phenotype poses two broad questions–what is the mechanism of protection, and what are the broader implications of viral protection for *Wolbachia*, host and pathogen? Here I will discuss current research that is addressing both of these important questions.

T4: Mapping and characterising Bergmanns genes in Drosophila

S. F. Lee, L. Rako, J. Axford, A. Varan, A. Hoffmann
Centre for Environmental Stress and Adaptation Research (CESAR), Bio21 Institute, Genetics Department, Melbourne University

Why are animals bigger in the cold? Many theories have been proposed but still little is known about its underlying genetic factors. In Australia, wing size of *Drosophila melanogaster* and *D. simulans* increases gradually from tropical Queensland to temperate Tasmania. Comparative QTL analysis indicates that the genes influencing wing size are different in these two species. In the model organism *D. melanogaster*, an INDEL polymorphism in the promoter of Dca is associated with wing size variation in natural populations (McKechnie et al 2010). We present here our recent investigation into the patterns of polymorphism around the Dca locus, allele genealogy, cellular basis of wing size variation, and clinal expression profiling. We also describe ongoing transgenic experiments to compare promoter activities among different allelic variants.

T5: Nutrient stress exacerbates genetic variance for development in a butterfly

D. J. Kemp
Department of Biological Sciences, Macquarie University, Sydney NSW, Australia

Models of adaptive evolution are based on the premise that the different genomes spread across individuals in a population are differentially viable. By definition, individuals in possession of a genotype of high average viability (*i.e.*, "good genes") should, on average, achieve higher levels of performance in fitness–related tasks such as growth and development. However, performance may depend on the environment in a way that no one genotype is the best under all conditions. Alternatively, the relative scaling of performance may vary across environments, such that the difference among genotypes is exacerbated under some conditions. I set out to investigate these issues by rearing full sibling families of the butterfly *Eurema hecabe* under

several environments of differing nutritional quality, and examining their growth and development. The data show a striking interaction between genotype and environment. Whereas all genotypes developed relatively fast under the good and medium quality environment, only some could achieve similar rates of development under the lowest nutritional treatment. Variance in a key component of larval performance in this species is therefore exacerbated under stress, which suggests that the opportunity for natural selection to act on viability in the wild would be greatest under this environment.

T6: Selective sweeps at the insecticide resistance locus, Rop–1, have impacted variation across and beyond the α–esterase gene cluster of the Australian sheep blowfly, *Lucilia cuprina*

C. J. Rose[1,2], J. Chapman[1], S. D. G. Marshall[1], S. F. Lee[3], P. Batterham[3], H. A. Ross[2], R. D. Newcomb[1,2]
[1]The New Zealand Institute for Plant and Food Research Limited, Auckland, New Zealand, [2]School of Biological Sciences, University of Auckland, Auckland, New Zealand, [3]Department of Genetics, University of Melbourne, Melbourne, Australia

At the Rop–1 locus of the sheep blowfly, *Lucilia cuprina*, polymorphisms at two sites within the LcαE7 gene encode proteins that confer organophosphorus insecticide resistance. To assess the impact of selection at these sites on variation around LcαE7 we sequenced regions within six other genes on chromosome IV across a set of isogenic (IV) strains. High levels of linkage disequilibrium, together with low numbers of haplotypes and haplotype diversity, were observed for two genes, LcαE1 and LcαE10, both members of the same α–esterase gene cluster as LcαE7. Significant linkage disequilibrium was also observed between LcαE7 and the next closest gene AL03. Since the selective sweeps, two forms of likely returning variation were observed, including variation in a microsatellite in an intron of LcαE10 and a recombination event between LcαE7 and LcαE10. These data suggest that within the last 60 years two incomplete soft sweeps have occurred at LcαE7 that have significantly impacted variation across, and beyond, the α–esterase gene cluster.

T7: Characterizing the genetic basis of insecticide resistance using population association studies

J. Schmidt and C. Robin
Department of Genetics, The University of Melbourne

Insecticide resistance provides an opportunity to understand genetic responses to extreme selection. Most insecticide resistance studies have focussed upon single genes many of which were originally identified by linkage mapping studies. These linkage studies have generally focussed on the gene explaining the major variance in resistance between two strains. To quantify the effect of a genetic variant, in a whole population we have been using an association study design, in which a large population of insects is screened at a dose of insecticide, and the frequency of a putative resistance alleles is compared between those that survive and those that die. In the case of DDT resistance in Drosophila melanogaster association studies confirm that the Cyp6g1 locus has undergone multiple adaptive allelic substitutions since DDT was first used in the mid 1940s. A recently derived allele, found at high frequencies in Northern Queensland, but which is at very low frequencies in other populations in the world, can explain a large fraction of the phenotypic variance within populations. Thus genome wide association studies offer a new and powerful way to quantify the number of genes that contribute to insecticide resistance, their individual phenotypic effects and population frequencies.

Comparative genomics: reptiles

T8: Sex in lizards: multiple transitions and independent evolution of sex chromosomes

T. Ezaz, A. Georges, A. Quinn, D. O'Meally, J. A. Marshall Graves, T. Gamble, S. Sarre
Institute for Applied Ecology, University of Canberra,Research School of Biology, Australian National University, Department of Genetics, Cell Biology and Development, University of Minnesota

Lizards epitomize the variability of sex determining modes and sex chromosome systems among amniotic vertebrates. These include genotypic sex determination (GSD) with male (XX/XY) and female (ZZ/ ZW) heterogamety; and temperature–dependent sex determination (TSD). In particular, the distribution of sex determining mechanisms shows no clear phylogenetic segregation among lizards. This implies that there have been multiple transitions between TSD and GSD, and between XY and ZW sex chromosome systems. Using molecular cytogenetic tools and comparative genomic analyses, we have shown that transitions between sex chromosomes and sex determining modes have occurred multiple times in Australian lizards . Our study revealed that ZW sex chromosomes have evolved independently at least twice in Australian dragon lizards (Agamidae). In addition, the gene content of ZW sex chromosomes of Australian agamids does not show homology to the ZW sex chromosomes of snakes, chicken or geckos.

T9: Snakes and ladders: the evolution of amniote sex chromosomes

D. O'Meally[1,2], S. Sarre[2], A. Georges[2], J. A. Marshall Graves[1], T. Ezaz[1,2]
[1]Comparative Genomics Group, Research School of Biology, ANU, Canberra, [2]Institute for Applied Ecology, University of Canberra, Canberra

Several recent studies have produced comparative maps of genes on vertebrate sex chromosomes. These have revealed tantalising patterns in linkage homology across lineages as different as mammals and lizards. We examine the evidence for processes that may explain some seemingly contradictory patterns. For example, the apparent functional stability of the

chicken Z chromosome, which shares linkage homology with the sex chromosomes of monotremes, birds, geckoes and perhaps turtles, is a striking example of conservatism in genome organization. An alternative explanation could be that sex chromosomes are "recycled" because some chromosomes are predisposed to that role. In other lineages, such as snakes and therian mammals, well–conserved but independently evolved sex chromosome systems have arisen. Among lizards, novel sex chromosomes appear frequently, even in congeneric species. We suggest that evolution of alternate sex determination mechanisms and de novo sex chromosomes can act as radicalising forces in genome organisation.

T10: Understanding genome evolution–Characterising the chromosomes of the dragon lizard *Pogona vitticeps* (Agamidae)

M. J. Young, D. O'Meally, A. Georges, J. Ward and T. Ezaz
Institute for Applied Ecology, University of Canberra, ACT

Comparative genomic analyses, including mapping of functional genes are powerful methods for understanding genome evolution among a diverse range of vertebrates. For example, comparative mapping among the butterfly lizard *Leiolepis reevesii rubritaeniata* and the Japanese four-striped rat snake *Elaphe quadrivirgata* has identified homology and mechanisms of genome evolution among squamate reptiles. Also, comparative mapping of a sex chromosome specific marker from the central bearded dragon, *Pogona vitticeps* among closely related dragon lizards has shown that sex chromosomes are highly labile within this group, having evolved multiple times over a short evolutionary period. *P. vitticeps* is emerging as a promising reptilian model species owing to the availability of genomic resources, including a Bacterial Artificial Chromosome (BAC) library. In this study, fluorescence in situ hybridisation (FISH) was used to develop a BAC anchored physical map of the *P. vitticeps* genome. In addition, we will end–sequence selected BAC clones from across the *P. vitticeps* genome. Together with physical mapping, these sequences will be used to develop a comparative map among vertebrates to understand genome evolution and organisation not only in reptiles but also in other vertebrate orders.

T11: Are Reptiles Predisposed to Temperature–Dependent Sex Determination?

A. Georges, T. Ezaz. A.E. Quinn, S.D. Sarre
Institute for Applied Ecology, University of Canberra, ACT, Australia Sex determination in mammals and birds is extraordinarily conservative comparison compared to that of reptiles, amphibians and fish. Reptiles in particular show an astonishing array of sex determining mechanisms, including male and female heterogamety, multiple sex chromosome systems, environmental sex determination and parthenogenesis. We suggest that reptiles are predisposed to evolving temperature-dependent sex determination (TSD) from genotypic sex determination (GSD) by virtue of the uniquely variable thermal environment experienced by their embryos. Explicit mechanisms for canalization of sexual phenotype in the face of high thermal variation during development provide a context for thermolability in sex determination at extremes, and the raw material for natural selection to move this thermolability into the developmental mainstream when there is a selective advantage to do so. Release of cryptic variation when canalization is challenged and fails at extremes may accelerate evolutionary transitions between GSD and TSD. Rapid evolutionary responses may be one response of species with TSD to gradual climate change.

T12: Maternal–fetal exchange and uterine angiogenesis in the eastern water skink *Eulamprus quoyii*

B. F. Murphy[1], *S. L. Parker*[2], *C. R. Murphy*[3], *K. Belov*[4], *M. B. Thompson*[1]
[1] *Integrative Physiology Research Group, School of Biological Sciences, University of Sydney, Sydney, Australia,* [2] *Department of Biology, Coastal Carolina University, Conway, South Carolina, USA.* [3] *Discipline of Anatomy and Histology, School of Medical Science and Bosch Institute, University of Sydney, Sydney, Australia.* [4] *Australian Wildlife Genomics Group, Faculty of Veterinary Science, University of Sydney, Sydney, NSW, Australia.*

Our research about reproduction in live–bearing lizards has revealed an exciting new connection between the evolution of live-birth and increased cancer susceptibility. While investigating how blood vessels grow in the uterus of pregnant lizards, we discovered a new type of vascular endothelial growth factor (VEGF111) that was previously found only in human cultured cells damaged by UV light. VEGF111 may make tumours very difficult to treat, because it produces a lot of blood vessels very quickly and is resistant to the bodys natural protein breakdown system. In contrast, VEGF111 appears to be very important for normal uterine vascularisation during pregnancy in the three–toed skink (*Saiphos equalis*). More recently, we described a vascular modification of the uterus in another lizard (*Eulamprus quoyii*) that appears to facilitate maternal–fetal communication during pregnancy. A vessel–dense elliptical area (VDE) on the mesometrial side of the uterus expands as the embryo grows, providing a large vascular area for physiological exchange between mother and embryo. We propose that angiogenic stimuli are exchanged between the VDE and the chorioallantois in E. quoyii to allow the simultaneous vascularisation of both tissues during pregnancy. We intend to use qRT–PCR to measure gene expression of several angiogenic growth factors and we are currently optimising primers to measure the expression of VEGF and platelet derived growth factor (PDGF).

T13: Sex determination in reptiles: evolutionary transitions along the continuum

S. Sarre, T. Ezaz, A. Quinn, D. O'Meally, J. A. Marshall Graves, A. Georges

Institute for Applied Ecology, University of Canberra, Research School of Biology, Australian National University Reptiles display remarkable diversity in sex determining mechanisms exhibiting genotypic sex determination (GSD) with male or female heterogamety and single or multiple sex chromosomes, or one of three different patterns of temperature–dependent sex determination (TSD). In our view, this diversity, and the rather ad hoc way in which they are distributed among reptile groups, represents a continuum of sex determining mechanisms rather than the dichotomy of modes often presented and is suggestive of relatively rapid transitions among forms. We have used a combination of experimental and genomic approaches to demonstrate that high incubation temperatures can reverse genotypic males (ZZ) to phenotypic females in the dragon lizard (Pogona vitticeps), which, like birds, has GSD with female heterogamety. Temperature thus overrides gene(s) involved in male differentiation in that species. Simulation modeling based on these temperature-genotypic interactions suggests that a temperature sensitive threshold dosage system for determining sex can reproduce most TSD patterns in reptiles and can also provide a simple mechanism for evolutionary transitions between sex determining modes and heterogamety. We demonstrate that transitions between mechanisms of sex determination are not only possible, but that they can occur without substantive genotypic innovation. Under our model, the W and X chromosomes, and the Y and Z chromosomes, can be homologous, and share a master sex determining gene. Thus, the physical network of genes involved in sex determination remains largely unchanged during transitions between ZZ/ZW, XX/XY and TSD systems. Our model makes possible the development of specific hypotheses of the ancestry of sex determination among groups of reptiles and charts the approach necessary to determine ancestry in any specific instance.

Keynote: Monday afternoon

T14: Predicting the Risk of Outbreeding Depression: Critical Information for Managing Fragmented Populations

Richard Frankham[1,2], *J. D. Ballou*[3], *M. D. B. Eldridge*[2], *R. C. Lacy*[4], *K. Ralls*[3], *M.R.Dudash*[5], *C.B. Fenster*[5]

[1] **Department of Biological Sciences, Macquarie University, NSW,* [2] *Australian Museum, 6 College Street, Sydney, NSW,* [3] *Center for Conservation and Evolutionary Genetics, Smithsonian Conservation Biology Institute, Smithsonian Institution, Washington, DC, USA,* [4] *Chicago Zoological Society, Brookfield, IL, USA,* [5] *Department of Biology, University of Maryland, College Park, MD, USA*

Fragmentation of populations typically leads to genetic erosion and increased risks of population extinction. Whilst these effects can be alleviated by gene flow, such action is being impeded by fears about outbreeding depression. Two major processes may cause rapid development of outbreeding depression, adaptive differentiation due to selection or fixation of chromosomal variants. Fixed chromosomal variants can be detected empirically. Based upon an extension of the breeders' equation, we predict that the risk of outbreeding depression due to adaptive differentiation between recently isolated population fragments will increase with intensity of selection, genetic diversity, effective population sizes, and number of generations of isolation. Calibration of our theory against field and laboratory data indicates that populations in similar environments must be isolated for at least thousands of generations to develop outbreeding depression. We develop a decision tree to provide practical guidance for predicting the risk of outbreeding depression, based upon the considerations above, plus questions about taxonomy and historical gene flow. If two candidate populations for enhanced gene flow are good species, or show fixed chromosomal differences, or lack gene flow in the last 500+ years, or inhabit different environments, the risk of outbreeding depression is elevated and crossing should be avoided, or only done on an experimental basis. Conversely, the risk of outbreeding depression in crosses between two populations of the same species will be low for historically connected populations with the same karyotype, isolated within the last 500 years and occupying similar environments. Retrospective evaluations indicate that our guidelines correctly identify cases with high risks of outbreeding depression. Current concerns about outbreeding depression in recently fragmented populations are almost certainly excessive.

Conservation genetics: general

T15: Entropy and Information Approaches to Genetic Diversity: Genomic Geography

W. B. Sherwin

Evolution and Ecology Research Centre, Biological Earth and Environmental Science, University of New South Wales, Sydney

This talk highlights the advantages of entropy–based measures of genetic diversity. Shannons entropy–based diversity is the standard for ecological communities. Shannons and the related "mutual information" excel in their ability to express diversity intuitively, and provide a generalised method of considering microscopic behaviour to make macroscopic predictions, under given conditions. The hierarchical nature of entropy and information allows integrated modeling of diversity along one DNA sequence, and between different sequences within and among populations, species, landscapes etc. The aim is to identify the formal connections between genetic diversity and the flow of information to and from the environment.

T16: Genetic Responses to Climate Change in the Common Brown Butterfly (*Heteronympha merope*)

A. Lister[1], *N. Murray*[1], *P. Sunnucks*[2], *M. Kearney*[3],

W. Porter[4], *M. Norgate*[2], *V. Yazgin*[1], *M. Barton*[3]
[1]*La Trobe University, Bundoora, Victoria,* [2]*Monash University, Clayton, Victoria,* [3]*The University of Melbourne, Parkville, Victoria,* [4]*The University of Wisconsin, Madison, Wisconsin, USA.*
The extent to which climate change will affect organisms depends in part on their ability to adapt to those changes. To assess and predict this ability requires an integration of molecular ecology, functional genomics and functional ecology. The common brown butterfly, Heteronympha merope, provides a baseline for these studies as past research (Pearse 1978) allows investigations into how the species has responded to thirty years of climate change. Pearses studies included an enzyme–based population genetic structure across most of the geographic range of the species. In this component of the integrative project, we compare contemporary allozyme population structure with Pearses historical data. Of particular interest are geographically isolated populations from Queensland and South Australia which Pearse found to be genetically distinct from the homogeneous contiguous range. This pattern persists, although a new, apparently non–isolated, site without historical counterpart (Mount Remarkable, SA) has also been found to be highly distinct. Comparisons with DNA markers strongly suggest that patterns of allozyme differentiation are driven by adaptation. In conjunction with the other components of the project these findings will underpin a predictive model of adaptive responses to climate change.

T17: Fine–scale genetic structure in Little Penguins is influenced by climatic and oceanographic variables

J. J. Sinclair[1], *B. Cannell*[2], *S. Bradley*[2], *R. Wooller*[2], *W. Sherwin*[1]
[1]*Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, UNSW , Sydney NSW,* [2]*Sustainability, Environmental and Life Sciences, Murdoch University, Murdoch WA*
Little Penguins, the smallest member of the Penguin family, are endemic to Australia and New Zealand and are threatened by urbanisation and climate change. Human disturbance and the introduction of feral pests and invasive weeds have resulted in habitat reduction throughout the Little Penguins range. In Australia, populations are now restricted mainly to coastal islands free of predators and large human settlement. In Western Australia, the population at Penguin Island is the northernmost extent of Little Penguin distribution and hosts the largest colony of Little Penguins in WA. Located within the Perth metropolitan region, 600m offshore from the industrial precinct of Rockingham, the Penguin Island population is threatened by rapid urban expansion and climate change. At Penguin Island, mortality and reproductive success appear to be influenced by ENSO events. Source populations located near Esperance WA were identified by multilocus and mtDNA genetic analyses. However, genetic estimates of dispersal indicate that gene flow is now restricted by oceanographic currents and could be affected by increasing sea surface temperatures and ENSO events. These results indicate that conservation of the Penguin Island colony depends largely on ensuring management practices address factors influenced by climate variability and connectivity with neighbouring penguin colonies.

T18: Population Genetics of the Broadbill Swordfish within the Indian and Pacific Oceans

H. M. Bradman
Department of Genetics, University of Melbourne, Victoria
The Broadbill Swordfish, *Xiphias gladius*, is a migratory pelagic fish with a broad global distribution. They are commercially fished by many nations, including Australia. Accurate information regarding the stock structure of this species is required to plan effective management strategies. Previous research using molecular markers has divided Swordfish into four distinct stocks: Mediterranean, North Atlantic, South Atlantic and Indo–Pacific. However, the structure within the Indo–Pacific stock is not well understood, with very little research focusing on this region. Microsatellite markers and mitochondrial markers have been used to reveal structure within the Indo-Pacific stock.

T19: Genetic consequences of population loss and reintroduction in the rare geographically disjunct *Banksia brownii* following pathogen driven local extinction

D. J. Coates, S. McArthur, M. Byrne
Science Division, Department of Environment and Conservation, Kensington
Banksia brownii is a Critically Endangered small endemic tree restricted to the Stirling Range and Albany area of Western Australia. Sixteen populations, containing approximately 8,000 mature plants, are currently known to be extant and 12 populations have now been recorded as extinct due to Phytophthora dieback. The species is highly susceptible to this pathogen, with all extant populations infected and in decline. We assessed the genetic structure and patterns of genetic diversity in 384 plants from 11 extant and 5 extinct populations using 12 microsatellite loci. Material for extinct populations was based on seed collections carried out up to 20 years prior to population extinction. Population genetic structure studies showed that the three biogeographical groups within B. brownii are genetically discrete and appear to be the result of extended isolation and limited historical gene flow. Following rarefaction analysis we found that some 50% of the genetic diversity and more than 30% of private alleles are from the extinct populations. By determining the contribution of within population genetic diversity and population differentiation to the total genetic diversity for each population we were able to highlight both extinct and extant populations that contributed most to genetic diversity in this species. This provided a useful approach for assessing the significance of individual populations and the genetic diversity lost from within the species following local extinction. Three separate re–introductions corresponding to

the three biogeographical groups have now been established and we discuss our results in the context of these re–introductions and broader issues of restoration.

T20: Genetic guidelines for the ecological restoration of seagrass meadows

E. Sinclair[1,2], *J. Anthony*[1], *S. Krauss*[1,2], *Gary Kendrick*[2]

[1]*Botanic Gardens and Parks Authority, West Perth,* [2]*School of Plant Biology, University of Western Australia, Perth*

Seagrasses are the dominant marine angiosperm, providing important ecosystem functions. The restoration of impacted meadows is a major undertaking globally. Nearly half of the worlds 60 plus species are found along a 1500km coastline in south–west Australia, including 8 of 9 species of Posidonia. We developed a set of polymorphic microsatellite markers to characterise patterns of genetic variation in one of the most widespread and robust species used in restoration, *Posidonia australis*. Twelve meadows were sampled within Perth metropolitan waters to develop genetically–based guidelines for the restoration of Cockburn Sound, an area impacted by eutrophication, industrial development and sand–mining. Estimates of gene flow from population structure suggest a high level of admixture across the sampled range ($\rho = 0.068$). However, levels of within meadow genetic diversity vary greatly (genotypic diversity=0.12–0.96), suggesting local conditions appear to have a strong impact on the successful establishment of recruits and local genetic structure. From these results, practical genetic guidelines for the sourcing and establishment of propagules for restoration are being developed.

T21: Investigating gene flow within and between sympatric sexually–deceptive orchid taxa

M. R. Whitehead, C. C. Linde, R. Peakall

Division of Evolution, Ecology and Genetics, Research School of Biology, Australian National University, Canberra

The terrestrial orchid genus *Chiloglottis* secures pollination by attracting male thynnine wasp pollinators through chemical mimicry of the female wasp sex pheromone. The "outcrossing hypothesis" posits that by hijacking the sexual behaviour of their pollinator, sexually–deceptive orchids ensure their pollen is transported far enough away from the parent plant to promote outcrossing. Furthermore, divergent floral volatile chemistry linked to different pollinator species is predicted to act as a strong pre–zygotic reproductive barrier, thereby limiting hybridization. Data from hypervariable microsatellite loci are used in this study to provide detailed insights into both of these aspects of *Chiloglottis* biology. Outcrossing measures are obtained by genotyping orchid offspring collected as seed and germinated in culture. Measures of neutral genetic variation within and between sympatric orchid taxa provide insights into the occurrence of hybridization in this system.

T22: Comparison of demographic and genetic effective population size estimators

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The effective population size (Ne) is proportional to the loss of genetic diversity, the rate of inbreeding, and the rate of extinction. It has been estimated using both genetic and demographic approaches, but only rarely in combination. In this study, I integrate 10 year population demographic and genetic studies of two gecko species to compare the two approaches. Our goals were to estimate Ne for four populations of each species, and to calculate the Ne/Na ratios (Na = number of adults). To estimate mean life-time reproductive success and its individual variance I used a novel approach that combines a microsatellite DNA mating system study with a demographic estimate that relies on changes in population size. In this presentation, I will identify the most important factors that contribute to the reduction of Ne below Na. I will also demonstrate how the demographic estimates compare to the genetically derived estimates and discuss if these provide a reasonable alternative for Ne estimation in the absence of detailed demographic analysis.

Gene expression

T23: Genetic variation in inducible gene expression

H. J. French[1], *K. Hardy*[2], *M. F. Shannon*[2], *R. B. H. Williams*[1]

[1]*Molecular Systems Biology Group,* [2]*Gene Expression and Epigenomics Group, Department of Genome Biology, John Curtin School of Medical Research, Australian National University, Canberra.*

Because activation of the immune response is dependent on extensive changes in gene expression, it is likely that a major component of inter–individual variation in the immune response is ultimately mediated at the level of gene regulation. Here, we examine the influence of genetic variation on inducible gene expression in the murine immune response. We extracted primary CD4+ splenocytes from inbred strains A/J, C57BL/6J, BALB/c, DBA/2J, and 129x1/SvJ (> 3 animals/strain) and measured mRNA transcript levels using microarrays in both basal state and four hours after stimulation with PMA/Ionomycin. We defined an expression change occurring during activation as the difference in measured expression intensity between stimulated and basal conditions. We identified genetically influenced genes using a gene–wise single-factor (strain) ANOVA (B–H corrected $P < 0.05$). We identified 2607, 1145 and 506 transcripts whose expression levels are under potential genetic influence in basal state, stimulated state and during activation, respectively. Preliminary analysis using the FIRMA algorithm suggests a number of instances of genetically–variant differential splicing. These differentially activated genes are ideal candidates

for further study into the influence of genetic variation on the mechanisms of gene induction, and provide mechanistic insight into inter-individual variation in the host response to infection.

T24: Novel method for discovery of alternative splicing and tissue specificity of large transcripts

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Completion of genome sequencing projects and collection of huge amounts of mRNA–transcript data focusses attention on the problem of multiple transcript isoforms. Many putative alternative splice forms can be missed because EST sequences are biased towards the 5 and 3 ends of genes. In the case of large or rare transcripts, the coverage of the internal sequence might be insufficient to correctly predict splice forms. The issue becomes even more complex if we need to assess tissue distribution of alternative transcripts because of the uneven coverage of EST tissue libraries. To address this problem of tissue distribution analysis of large alternative transcripts we use ~12kb of the mouse BPTF gene as a model. NCBI annotated only two BPTF isoforms with two alternatively spliced regions. During initial RT–PCR analysis of mouse isoforms we discovered six additional splice variants. This diversity had remained undetected because previous experimental analysis had been done by northern blot and cDNA library screening. Although computational prediction would have identified a need to check for other variants, this was not done. This is one thus a good example of the risks of insufficient EST coverage to discover all alternative splice forms. To analyse the tissue expression of the newly predicted BPTF transcripts a novel method was designed. This may have wide applicability for large genes.

T25: New roles for neural receptors: the divergence and expression patterns of ligand–gated chloride channel subunits in insects

E. Remnant, P. Daborn and P. Batterham
Department of Genetics, The University of Melbourne

The role of ligand–gated chloride channels in inhibitory neurotransmission is well established in both vertebrates and invertebrates. In insects and nematodes, genes belonging to this family are major targets of antiparasitics and insecticides, and in humans offer targets of therapeutic significance. The *Drosophila melanogaster* genome contains 12 ligand–gated chloride channel subunits that are gated by neurotransmitters including GABA, Glutamate and Histamine. However three uncharacterised subunits form a separate clade and genome sequences have revealed that these genes have varying copy number in insect species. Furthermore, in Drosophila, all three subunits show expression in non–neuronal tissues, such as gut, salivary glands, and reproductive organs. The distinctive expression patterns as well as evolutionary divergence suggest a novel function of these subunits distinct from a role in neurotransmission. Expression has been localised to regions of tissues and specific cell types. This will contribute to understanding the role these subunits play in peripheral tissues. Using information about species–specific adaptations may reveal unique pest subunits for targeting by novel pesticides.

T26: The molecular genetics of vernalization–induced flowering in cereals

B. Trevaskis
CSIRO Division of Plant Industry, Canberra

Many wheats and barleys require prolonged exposure to cold in order to flower–vernalization. In these cereals vernalization–induced flowering is controlled by the VERNALIZATION1 (VRN1) gene; a promoter of flowering activated by low temperatures. VRN1 transcript levels increase gradually during vernalization, with longer cold treatments inducing higher levels of expression. Elevated VRN1 expression is then maintained in the shoot apex and leaves after vernalization, and the level of VRN1 expression in these organs determines how rapidly vernalized plants flower. Some alleles of VRN1 are expressed without vernalization due to deletions or insertions within the promoter or first intron of the VRN1 gene. Varieties of wheat and barley with these alleles flower without vernalization and are grown where vernalization does not occur. The first intron of the VRN1 locus has histone modifications typically associated with the maintenance of an inactive chromatin state, suggesting this region is targeted by epigenetic mechanisms that contribute to repression of VRN1 before winter. Other mechanisms are likely to act elsewhere in the VRN1 gene to mediate low–temperature induction. This talk will examine how understanding the mechanisms that regulate VRN1 can provide insights into the biology of vernalization–induced flowering in cereals and how this might contribute to crop improvement.

T27: From flowering time to human genetic disorders in *Arabidopsis thaliana*

S. Balasubramanian
School of Biological Sciences, The University of Queensland, St.Lucia, QLD

Natural variation in *Arabidopsis thaliana* provides an excellent resource to address fundamental questions of evolutionary significance. We have been exploiting natural variation in *Arabidopsis thaliana* to reveal molecular mechanisms regulating phenotypic variation. I will provide an overview of our work covering the molecular basis of phenotypic variation in flowering time, temperature responses as well as fitness trade offs in *Arabidopsis*. Through a combination of Quantitative Trait Locus (QTL) mapping, genome wide association mapping expression studies and functional analysis, we have identified genes underlying these traits. Finally, we have identified the first example outside humans for a triplet expansion associated genetic defect, which has opened up several new avenues including impacts on human genetic diseases and I will describe some of the current work currently being done in my lab.

T28: Identification and analysis of stable internal reference genes for qRT–PCR in tobacco

(*Nicotiana tabacum*) during development and abiotic stress

G. W. Schmidt[1,2] *S. K. Delaney*[2,3]
indexDelaney, S. K.—textbf
[1]*Friedrich Schiller University of Jena, Jena, Germany,* [2]*Discipline of Genetics, School of Molecular and Biomedical Science, University of Adelaide, South Australia,* [3]*School of Biotechnology and Biomolecular Sciences, University of NSW, Sydney*

Quantitative RT-PCR (qRT–PCR) is a powerful technique for the measurement of gene transcription, but its accuracy depends on the stability of the internal reference genes used for data normalization. Tobacco (*Nicotiana tabacum*) is an important model in studies of plant gene expression, but stable reference genes have not been well–studied in the tobacco system. We addressed this problem by analysing the expression stability of eight potential tobacco reference genes in a variety of tissues at different stages of development and in response to a range of abiotic stresses (*Molecular Genetics and Genomics*, **283**:253–241, 2010). Three genes (L25, EF–1α and Ntubc2) were identified as being sufficient for accurate normalization across all of the tissues tested, and an improved "high–throughput" methodology for qRT–PCR was developed and tested. The results of this study provide a foundation for further qRT–PCR studies in tobacco, and the methods developed are generally applicable to gene expression studies using the qRT–PCR technique.

T29: Conserved and divergent elements of the *Caenorhabditis elegans* dauer signaling pathways in *Parastrongyloides trichosuri*

W. N. Grant[1], *M. Crook*[2], *S. Stasiuk*
AgResearch Ltd., Palmerston North, New Zealand ([1]*Genetics Dept., La Trobe University, Bundoora;* [2]*Pennsylvania State University, University Station, U.S.A.)*

Dauer formation in *Caenorhabditis elegans* and other free–living nematodes is initiated in response to the accumulation of a cocktail of fatty acid–derived metabolites in the environment (termed "dauer pheromone") in conjunction with other environmental signals, most notably temperature and the availability of food. Under a given set of food and temperature conditions, exceeding a threshold concentration of "pheromone" initiates a signal that is transduced via highly conserved cyclic nucleotide, insulin/IGF and TGF–β pathways. These pathways converge at DAF–12, a nuclear hormone receptor activated by dafachronic acid. It has long been hypothesised that dauer development was an important factor of the evolution of parasitism, and that the infective stage of at least some parasitic nematodes is derived directly from the dauer of free–living nematodes. Parasites of the genera *Strongyloides* and *Parastrongyloides* have complex life history strategies in which it is proposed that the switch between free–living and parasitic development has evolved directly from the dauer switch and signal transduction pathways in *C. elegans*. We have shown that in *P.trichosuri*, there is conservation of a "pheromone" signal, and that the setting of the threshold at which free–living to parasitic life history switching occurs has a strong genetic component. Furthermore, we have cloned the major components of the insulin/IGF pathway, and have tested their function in transgenic *C. elegans*, to show that some functions of this pathway appear to be well conserved and others are not. Similarly, we show that TGF–β signaling is divergent between *P. trichosuri* and *C. elegans* but that dafachronic acid signaling is likely conserved. Thus, the free–living/parasite switch in *P. trichosuri* is likely to be evolutionarily derived from the dauer switch in free–living nematodes in a broad EvoDevo sense, but the details of the function of the signal transduction pathways clearly differ.

T30: Identification of olfactory genes in *Drosophila melanogaster*

Y.–C. Liu, M. Beale, M. de Bruyne, C. G. Warr
School of Biological Sciences, Monash University, Australia

In *Drosophila* odours are detected by a large family of odorant receptors (Ors). Unlike mammalian Ors which are G protein–coupled receptors, the insect Ors appear to encode directly ligand–gated ion channels. To identify genes involved with the Ors in peripheral olfactory function in *Drosophila* we have performed genetic screens using electrophysiological recording techniques. This approach has identified an EMS–generated mutant strain which has an interesting electrophysiological phenotype that suggests it may affect the function of the accessory cells that support the ORNs. We have deficiency mapped the mutation to a region in 33E containing 6 annotated genes. We are using multiple approaches to identify the gene affected in the mutant: candidate gene sequencing, expression analysis, RNA interference experiments, and rescue experiments. Recent in vivo RNAi experiments indicate that the gene affected is *bru–2*, an essentially uncharacterised member of the bruno family of RNA-binding proteins, and that its function is required in the accessory cells. Rescue experiments are currently in progress to confirm this.

T31: Candidate Genes for Cotton Root System Architecture

Md Asaduzzaman Prodhan, Peter A McGee, Jennifer A Saleeba School of Biological Sciences, The University of Sydney, NSW

The root system offers many functions including anchoring support and water and nutrient absorption by the plant. As such, root system architecture (RSA), the spatial arrangement of the root system within the soil, bears tremendous importance in a plants stability and adaptability to the adjacent environment. In many agricultural environments plants suffer restricted water and nutrient availability. One potential approach to improving plant yield under these harsh environmental conditions is to define the appropriate RSA of an individual crop in a target environment and then manipulate it towards an optimization of water and nutrient uptake from the soil. Here we discuss the assay of phenotypic variation in RSA in cotton cultivars

and the use of genomic resources and knowledge of candidate genes gained in Arabidopsis thaliana for the identification of candidate genes of cotton RSA.

Keynote: Tuesday morning

T32: Going it alone from time to time: Mate limitation, sex allocation and the evolution of hermaphroditism in plants and animals

John R. Pannell
Dept of Plant Sciences, University of Oxford UK

The remarkable diversity of plant sexual systems points to repeated evolutionary transitions between contrasting strategies. One frequent such shift is the evolution of self–fertilisation. Another is the transition between hermaphroditism and dioecy (the possession of separate sexes). In my presentation, I will explore the implications of demographic fluctuations, operating at a number of spatial scales, for transitions from dioecy to self–compatible hermaphroditism. Although I will focus particularly on a series of detailed studies of an uncharismatic European herb, my main aim will be to illustrate the striking parallels displayed by plants and animals that have undergone the same evolutionary transitions for apparently similar reasons–selection for reproductive assurance when mating partners are scarce.

Population Genetics

T33: Can pollinator behaviour maintain divergent floral morphs in sympatry?

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Pollinators play a central role in the origin and maintenance of floral variation in natural populations, as well as in speciation, acting as vectors for gene flow and selection. Here, the mating patterns and phenotypic selection on floral traits were characterised during two flowering seasons for *Gladiolus longicollis* (Iridaceae) morphs. A mating model, with genetic and phenotypic predictors, was developed to identity the paternity of seed. A multivariate analysis was used to estimate selection on correlated floral traits based on male and female fitness. Mating patterns among floral morphs were density dependent, resulting in assortative mating at low plant densities and considerable mating among morphs at high densities. Weak disruptive selection on tube length was detected in one of the two seasons for female fitness. Plant height was under opposing directional selection for female (+) and male (−) fitness. These results indicate that *G. longicollis* morphs will introgress rather than diverge towards speciation. Density dependent mating is predicted to result in the loss of rare morphs within populations.

T34: Pollinator–driven speciation—Insights from Australian orchids

R. Peakall[1], *D. Ebert*[1], *C. Hayes*[1], *J. Poldy*[2], *R. Barrow*[2]
[1]*Research School of Biology.* [2]*Research School of Chemistry, ANU, Canberra*

Australian sexually deceptive orchids may offer an ideal system for testing the hypothesis of pollinator-driven speciation. These orchids lure their specific male pollinators to the flower by emitting semiochemicals that mimic the sex pheromone. In a multidisciplinary study we evaluated wasp pollinator specificity; identified the novel compounds involved in pollinator attraction; and mapped our chemical findings onto the phylogeny. While orchid speciation was usually underpinned by chemical change, it often occurred with little morphological change. In order to define cryptic orchid species boundaries we combined chemical and population genetic analysis. A survey of 41 chloroplast simple sequence repeat loci (cpSSRs) revealed more differentiation among taxa than 13 nuclear SSR loci (mean 57% versus 27%). Furthermore, among a chemically defined pair of cryptic sympatric taxa with trivial cpSSR and nSSR differentiation (6% versus 1.4%), haplotype sharing was virtually absent, confirming little or no bi–directional gene flow. Chloroplast SSRs have been largely neglected in studies of wild species, yet as evident from this study they can offer unique insights into evolutionary processes.

T35: Characterizing the radiation of subspecies of polyploid Atriplex nummularia Lindl. (Chenopodiaceae) using microsatellite markers

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[1]*Science Division, Department of Environment and Conservation, Bentley, WA, Australia,* [2]*CRC for Plant Based Management of Dryland Salinity, The University of Western Australia, Nedlands, WA, Australia*

There is increasing interest in the roles of polyploidy and hybridization in the radiation and divergence of plant species. The Australian perennial species of Atriplex have a complex evolutionary history that includes polyploidy and hybridization but evidence so far has been derived from morphology and cytology. The octoploid, dioecious species *A. nummularia* is proposed to have evolved from an octoploid ancestor in the coastal semi–arid fringe of south–western Australia, then spread east and diverged into taxa which occupy edaphically different environments. Nuclear microsatellite markers were used to investigate population genetic structure and taxonomic relationships of two common subspecies of *A. nummularia*. Analysis of relationships showed differentiation between subspecies and separation of the population from the putative ancestral area. Genetic diversity was high overall (A=509, A'=42.4, H' =2.8) and differentiation of population was low within subspecies (FSC=0.048), indicating that drift/selection has not produced substantial divergence of populations. These findings are consistent with radiation of *A. nummularia* subspecies through homoploid hybridizations from a taxon in the

western semi–arid fringe rather than from divergence through selection/drift.

T36: Genetic basis of chemotype variation in Australian Melaleuca

A. Padovan[1], *A. Keszei*[1], *H. Webb*[1], *C. Kulhiem*[1], *Y. Hassan Sharkey*[1], *T. G. Köllner*[2], *J. Degenhardt*[2], *W. J. Foley*[1]

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One of the most striking aspects of Australian Myrtaceae is the variation in terpenes within and between species. Terpenes are responsible for the aroma and biological activity of many plant products and also play an important ecological role. Chemotypic variation of terpenes is under strong genetic control. Terpenes are synthesised by terpene synthases, which can produce multiple products from a single substrate and just a few enzymes can be responsible for the synthesis of up to 30 different compounds. We investigated the genes that code for terpene synthases in Australian *Melaleuca* (paperbarks and tea–trees) to explain the molecular basis of their foliar terpene chemotypes. In *Melaleuca alternifolia* (medicinal tea–tree), we know of six distinct chemotypes. These are characterized by different ratios of 1,8–cineole, terpinen–4–ol and terpinolene. These chemotypes arise from differences in the genomic presence and expression of three major characterized monoterpene synthases. Using this information, we can increase the competiveness of the Australian tea–tree industry by improving quality of leaf oil. In *Melaleuca quinquenervia*, differences in the expression of sesquiterpene synthases produce different chemotypes which differentially influence herbivory by several insect species. We can use this information in conservation practices in Australia and eradication practices in Florida.

T37: Coevolutionary implications of variation in mating systems of flax (*Linum marginale*) and flax rust (*Melampsora lini*)

A. Nemri, L. G. Barrett, P. H. Thrall, J. J. Burdon
CSIRO Plant Industry, Canberra

Coevolution between hosts and parasites is a major driver of the evolution of species. An essential part of this coevolution consists of the generation and maintenance of diversity in resistance and virulence genes for host and parasite respectively. This is achieved by mutation and recombination and therefore relies heavily on the reproductive system. The *Linum marginale–Melampsora lini* interaction exhibits regional variation in host mating system and parasite sexuality. We have investigated how inbreeding vs outbreeding affect the distribution of host resistance in different environments. Similarly, we studied the potential effect of sexual vs asexual reproduction on parasite virulence and diversity in environments in which weather conditions favour one or the other. We have found that recombination is associated with increased diversity in both host and parasite and increased host resistance. Conversely, inbreeding and asexual reproduction are associated with a reduction in host and parasite diversity seemingly advantageous for the parasite. Our findings are consistent with the Red Queen Hypothesis that postulates that sexual reproduction confers an advantage to hosts in the arms race against parasites. Our work also emphasizes the importance of life–history traits and heterogeneous environments on the interaction between hosts and their parasites.

T38: Design–R–genes: chimeric disease resistance proteins reveal new insights into inter– and intramolecular interactions in the flax–flax rust pathosystem

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CSIRO Plant Industry, Canberra, ACT, Australia

Identification of directly interacting fungal effector and plant disease resistance proteins has facilitated the study of both the physical nature of these interactions and the evolutionary forces that have resulted in a molecular arms race between these organisms. Disease resistance in this pathosystem is determined by a gene–for–gene recognition where flax immune receptors interact with rust effector proteins. In one such example, members of the AvrL567 effector protein family in flax rust interact with members of the L receptor protein family (L5, L6, and L7) in flax. Amino acid polymorphisms occur on the surface of AvrL567 variants and determine their recognition specificities. An integrated analysis of the L gene family, combining tests for positive selection with hypothetical structural models, has revealed a number of positively selected amino acid residues that may mediate interactions with AvrL567 variants. Based on these results, chimeric L proteins have been constructed. Evaluation of direct interactions between these chimeric L proteins and AvrL567 protein variants in the yeast–2–hybrid assay has identified specific regions within a leucine–rich repeat domain of L proteins that mediate interactions with AvrL567 variants, and has also revealed evidence of domain co–adaptation within L proteins.

Human Genetics and Genomics

T39: Dynamics of Plasma Proteome of Genetically Leptin-deficient Patients during Leptin Replacement Therapy

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The mechanisms of leptin resistance, anti–lipotoxicity, and cross–talk between leptin–insulin are incompletely understood. To investigate the dynamics of plasma

proteome of three genetically leptin-deficient adults, we compared protein abundance levels at four stages of treatment with recombinant methionyl human leptin: leptin–naive, after 18 months and 6 years of treatment, and 7 weeks after temporary leptin withdrawal. We used proteomics analysis with iTRAQ approach, and employed a two–dimensional HPLC–ESI/MS scheme for bottom–up proteomics analysis. Protein identification was done by the X!Tandem search engine. Pathway and network analyses were performed using proteins identified with E–value $\leq 10^{-3}$. Heat maps and hierarchical clustering of protein abundances were generated. We identified with high significance (median logE$\leq$ 24.7) and quantitated 556 proteins (31% extracellular, 69% intracellular). Treatment up–regulated pathways related to cell adhesion, cytoskeleton remodelling, cell cycle, coagulation, glycolysis and gluconeogenesis. When off–leptin, up–regulation of inflammation, lipoprotein and lipid metabolism pathways was observed only for the oldest and most obese patient. In the insulin resistance network, adiponectin and sex hormone–binding globulin were overabundant for all patients in all treatment stages. In conclusion, leptin treatment/withdrawal is associated with the up–regulation of specific pathways. Those results are clinically relevant, and should be taken into account when treating patients with leptin.

T40: The Gentrepid candidate gene prediction system

J. Y. Liu[1], *S. Ballouz*[1], *M. Oti*[1], *D. Fatkin*[2,3], *M. A. Wouters*[1,3]

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Gentrepid (`http://www.gentrepid.org/`) is a web resource which predicts and prioritizes candidate disease genes for both Mendelian and complex diseases. It uses two approaches to prioritize candidate disease genes, Common Pathway Scanning (CPS) and Common Module Profiling (CMP). CPS assumes specific phenotypes are associated with dysfunction in proteins that participate in the same complex or pathway and applies network data derived from protein–protein interaction and pathway databases to identify relationships between genes. CMP is based on the hypothesis that disruption of genes of similar function will lead to the same phenotype and identifies likely candidates using a domain–based sequence similarity approach. In a benchmark using Mendelian diseases, the two methods had a combined sensitivity of 0.52 and specificity of 0.97, reducing the candidate list by 13–fold. These approaches are also applicable to candidate gene prediction in other species. Here I describe the Gentrepid system and its web interface.

T41: Application of the Gentrepid candidate gene prediction system to Genome-Wide Association Studies

S. Ballouz[1,2], *J. Y. Liu*[1], *M. Oti*[1], *B. Gaeta*[2], *D. Fatkin*[1,3], *M. Bahlo*[4], *M. A. Wouters*[1,3]

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Genome–wide association studies (GWAS) aim to identify the genetic architecture of complex diseases by testing a large number of SNP markers for disease correlation. However identifying the relevant disease gene is not straightforward. Here we analysed data from the Wellcome Trust Case Control Consortium (WTCCC) on seven disease phenotypes: bipolar disorder, coronary artery disease, Crohns disease, hypertension, rheumatoid arthritis, and type I and II diabetes. We used the Gentrepid candidate gene prediction system to select likely gene candidates associated with SNPs of less stringent statistical thresholds than applied in the original analysis, using multiple SNP/gene mapping assumptions. Mapping assumptions resulted in search spaces ranging from 2 to 4431 genes. Under the common nearest neighbour SNP mapping, only 76% of characterized genes are associated with Affymetrix500k SNPs. Gene coverage increases to 99% in other tested mappings. However, even when the entire genome is considered, only 57% of characterized genes have Gentrepid annotations and are thus potentially predictable as candidates. Predictions were made using protein domains and pathway information via known disease genes or multiple loci. Gentrepid was able to extract known disease genes and predict novel plausible disease genes in known and novel, WTCCC–implicated loci, demonstrating its value for GWAS analysis.

T42: Genetic variation in the Dopamine D4 receptor protects against the adverse effect of childhood adversity on emotional resilience

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Dopamine receptor D4 (DRD4) allelic variant moderates the impact of childhood environment on behaviour and other health related outcomes. But its effect on emotional resilience (ES), an important determinant of mental health, is unclear. In this study we tested the effect of the DRD4 alleles containing 4 or 7 repeats in exon III on ES of a representative population sample of 1217 individuals in their early 30s. We observed no main effect but an interactive effect of the DRD4 genotype and childhood adversity (CA) on ES, when effects due to age, gender and education were controlled for (β=0.125; P=0.003). The 7r allele appears to protect against the adverse effects of CA since the decline in ES associated with increased adversity was not evident for individuals with the 7r allele. The interactive effect of DRD4 genotype and CA on ES appears to be mediated through behavioural activation

system (BAS) components of Grays personality scale. Childhood stressors reduce ES and increase the risk of mental health disorders in later life. It appears that when stressors are present, DRD4 genotypes containing the 7r allele influence the development of personality in a way that provides protection against these adverse outcomes.

T43: Using genetic evidence to evaluate four palaeoanthropological hypotheses for the timing of Neanderthal and Modern Human origins

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A better understanding of the evolutionary relationship between modern humans and Neanderthals is essential for an improved resolution of the hominin tree in general. Palaeoanthropology currently offers four distinct chronologies for the timing of population divergence, ranging from the late Middle Pleistocene to the late Early Pleistocene, each based on different interpretations of hominin taxonomy. Genetic data can present an independent estimate of the evolutionary timescale involved, making it possible to distinguish between these competing models of hominin evolution. We analysed five dated Neanderthal mitochondrial genomes, together with those of 54 modern humans, and inferred a genetic chronology using multiple age calibrations. The means of our date estimates are consistent with a process of divergence within an ancestral population commencing around 410–440 ka, suggesting that a reappraisal of key elements in the Pleistocene hominin fossil record may now be required.

T44: A genome–wide scan reveals candidate loci associated with sex–differential natural selection in humans

J. Feng, S. Easteal, G. A. Huttley
The John Curtin School of Medical Research, ANU, Canberra

The differential action of natural selection on male and female members of the same species is an important evolutionary force in a number of species but it has not been extensively investigated in humans. The extent to which this pattern of natural selection is shaping the human genome is thus unknown. We will present evidence of ongoing sex-differential natural selection in humans, by comparing the genotype distributions in males and females in a British sample of ∼5 thousand healthy (control) individuals, with ∼2 million single–nucleotide polymorphisms distributed across the human genome. Candidate regions with significant support for sex–differential selection will be identified and evidence presented that they are associated with sex-differential morbidities of one or more diseases. They may also be associated with sex differences in prenatal viability and thus with spontaneous abortion, and infant health.

T45: Can Indirect Tests Detect a Known Recombination Event in Human mtDNA?

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It is becoming increasingly more obvious that exceptions to the strictly clonal evolution of mtDNA exist across the animal kingdom. Substantial evidence now exists to suggest that mitochondrial recombination is not only possible, but is actively occurring in several species of vertebrate. However, evidence has been limited to hybrid zones and interspecific crosses, or in genetically dysfunctional mtDNA. The consensus for human populations is that mitochondrial recombination is not occurring at a biologically relevant level, although such evidence does exist for animal mtDNA. Here, we question the ability of the current tools for detecting recombination at a population level in human mtDNA, namely the computationally oriented indirect tests of recombination. Six well–established indirect tests of recombination (r^2 vs. distance, D' vs. distance, the Homoplasy test, the PHI, NSS, and Max χ^2) were assessed in a human mtDNA data set, in which recombination had been empirically confirmed. The upper limits of detection of these tests were also estimated for human mtDNA. Our results show that half of these tests were not able to successfully detect recombination, indicating that recombination between distinct mtDNA haplotypes may occur in human populations. If so this would impact not only on studies that use mtDNA to explain human diversity, but on the evolution of the molecule itself.

Kann et al 1995 PNAS
Engl J. Med 347 (2002) ?
2004. Piganeau et al. Schwartz & Vissing.

Keynote: Tuesday afternoon

T46: Issues in Forensic Science

John Buckleton
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Over the last few years there have been a number of significant events in forensic science. These include the trial and acquittal of Sean Hoey for the Omagh bombings, followed by the suspension and subsequent reinstatement of 34 cycle work in the UK, the almost analogous suspension and reinstatement of DNA reporting in an Australasian lab and a new virulence to the letters to the editor column in many forensic journals. Having been involved in these events a parallel becomes obvious with the issues arising from the OJ Simpson case in 1994. It is timely for us to review the lessons that could have or should have been learned in that earlier case and from recent events. These lessons need to be taken in mind by laboratories and academic institutions alike, in moving forensic science forward.

Science 2004

Emerging Technologies

T47: The next generation of DNA sequencing–a first for the scale insects (Coccoidea)

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Scale insects are difficult to work with molecularly. Only a few gene regions have been successfully sequenced for multiple scale insect families, and success is even less at the genus level. It is thought that some of the problems in amplifying mitochondrial DNA in scale insects might result from unusual base compositions within and among taxa, leading to primer mismatch and difficult sequencing templates. In the nuclear genome, there appear to be multiple copies of some genes that seem to be low–copy in other insects. To try to overcome some of the problems with conventional sequencing, we have obtained data using a next–gen sequencing approach for scale insects–a first for this group of insects. The information obtained will be used for multiple purposes, including identifying gene regions for higher level systematics, and developing markers suitable for population–level analyses.

T48: Rise of the machines–simultaneous development of multiple genetic markers for varied evolutionary applications using second generation sequencing with notes on microsatellite motif family proportions in eukaryote genomes

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The advent of second generation sequencing greatly enhances the potential to develop multiple molecular genetic markers for non–model organisms. Here we report the large scale utility of this technology to develop microsatellites, a commonly used class of genetic markers in ecological studies, in over 60 non–model eukaryote species. We outline a workflow for the simultaneous development of other markers for phylogeographic and phylogenetic studies. The average size of fragments was 350 base pairs and the average number of sequences obtained per species was about 120 000. The method gave approximately 0.013× coverage per species. We present a combined analysis of microsatellite abundance from eukaryote species of plants, invertebrates and vertebrates for partial unpublished genome sequences from these runs. We also include, for the first time, an analysis of the proportions of different motif families with genome size and GC content. We found that the proportions of different families of microsatellite motifs exhibit very different patterns with genome size. For example, across combined species there was a significantly negative relationship of trinucleotide abundance and genome size, and this negative relationship was reflected in analyses within all taxonomic groups.

T49: Low abundance transcripts in whole blood using RNAseq: How does globin expression influence detection?

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Whole blood provides a readily accessible tissue with the capacity to integrate environmental factors such as immune, nutritional, physiological and psychological states. Gene expression profiling in blood is complicated by biological factors related to tissue homogeneity and is characterized by a high abundance of globin (HBB) expression which can impact the ability to detect rare or low abundance transcripts in hybridization–based gene expression assays. Next generation sequencing of steady–state RNA derived from blood offers the potential to refine gene expression signatures for this tissue. With RNAseq, a titration was performed to understand the relationship between three sequencing depths and the ability to detect less abundant transcripts as compared to microarrays. Sequencing reads mapped across the genome but as expected, a high density of reads mapped to the globin cluster on chromosome 11. A preliminary analysis has demonstrated adequate sequencing depth can be achieved to allow detection of rare transcripts that are expressed at very low levels in blood. RNAseq has the potential to offer whole transcriptome analysis and biomarker discover in whole blood with applications in clinical and a wide number of other applications. (Supported by the Commonwealth Department of Health and Aging, Anti–doping Research Program)

T50: Identification and Characterisation of Odorant Receptors Epiphyas postvittana

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Most of the worlds major crop pests are members of the Lepidoptera. Understanding how these insects are attracted to their target crops and con–specific mates may enable the development of new pest control strategies. An obvious starting point for such a strategy is the isolation and characterisation of pest odorant and pheromone receptors (ORs and PRs). The light brown apple moth (LBAM) is a major pest for Australasias horticultural industries. Previously three OR genes were identified from ESTs through similarity to known ORs. In order to increase the representation of ORs from LBAM, high throughput 454 sequencing of male antennal transcripts was conducted, revealing 26 new putative ORs. In addition, 10× Solexa whole

genome sequencing identified genomic regions for the previous 29, and parts of 24 further OR genes bringing the total to 53. Of these three are candidates for encoding PRs. Future plans towards a full assembly of the LBAM genome and characterisation of its ORs and PRs will also be presented.

T51: Comparative mapping with high marker density reveals high synteny and colinearity among *Eucalyptus* genomes

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The completion of the *Eucalyptus grandis* reference genome sequence reinforces the need for a better understanding of genome homology in the genus. Most commercially important eucalypts belong to the largest subgenus: *Symphyomyrtus*. Comparative mapping using high-density linkage maps revealed high genome homology among *E. grandis*, *E. urophylla* (both in section *Latoangulatae*) and *E. globulus* (section *Maidenaria*). In two intra-section comparisons, 99% of common markers (234 and 245 respectively) were syntenic (mapped to equivalent linkage groups) and >94% of syntenic markers were colinear (same rank-order position within a linkage group). A slightly lower degree of synteny (91.6% of 463 common markers) and colinearity (90.6%) was observed in an inter-section comparison between *E. globulus* and an *E. grandis-E. urophylla* consensus linkage map. Nevertheless, marker synteny and colinearity between species were remarkably good, indicating high genome homology and high transferability of the *E. grandis* genome sequence information across the subgenus. This transferability will facilitate the identification of genes underlying phenotypic traits and evolutionary studies in *Eucalyptus*.

T52: Genetic diversity and the mobile genome

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Generating DNA sequence data is now simple. Bacterial genomes can be sequenced overnight, and routine genome sequencing of eukaryotes will soon be possible. Genome sequencing of an individual eukaryotic organism recovers representatives of most, if not all, the genes in that species. However, genome sequencing of a single Bacterial strain recovers only a portion of the genes in that species, because different strains can differ by up to 20% in their gene content. The characterization of such intra-specific variation is central to assembling the "species genome", the individual elements of which do not reside in any one cell, but are dispersed amongst clonal lineages within species. Consequently, genome sequencing of multiple bacterial strains is not an efficient way to explore Bacterial genomes, since the majority of genes will already have been characterized. As more genomes are sequenced, the discovery curve for new genes and hypothetical proteins flattens out. In contrast, if we concentrate on recovering and characterizing laterally transferred genetic elements, the rate of discovery of new genes remains above 90%. I will illustrate the extent of "species genome" diversity and methods for selectively recovering mobile DNAs using integrons and their gene cassette arrays as examples.

Teaching with *C. elegans*

T53: Class practical: using RNA interference to disrupt gene function in *C. elegans* vulval development

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This practical was designed to introduce 3rd-year molecular genetics students to *C. elegans* as a model, to introduce them to the EGF signalling pathway and its role in vulval development, to teach students how to perform and interpret RNA interference (RNAi) experiments in C. elegans and to further their understanding of RNAi. The students use the "feeding" method of introducing dsRNA into the nematodes, targetting the following genes: *lin-1* (ETS transcription factor essential for vulval development), *lin-3* (EGF family growth factor essential for initiation of vulval development), *unc-22* (positive control for RNAi), and an Arabidopsis gene, *Atlhc* (negative control for RNAi). The phenotypes scored are vulvaless (*lin-3*), multivulva (*lin-1*), twitching/paralysis (*unc-22*) and wild type (*Atlhc*). We used an RNAi-hypersensitive strain of *C. elegans*, either *rrf-3* or *eri-1/lin-15b*, to enhance the RNAi phenotypes. In week 1 the students prepare worms from a synchronised worm population and transfer them to pre-seeded RNAi plates; in week 2 they observe and analyse the phenotypes.

T54: Caenorhabditis in the teaching lab

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Caenorhabditis elegans has risen to be one of the premier model organisms due in large part by the ease with which genetic analysis using either forward or reverse genetic approaches can be carried out, but there appear to be relatively few publicly available protocols for using *C. elegans* for teaching purposes. We have developed and delivered 4 exercises suitable for 2nd and 3rd year genetics or molecular biology undergraduate classes. These illustrate the use of RNA interference, the positioning of genes in signal transduction path-

ways by tests of epistasis, basic complementation and linkage analysis (using either molecular or phenotypic markers) and also some basic concepts in population genetics such as genetic drift. The exercises require 2 to 4 weeks to run, are relatively inexpensive (particularly for the non–molecular exercises), and have been scaled up to be carried out in classes of up to 200 students. The equipment requirements are modest (access to stereomicroscopes with transmitted light is the most likely technical barrier), seem robust enough to "work" even with students who had not seen a nematode previously, and have generally been well received by the students.

Systematics and phylogeography

T55: Life History Determines Biogeographical Patterns Of Soil Microbial Communities Over Multiple Spatial Scales

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The extent to which the distribution of soil bacteria is controlled by local environment versus spatial factors (*e.g.* dispersal, colonisation limitation, evolutionary events) is poorly understood and widely debated. Understanding of biogeographic controls in microbial communities is hampered by enormous environmental variability encountered across spatial scales and the broad diversity of microbial life histories. We constrained environmental factors to investigate the specific influence of space on bacterial communities in soils over distances from m to 102 km. We analysed whole bacterial community structure with Terminal Restriction Length Polymorphism (TRFLP), structure of bacterial groups with different life histories using PhyloChip microarrays and functional diversity with Biolog substrate utilisation plates. We found strong evidence for a spatial component to bacterial community structure that varies with scale and organism life history. Geographic distance had no influence over community structure for organisms exhibiting survival–stages, but the converse was true for less hardy organisms. Community function was shown to be highly correlated to community structure, but not to abiotic factors, suggesting non–stochastic determinants of community structure are important. Our results support the view that bacterial communities are constrained by both edaphic factors and geographic distance, and show that the relative importance these constraints depends critically on taxonomic resolution used to evaluate spatio–temporal patterns of diversity, as well as life–history the investigated groups, much as is the case for macro–organisms

T56: Molecular markers for plants biodiversity allocation and species evolutionary history

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Understanding the origin of Australian flora is a continuing scientific challenge. To build on this understanding, an attempt to identify geographic regions with significant genetic and species diversity was conducted. We investigated the use of molecular markers for exploring the spatial distribution of biodiversity and their evolutionary history in Australia. We used species of the genus *Glycine* (Leguminosae) to illustrate the case. The phylogenetic analysis of endemic areas employed the newly developed Phylogentic Endemism (PE) measure, based on molecular phylogeny of histone H3D sequences, and geographic records of species occurrence in Australia. This technique could prove useful in identifying biodiversity hotspots and aid the development of management and conservation plans.

T57: Phylogeography of two related Gondwanan Orthocladiine chironomids (Diptera) from New Zealand and Patagonia

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Comparative phylogeography traditionally considers a given geographical region to evaluate the relative effect of the regions biogeographical history on co–distributed taxa. This study however, compares the response of three closely related species to population fragmentation on separate continents. Krosch *et al.* (2009) revealed several divergent geographically restricted mitochondrial lineages among Wet Tropics populations of the Australian closed forest endemic *Echinocladius martini* which corresponded to historical rainforest refugia. The current study aims to test population structure in *Naonella forsythi* in New Zealand and *Ferringtonia patagonica* in Patagonia, both of which are known relatives of *Echinocladius*. A 734bp fragment of the COI gene was amplified from 184 individuals of *N. forsythi* from seven sites in the northwestern South Island and 62 individuals of *F. patagonica* from eight sites across the Andes barrier. Phylogenetic analysis revealed four highly divergent lineages of late Miocene–Pliocene age in both taxa which did not correspond to geography and likely represents post–isolation range expansion. This study is the first analysis of population genetic structure in New Zealand and Neotropical chironomids, and is a novel application of comparative phylogeographic theory to investigating population structure among closely related species inhabiting different landmasses.

T58: Phylogeography of the rainforest–restricted fawn–footed *Melomys*, *Melomys cervinipes* (Rodentia: Muridae)

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The mosaic–tailed rat *Melomys cervinipes* (Rodentia: Muridae) is distributed throughout closed forest along the east coast of Australia from northern New South

Wales to Cape York in north Queensland. This distribution spans a number of potential biogeographical barriers to closed forest restricted taxa including the Black Mountain Corridor, the Burdekin Gap, and the St. Lawrence Gap. Previous analysis has suggested that at least two polyphyletic lineages could exist within *M. cervinipes* and it was suspected that the aforementioned potential biogeographic barriers may have influenced phylogeographic patterns in this taxon. In order to test this, samples were obtained throughout its entire distribution and phylogenetic analysis was undertaken using both mitochondrial (16S) and nuclear intron (AP5) sequence data. The analysis revealed three divergent lineages corresponding to northern, central and southern clades and also revealed polyphyly with regard to *M. capensis* and *M. rubicola*. Unexpectedly, however, these divergent lineages did not correspond directly to the aforementioned potential barriers to gene flow and the highly divergent (~2.1% 16S) northern and central lineages occur in sympatry in a narrow suture zone, suggesting a more complex evolutionary history within the archipelago of closed forest present along Australia's north-east coast.

T59: Molecular phylogenetics of Acacia

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The genus *Acacia* is the largest plant genus in Australia and is a key component of many ecosystems, especially in arid regions. We present molecular phylogenetic results from plastid and nuclear DNA regions for over 200 species. These data resolve major lineages of *Acacia* that have not been previously reported. We will present the phylogenetic data and present a foundation for a new informal classification of *Acacia*.

T60: An evolutionary history of gummy sharks (Mustelus): a peculiar genus

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Mustelus (gummy sharks) are coastal benthic sharks, abundant in temperate and tropical localities and important in fisheries worldwide. Mustelus species are notoriously difficult to distinguish using morphological characters as many distinguishing characters are internal, common to more than one species and/or show considerable variation within species. The difficulties in using morphology to distinguish species and limited attention that has been paid to Mustelus in developing countries suggest that species composition of the genus may differ markedly from what is currently recognized and warrants investigation using a molecular approach. Furthermore, the genus provides a unique opportunity for insight into the evolution of reproduction as it encompasses species with two distinct reproductive strategies, placental and aplacental viviparity. In this study we use genetic markers to explore the evolution of Mustelus. We combine our molecular phylogeny with physical, ecological and behavioural data to elucidate the evolutionary history of Mustelus in Oceania. Finally, we explore the evolutionary relationships between placental and aplacental Mustelus and discuss what these relationships imply about the evolution of reproductive modes.

Posters

P1: Development of a database of Rubisco sequences

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The large subunit of Ribulose–1,5–bisphosphate carboxylase/oxygenase (Rubisco) is arguably the most sequenced gene with >50,000 sequences available in NCBI from all three forms of life, eukaryotes (plants and algae), archaea and prokaryotes (autotrophic bacteria). Although Rubisco is the key enzyme in photosynthesis that catalyzes the fixation of CO2 into energy–rich molecules that underpin all life, it is inefficient and there is intense interest in improving it to develop better crops etc. Rubisco from diverse sources show distinct catalytic properties, and several analyses have shown the sequence is under evolutionary selective pressure. However, definition of the sequence traits that confer superior catalytic activity observed in some naturally occurring Rubiscos has not been achieved. The availability of a large number of Rubisco sequences, as well as x–ray crystal structures, provides the opportunity to map catalytic properties to sequence and structure. We have created a curated database of Rubisco sequences (both nucleotide and protein) that enables us to perform a range of studies, such as phylogenetic and comparative sequence analysis and sequence–structure–function relationships, to identify positions conferring distinctive Rubisco kinetic properties and to decipher their natural variation. The relational database uses automated python scripts to download Rubisco sequence data from public databases.

P2: Colonization of Lake Eacham by the Rainbowfish *Melanotaenia splendida*

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Lake Eacham is an isolated crater lake in a world heritage national park in northeast Queensland. It was home to the only known population of the Lake Eacham Rainbowfish, *Melanotaenia eachamensis*, declared extinct in the wild in the late 1980s following the translocation of several predators into the lake. Repeated attempts at reintroduction using captive bred stocks failed dramatically. Further populations of *M. eachamensis* have now been discovered elsewhere, but the crater lake remained free of rainbowfish until the late 1990s when rainbowfish reappeared. During a field survey in late 2009, a single individual was recovered from the lake. Photos of the fish and a small

fin clip were taken before the fish was released and this tissue subsequently used for molecular analysis. A portion of the mitochondrial D–loop was sequenced and compared to sequences generated from other rainbowfish in the surrounding area and to those in DNA databases. This comparison identified the unknown fish as *M. splendida*, which is common in the surrounding area. The mitochondrial haplotype of this individual was central to the haplotype network of the species. Whether the lake has been restocked by human intervention or by some unidentified natural means, remains unknown.

P3: Physical and Functional Interactions between Pathogen–Induced Arabidopsis WRKY18, WRKY40, and WRKY60 Transcription Factors

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WRKY proteins are a group of plant specific transcription factors involved in plant stress response and plant development. Here we report physical and functional interactions between structurally related and pathogen–induced WRKY18, WRKY40, and WRKY60 transcription factors in *Arabidopsis thaliana*. The three WRKY proteins formed both homocomplexes and heterocomplexes and DNA binding activities were significantly shifted depending on which WRKY proteins were present in these complexes. Single *WRKY* mutants exhibited no or small alterations in response to the hemibiotrophic bacterial pathogen and the necrotrophic fungal pathogen. However, *wrky18/wrky40* and *wrky18/wrky60* double mutants and the *wrky18/wrky40/wrky60* triple mutant were substantially more resistant to bacterial pathogen but more susceptible to fungal pathogen than wild–type plants. Thus, the three WRKY proteins have partially redundant roles in plant responses to the two distinct types of pathogens, with WRKY18 playing a more important role than the other two. These results indicate that the three WRKY proteins interact both physically and functionally in a complex pattern of overlapping, antagonistic, and distinct roles in plant responses to different types of microbial pathogens.

P4: A quantitative genetic analysis of ecological limits to species distribution in *Eurema* butterflies

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Species often do not extend as far as geographical barriers allow. There is a positive correlation between latitude and species latitudinal range which has been termed Rapoports rule. Where this phenomenon occurs climatic conditions may be the limiting factors. The climatic variability hypothesis contends that species at higher latitudes need greater ability to cope with a wider range of climatic conditions. If this is true then some species may be limited in their capacity to adapt to ecological conditions beyond their range. Tropical species, in particular, are thought to be less tolerant of climatic changes than temperate species, which may be due to specialisation to a constant environment, and which would be evident in low genetic variation for ecological stress traits. To test this we aimed to compare the cold tolerance and desiccation resistance of several species of butterflies of the genus *Eurema* from Cairns, North Queensland. We compared phenotypic values across a range of species, and then used a quantitative genetic analysis to compare genetic variances for cold tolerance between a broadly distributed (*E. hecabe*) and a restricted tropical species (*E. laeta*). Here we present preliminary data on our contrasts and interpret them in relation to key predictions of the climate variability hypothesis.

P5: Population genetics of the coppertail skink, *Ctenotus taeniolatus*, in the Sydney sandstone environment

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Exfoliated sandstone rock is utilized as a retreat site by a rich diversity of fauna on ridge tops surrounding Sydney. Illegal collection of sandstone rock has led to reduced numbers of some species and the use of artificial rock has been trialed. The success of this sort of habitat remediation is influenced by levels of dispersal from undisturbed areas. We assessed dispersal of the rock–dwelling coppertail skink (*Ctenotus taeniolatus*). Mitochondrial and microsatellite data was generated and levels of gene flow within and among ridge-tops in Ku–ring–gai Chase National Park were examined to establish whether intervening gullies act as dispersal barriers. Results indicate widespread dispersal between populations despite apparent barriers. Contrasting levels of nuclear and mitochondrial differentiation among sampling localities is suggestive of male–biased dispersal. However, levels of dispersal for each sex appear sufficient to allow natural recruitment into ridge tops where rocky retreats have been re–established.

P6: Protection of marsupial pouch young: from genomics to functionality

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Marsupial young are unable to elicit a functional immune response until about one third of their way through pouch life, yet they are born into a non–sterile environment containing a range of potentially pathogenic bacteria. Over the last 20 years, the mechanisms employed by marsupials to protect their young have received some attention. Of particular interest have been immune compounds transferred to the young in colostrum and milk, and those of

the innate immune system. The sequencing of the tammar wallaby genome has enabled a number of marsupial immune genes to be identified; however, further research is required to determine which of these plays a role in the protection of pouch young. We have started to identify additional innate immune genes such as mucins and lysozymes and will examine the level of expression of these genes in tammar wallaby young. Future work will determine the biological activity of these gene products on bacterial strains which are associated with the developing young.

P7: Characterisation of endogenous retroviruses in the Australian saltwater crocodile (*Crocodylus porosus*)

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Endogenous retroviruses (ERVs) are inherited copies or remnants of exogenous retroviruses derived from past infections of germ cells with subsequent integration into the host genome, which are passed on to subsequent generations. ERVs are thought to be ubiquitous in the genomes of most, if not all, vertebrate species. Knowledge of endogenous retroviruses in Crocodylians is limited, and their diversity within saltwater crocodiles is unclear. A previous study has revealed that two groups of ERVs exist within the Crocodylia, one of them specific for crocodiles. Here we present preliminary data from studies into the distribution and potential functionality of ERVs in saltwater crocodiles of the Northern Territory. Primers have been designed to amplify regions of the group specific antigen, protease–reverse transcriptase, and envelope genes of the seven major retroviral families. Characterisation of these retroviruses will allow functional and non functional retro-elements to be identified. Determining the diversity and distribution of functional retro–elements is the first stage in the identification of functional and potentially transmissible ERVs, allowing possible associations to be drawn between crocodile ERVs and the incidence of disease.

P8: Characterisation of MHC Class I genes in wild suids and peccaries

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Many wild species of Suids (pigs and hogs) and Tayassuids (peccaries) are under threat from human activities and habitat degradation. Consequently, a number of species are now listed as endangered and critically endangered. With growing interest in wildlife immunogenetics and also farming and breeding programs, it is important to establish the level of immunogenetic diversity in existing populations. It has been demonstrated that available genetic resources can be useful for cross-species characterisation of coding and non-coding DNA sequences of closely and distantly related species. Here we implement this approach by using domestic pig genetic resources to study the Major Histocompatibility Complex (MHC) class I genes of eleven wild species of Suidae from Africa, Asia and Europe, and Tayassuidae from the Americas. The primers presented here have been designed to amplify exons 2, 3 and 4 of porcine MHC Class Ia and Ib family genes. We will describe the number of variants obtained from these primers among species as well as those from an additional primer set which was tested on various individuals from one of the peccary species. Finally, we will discuss the importance of this study to approach the heterologous microarray capture and sequencing of the MHC region among these species.

P9: Microsatellite DNA markers associated with resistance to lymphocystis and *Edwardsiella tarda* disease in olive flounder (*Paralichthys olivaceus*)

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The olive flounder (*Paralichthys olivaceus*) is an economically important fish as a food, and has been widely cultured in Asian countries such as Korea, Japan and China. We chose to study lymphocystis disease (LD) and *Edwardsiella tarda* disease because it has become widely spread in Korea and seriously damaged fish farms. We developed 227 microsatellite markers from the genome of *P. olivaceus* and used these markers to search for a locus associated with resistance to LD or *E. tarda* disease in this species. The total of 1,000 individuals from the F2 family was challenged with lymphocystis disease virus or E. tarda by immersion in an indoor tank. We selected disease–susceptible ($n = 100$) and resistant olive flounder individuals ($n = 100$). Associations of marker locus with resistance to LD or *E. tarda* disease were analysed by ANOVA. 43 microsatellite loci were positively associated with resistance to LD ($P < 0.01$) and 16 loci were positively associated with resistance to *E. tarda* disease ($P < 0.01$). Identification of a larger number of DNA markers linked to QTL controlling the resistance to LD or *E. tarda* disease will contribute to the application of DNA marker-assisted selection in aquacultural breeding of olive flounder.

P10: Gene expression comparison between brain and heart during inflammatory stress

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With the aim of comparing central and peripheral pathways elicited during Gram–negative bacteria lipopolysaccharide (LPS)–induced immunological challenge, we compared two microarray studies that were previously performed in the brain and heart of rodents treated with the same dose and serotype of *E. coli* LPS (25mg/kg). We used young adult mice (wild type males C57BL/6) and rats (males Sprague–Dawley). The rodents were given intraperitoneal injections of either saline or LPS and sacrificed later at 6h (mice) or 6 and 24h (rats). Total RNA was extracted from each organ to generate two pools (5–6 animals / pool) of $10\mu g$ and processed following Affymetrix guidelines. All of the genes whose expression was significantly altered ($P < 0.05$) displaying an FC $+/-1.5$ were considered for the present analyses. We found that within the heart ventricles a total of 240 transcripts were significantly increased, whereas only 32 transcripts were significantly increased in the brain. Among the 32 genes which expression increased in the brain at 6h only 10 were also significantly increased within the heart. In summary, these results suggest that during inflammation there may be specific spatial–temporal patterns of gene expression within the brain and the periphery.

P11: Life, death, and diversity in a biofilm model of Pseudomonas aeruginosa infection

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Pseudomonas aeruginosa lung infection remains the leading cause of death in cystic fibrosis patients. High genetic diversity of the colonising population is thought to contribute to both infection success and antibiotic resistance. The mechanisms responsible for generating this diversity are not well understood, although elevated mutation rates in clinical P. aeruginosa strains are considered important. Further elucidating the processes contributing to diversity is crucial for novel treatment development. Our lab has reproduced diversification using biofilm models of lung infection. When grown as a biofilm, the clinical *P. aeruginosa* isolate 18A develops greater phenotypic and genetic diversity than the laboratory strain PAO1. Using Luria–Delbruck fluctuation tests, we show this is not due to an intrinsically elevated mutation rate in 18A. Interestingly, we observed an elevated death rate in 18A. Mathematical modeling predicts that in populations with a fixed size limitation, elevated death rates can lead to greater diversification. The increased death rate of 18A may therefore contribute to its high level of diversity, both in biofilm models and in the constrained environment of the lung. Our simulations of bacterial populations with varying death rates support this hypothesis. These findings are discussed in light of recent textitP. aeruginosa comparative whole–genome sequencing.

P12: Genetic adaptation to drought in Eucalyptus camaldulensis (river red gum) along the Murray River

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Australia has been experiencing one of the worst droughts on record. In order to survive, sessile organisms must either be resilient to, or adapt to, new environmental conditions. Eucalyptus camaldulensis inhabits watercourses and floodplain ecosystems, which may make it sensitive to the effects of drought. Results from a previous study indicate that natural and human induced drought has imposed a strong selective pressure on red gum populations in Yanga National Park. Evidence of selection was identified in water related candidate genes in populations under contrasted levels of water stress inYanga and Australia–wide. In a new study, we will probe for signatures of adaptation among Single Nucleotide Polymorphisms (SNPs) in environmentally contrasted populations of red gum along the Murray River. Putatively adaptive and neutral SNPs are being selected from candidate genes involved in drought, salt and herbivore tolerance. The functional basis of adaptive SNPs and their effects on fitness will also be investigated. We hope to identify: 1. the dynamics of genetic adaptation to water availability in red gum populations over different time scales; 2. whether adaptively important SNPs improve fitness under increased water stress; and, 3. whether existing levels of population genetic diversity are sufficient to afford resilience under projected climate change scenarios.

P13: MHC diversity in ancient Tasmanian devils

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The Tasmanian devil (*Sarcophilus harrisii*) is at risk of extinction due to the emergence of a contagious disease known as Devil Facial Tumour Disease (DFTD). The emergence of this disease has been linked to a lack of diversity in the Major Histocompability Complex (MHC) genes of the devil. Devils have survived several population crashes in the last two centuries which may have caused a loss of MHC diversity. Alternatively, MHC diversity may have been lost prior to European colonisation. To determine when Tasmanian devils lost MHC diversity we aim to sequence the class I and class II MHC alleles from Tasmanian devil samples collected during the 19th and 20th centuries (during which devil population fluctuations were occurring), as well as samples from pre–European colonisation (both Tasmanian and mainland Australia). Here we present preliminary data of MHC class I and class II sequences from devil samples collected at several time points

during the 20th century. No additional MHC diversity has been found in these devils suggesting that loss of MHC diversity occurred prior to the 20th century.

P14: Sex Determination Genes in Queensland Fruit Fly, *Bactrocera tryoni*

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Bactrocera tryoni is Australias most destructive horticultural pest. Suppression methods, including the release of irradiated, sterile individuals or individuals infected by Wolbachia (intracellular bacteria of insects that can cause crossing incompatibilities), are techniques that will become increasingly useful with the introduction of a male–only strain. To ultimately achieve a male–only strain of B. tryoni, a thorough understanding of the genes that form the sex determination pathway is necessary. The pathway has been well studied in *Drosophila melanogaster*, and this has provided a starting point for characterising genes involved in sex determination in other Diptera. However, the initial signal in male Bactrocera is communicated by the Y–located dominant male determiner, M, which is absent in Drosophila species. The mode of action of M, and its direct targets have yet to be characterised in fruit flies or in any other Y–determined insect. I am using quantitative PCR to study expression of important sex determination genes in sexed single embryos, with and without infection by Wolbachia. This may clarify the timing and operation of M, as well as reveal any disruption of expression of sex determination genes by Wolbachia in early development.

P15: Can genetic signatures of coevolution be detected for Eucalyptus and their leaf–spot fungal pathogens?

P. D. Rymer, B. A. Summerell

Botanic Gardens Trust, NSW Herbarium, Sydney

Antagonistic interactions can lead to an evolutionary arms–race, resulting in local–adaptation and potentially clear genetic signatures of co–speciation when host–shifts are infrequent. Dispersal events may encourage host–shifts with the potential for diversification into the new niche, while rampant dispersal will blur the signal of co–speciation and diversification. In this study we explore patterns of diversification in a well described host-pathogen system: leaf spot fungus, Mycosphaerella, on *Eucalyptus* (Myrtaceae). We utilise publicly available DNA sequences to re–construct molecular phylogenies of the host and pathogen. The host–pathogen interactions, collated from published literature, facilitate trait mapping. We test if the fungal and plant phylogenies are congruent, supporting a co–evolutionary process, or incongruent due to imbalances, indicating diversification with host shifts. Linage and geographic specific signals are explored.

P16: A molecular population genetic survey of the Z–chromosome of the cotton boll worm, *Helicoverpa armigera*

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An understanding of the genetic basis of insecticide resistance can inform pest management strategies and provide insights into the molecular basis of adaptive evolution. Advances in sequencing technologies provide new ways to identify insecticide resistance genes and allow an integrated genome–wide perspective. We are interested in two new approaches: the first involves scanning the genome for signs of selection in the patterns of molecular polymorphism while the second involves genome–wide association studies. However before we can interpret population genomic data sets certain population parameters need to be understood for the organism of interest–which in our case is the cotton bollworm *Helicoverpa armigera*. So in a preliminary survey we have set out to characterize the extent of polymorphism and linkage disequilibrium of loci on the moth Z chromosome. We have chosen this chromosome because hemizygosity in females allows us to avoid the confounding influences caused by the diploid state. We find high levels of polymorphism including abundant indel variation. We also present data suggesting that one of the Z–linked loci displays patterns of polymorphism inconsistent with the neutral model and suggests recent positive selection acting on this gene.

P17: A search for inter–chromosomal disequilibrium in Hapmap

J. Sved

Biological Sciences, Sydney University and BEES, University of NSW

Genes on different chromosomes are assumed to segregate independently, although this assumption has never been tested on a broad scale. Independent segregation is expected to lead to independence of population frequencies of genes on different chromosomes (gametic equilibrium). Large scale tests for discrepancies from such equilibrium are now possible using SNP databases such as Hapmap. The number of tests may, however, be daunting. For the CEU European Hapmap population, for example, a complete test of all SNP pairs on all chromosome pairs would require 334,212,517,340 linkage disequilibrium (LD) calculations. In addition to the large number of such calculations, it is not clear that inter–chromosomal effects are most efficiently detected in this way. Within chromosomes, high levels of LD are expected between closely linked SNPs. Any inter–chromosomal departures from equilibrium are expected to be accompanied by similar departures at neighbouring SNPs on both chromosomes. Therefore it makes sense to test for departures from equilibrium of blocks of SNPs rather than individual SNP pairs. In this presentation I outline such a test. No significant departures from equilibrium expectations have yet been detected.

P18: High Throughput Amplicon Sequencing

of Complex Tandem Repeats in AVPR1a

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Short tandem repeat polymorphisms (STRPs) in the promoter region of AVPR1a gene are associated with brain functions. The human STRP locus (RS3) contains two variable motifs: (CT) and (GT). Differences in their relative numbers may have important functional consequences since one (GT) but not the other (CT) is a purine–pyrimidine tract with potential to induce Z–DNA formation, but these differences cant be detected by genotyping based on PCR fragment size. Thus, for example, the 334 allele with 33 repeats, may include eight cryptic alleles ranging from (CT)6(GT)27 to (CT)13(GT)20. We have developed a method of STRP genotyping based on amplicon sequencing on the Roche GS FLX platform that is capable of distinguishing the full range of variation at complex STRP loci. The method uses the Parallel Tagged Sequencing approach. In an initial application we genotyped RS3 and three other STRP loci in the AVPR1a gene in 59 individuals, who had been previously typed on the basis of PCR fragment size. Using 1/4 of a sequencing plate, 125,551 sequence reads were generated from 58 individuals, 111,462 (89%) of which mapped to the 4 AVPR1a loci giving an average of 480× coverage.

P19: Next–generation sequencing for efficient microsatellite marker development, as demonstrated in two Australian freshwater turtles

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Microsatellite marker development has traditionally been a costly, laborious and often inefficient process, and accordingly one that has limited the accessibility of these high–resolution markers for researchers working with non–model taxa in small laboratories. We describe the successful application of a new cost–effective technique, which employs low–coverage next–generation genomic sequencing to randomly sample the genome for microsatellite loci. The Roche 454–FLX pyro–sequencing platform was used to produce >36 million bases of DNA sequence data (>100,000 reads) for two species of Australian freshwater turtles: *Elseya albagula* and *Emydura macquarii krefftii*, representing ~1–2% of the genome of each species. Freely available bioinformatics software was then used to mine sequence data, identifying large numbers (200–300) of microsatellites in each species suitable for amplification by PCR. Previously, a lack of high–resolution genetic tools has limited fine–scale genetic studies in Australian freshwater turtles. Our experience demonstrates that next-generation sequencing is a fast, efficient and relatively inexpensive way of identifying large numbers of loci useful for such studies.

P20: Tracking the Dispersal of Invasive *Gambusia holbrooki* in Australia Using Genetic Markers

H. Varinli, Y. A. Aksoy, C. Brown and M. R. Gillings Department of Biological Sciences, Macquarie University, NSW, Australia

Gambusia holbrooki is the most widespread freshwater fish in the world as a result of its introduction into many countries to control mosquito borne diseases. It is a serious pest species, causing decline of native fishes and frogs, and is strongly implicated in the decline of freshwater ecosystems wherever it is introduced. To understand the ongoing dispersal of Gambusia in Australia, we examined mitochondrial DNA markers in over 500 individual fish from 40 locations. Populations in NSW, Queensland and Tasmania consisted of a single haplotype across 320 bp of the D–loop. Sequencing of 340 bp of the cytochrome b gene revealed three haplotypes. All three haplotypes were found in NSW, two were found in Tasmania but only one was found in Queensland. Previous studies claim that Gambusia was introduced to Australia from Spain or Italy; however, these countries have only a single cytB haplotype. The diversity of haplotypes in Australia, and the restriction of some haplotypes to small geographic regions, suggests that there were multiple introductions of Gambusia into Australia. Nevertheless, Australian Gambusia must have undergone a series of genetic bottlenecks. Their adaptability and success despite significant reductions in genetic diversity is an interesting evolutionary and management problem.

P21: SNP genotyping of samples from Riverine with mixed Australian indigenous and non-indigenous ancestry

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Comparison of Australian indigenous population has been compared to other ethnic population for mitochondrial DNA and shown distinct Australian specific haplogroups which confirm the long period of genetic isolation of the population. However, relatively little information has been available for large numbers of nuclear loci in this population. This is in part due to the difficulty in satisfying the ethical issues in using samples for genetic testing and the general distrust of scientist because of past treatment. Dr van Holst has developed a close relationship with people of the Darling River region of NSW over the past 17 years through the project, "Indigenous Australian families, genes, health and well–being". Affymetrix SNP 6.0 chips from UC Irvine were processed for 38 samples at the Ramaciotti Centre. A preliminary comparison to 51 populations for 166K SNPs clusters these Riverine people in an Oceania group with Papuans and Melanesians. Although all maternal lineages are aboriginal,

the mixture of indigenous and non–indigenous ancestry is evident in principal components. Comparison to 11 populations from HapMap3 for 576K SNPs separates these Riverine samples from others and confirms their mixed ancestry. We hope to extract some baseline data about variation in some Australian aborigines.

P22: Changes in wing pattern morphometrics of the Common Brown Butterfly (*Heteronympha merope merope*) over a thirty–year period

V. Yazgn[1], *N. Murray*[1], *P. Sunnucks*[2], *M. Kearney*[3], *W. Porter*[4], *M. Norgate*[2], *A. Lister*[1], *M. Barton*[3]
[1]*La Trobe University, Bundoora, Victoria,* [2]*Monash University, Clayton, Victoria,* [3]*The University of Melbourne, Melbourne, Victoria,* [4]*The University of Wisconsin, Madison, Wisconsin, USA*

Global surface temperature has increased substantially over the past 30 years and both rainfall and humidity have erratically altered. Hence it is expected that genetically based and geographically variable characters would alter as adaptive responses to environmental change. A study performed in the 1970s by Kay Pearse (Pearse and Murray, *Evolution*, 1982) documented geographic variation of genetically determined wing–pattern characters of female Common Brown Butterflies (*H. m. merope*) in south–eastern Australia. The study of seven independent wing characters demonstrated climatic associations with phenotypic morphology. If these associations are causal ones, *i.e.* if the characters are indeed adaptive, it can be predicted that these geographically variable characters should have altered given the climatic changes over the past 30 years. This study analyses geographic variation in contemporary *H. m. merope* populations and finds evidence that since the 1970s fundamental changes have indeed occurred in individual characters and in the character–combinations that contribute most to the variation between populations. These changes, as well as contemporary geographic patterns of variation and their relationships to climatic variables, will be described and discussed.

P23: The Arabidopsis B–sister MADS–box gene AGL63 controls fruit development via cell expansion

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The MADS–box family of transcription factors have diverse functions in flower pattern formation, gametophyte cell division and fruit differentiation. The B–sister MADS–box proteins are most similar to the B–class floral homeotic proteins, and are expressed in female reproductive organs. The Arabidopsis B–sister MADS–box protein, TT16, is necessary for inner integument differentiation. We have functionally characterized the only other B–sister MADS–box gene in Arabidopsis, AGL63. A loss–of–function mutation in AGL63 or reduction of endogenous AGL63 expression results in larger fruits, illustrating its novel function in regulating fruit growth. Consistent with its function, AGL63 expression is detected in the walls of the valves and throughout the replum of the fruit. Our phenotypic and molecular analyses of 35S::AGL63 and agl63 plants show that AGL63 controls organ size via cell expansion. Further, functional studies of agl63tt16 double mutants have shown their additive role in controlling seed coat development, and have revealed the importance of AGL63 expression in the outer integument. Together, our studies provide evidence of a new regulatory role for a B-sister MADS-box gene in the control of organ growth.

Keynote: Wednesday morning

T61: A New Class of Small RNAs: unlocking the secrets of the tammar genome

Rachel O'Neill
Department of Molecular and Cell Biology, University of Connecticut

Although only recently discovered, small RNAs have proven to be essential regulatory molecules encoded within eukaryotic genomes. These molecules, represented by four major class sizes ranging from 20nt to 42nt, are participants in a diverse array of cellular processes including gene regulation, chromatin dynamics and genome defense. The genome sequencing initiative for the tammar wallaby has afforded an opportunity to explore the evolution of each of the major classes of small RNAs, siRNAs, miRNAs, piRNAs, and the newest class of small RNAs, crasiRNAs (centromere repeat associated short interacting RNAs), first discovered in the tammar wallaby. Our analysis includes the detailed examination of these small RNAs, salient features that define their canonical members and the constitution of species–specific members derived from mobile genetic elements. Understanding the evolution of these important regulators and surveyors of the genome in this unique marsupial model brings valuable insights into the processes of genome and chromosome evolution.

Mammalian Genetics I

T62: Genome rearrangements in Devil Facial Tumour Disease

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The Tasmanian devil is now listed as an endangered species due to a contagious cancer. Devil Facial Tumour Disease (DFTD) is ravaging the Tasmanian devil population and could lead to extinction of the species in the wild within the next few decades. Cytogenetic analysis of DFTD tumours provided the first evidence for the clonal transmission of the disease, with tumours from different individuals possessing identical chromosomal rearrangements (Pearse and Swift, *Nature*, 2006). The highly rearranged tumour karyotype consisted of 13

chromosomes including four marker chromosomes, the origins of which could not be determined with standard karyotyping techniques. It has been of great interest since the initial characterisation of DFTD tumours to determine the origins of these marker chromosomes and more accurately assess the extent of rearrangement between normal and tumour chromosomes. Physical mapping of over 100 genes onto normal and DFTD tumour chromosomes has provided further insight into the extent of rearrangement between normal and DFTD chromosomes and determined the origin of the marker chromosomes. These gene maps will be an extremely useful resource for the assembly of genome sequences and the detailed characterisation of this unusual disease.

T63: You don't know Y–the marsupial Y story

V. J. Murtagh

Evolution, Ecology and Genetics, Research School of Biology, ANU, Canberra

The marsupial Y chromosome has traditionally been portrayed as a degraded relic of the original therian mammalian Y, lacking the large autosomal addition that revitalised the sex chromosomes of eutherian mammals. Our interest in the marsupial Y lies in its ability to provide insight into the early events in the degradation of the therian Y chromosome. Here I compare marsupial and eutherian Y chromosomes and describe five novel marsupial Y genes isolated from the tammar wallaby. QPCR analysis illustrates that all five genes are ubiquitously expressed. These results also indicate a lower level of expression for all but one of these genes compared to their X partners, which is indicative of sex chromosome dosage compensation. By isolating sequences from other model marsupial species, representing dasyurids and american marsupials, I provide evidence that these genes were present on the original therian Y. We now know that only five of the eleven genes known on the marsupial Y are still present in eutherians. This surprising difference could mean either that the marsupial sex chromosomes differentiated more slowly than eutherian sex chromosomes, or that Y genes were retained in marsupials due to acquisition of a selectable function in males.

T64: X chromosome inactivation: expression, escape and evolution

S. Al Nadaf[1], P. D. Waters[1,2], E. Koina[1,2], J. E. Deakin[1,2], K. S. Jordan[1], J. A. Marshall Graves[1,2]

[1]Research School of Biology, The Australian National University, Canberra, Australia; [2]ARC Centre of Excellence for Kangaroo Genomics

X chromosome inactivation (XCI) is arguably the most striking example of epigenetic regulation in mammalian genomes. To examine the evolution of XCI we investigated X–borne gene expression in a model marsupial, the tammar wallaby. Unlike the best-studied models of X inactivation, in humans and mice, information on marsupial XCI comes largely from five X–borne genes. Our study focused on examining gene dosage at the cellular level, by examining expression of 25 genes mapped to the tammar X. Our results show that loci on one X (the active X; Xa) were co–ordinately expressed in every cell, but loci on the other X (the inactive X; Xi) were independently expressed at locus-specific frequencies. This suggests that stochastic transcriptional inhibition (on Xi) is the basis for XCI in marsupials, similar to that observed in platypus where dosage compensation is controlled by regulating the probability of transcription, rather than the amount of transcription. Furthermore, the activity map of the tammar X reveals no correlation between gene location and XCI status, implying that unlike mouse and human, there is no regional control of XCI and, therefore, no XCI centre. These findings, along with the monotreme results, raise the possibility that XCI may have evolved more than once during the course mammalian evolution, probably from stochastic monoallelic expression. Our results also provide evidence for remarkable diversity in the mechanisms underlying dosage compensation mechanisms between mammalian groups.

T65: Dosage compensation for the multiple platypus X chromosomes

A. M. Livernois, P. D. Waters, J. E. Deakin, J. A. M.Graves

Comparative Genomics Group, Research School of Biology, The Australian National University, Canberra ACT, Australia.

The unique phylogenetic position of the egg–laying monotremes, along with the surprising discovery that their sex chromosomes share considerable homology with the chicken Z chromosome, but not the therian X chromosome, makes platypus a fascinating species in which to study dosage compensation. Necessity of dosage compensation would be expected in the platypus because of their complex sex chromosome system; with males having five Xs and five Ys, and females having five pairs of Xs. However, it appears that some genes on the sex chromosomes are not dosage compensated between males and females, whereas others display varying degrees of compensation through stochastic transcriptional inhibition. Transcription of 27 BACs, representing 22 X–specific, and 11 pseudoautosomal genes was examined by RNA–FISH in male and female fibroblast cells. Most pseudoautosomal BACs were expressed from both alleles in female and male fibroblasts, implying the Y alleles are active. I will discuss the partial and stochastic dosage compensation of platypus X genes and explore why dosage compensation is not tightly controlled in platypus. Finally, I will touch upon the evolution of stable and complex X chromosome inactivation, typical of most therian X–borne genes, from random monoallelic expression, not unlike the partial dosage compensation we observe in the platypus.

T66: Evolution of atypical T cell receptor loci in marsupials and monotremes

R. D. Miller

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A novel T cell receptor locus, called TCRμ, was discovered in marsupials and a homologue has been found in monotremes. The genomic organization of TCRμ is unusual and is consistent with its origins through recombination between conventional TCR genes and those encoding the immunoglobulin heavy chain locus. Although marsupial and monotreme TCRμ are clearly homologous, they have followed different evolutionary paths. The marsupial TCRμ has been subject to retro–transposition events that have eliminated the need for somatic DNA recombination of the genes encoding an invariant variable–type domain. The platypus TCRμ, on the other hand, still undergoes somatic recombination like conventional T cell receptors, albeit in a way that limits diversity resulting in a similarly invariant variable–type domain. The predicted structure of TCRμ resembles that of atypical TCR found in amphibians and sharks and suggests a widespread distribution across the jawed vertebrates. Nonetheless, TCRμ appears to have been lost in the eutherian lineage of mammals.

T67: Transcriptomic analysis and gene identification in the cervical and thoracic thymuses of the tammar wallaby

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The thymus plays a critical role in the development and maturation of T–cells. Humans have a single thoracic thymus and presence of a second thymus is considered an anomaly. However, many vertebrates have multiple thymuses. The tammar wallaby has two thymuses: a thoracic thymus (typically found in all mammals) and a dominant cervical thymus. Researchers have known about the presence of the two wallaby thymuses since the 1800s, but no genome–wide research has been carried out into possible functional differences between the two thymic tissues. Here, we used pyrosequencing to compare the transcriptomes of a cervical and thoracic thymus from a single 178 day old tammar wallaby. Using a dataset of wallaby genes including a curated immune gene set which I constructed, we show that both the tammar thoracic and the cervical thymuses displayed gene expression profiles consistent with roles in T–cell development. Both thymuses expressed genes that mediate distinct phases of T–cells differentiation, including the initial commitment of blood stem cells to the T–lineage, the generation of T-cell receptor diversity and development of thymic epithelial cells. Comparable patterns of expression of non–coding RNAs were seen. 67 genes differentially expressed between the two thymuses were detected, and the possible significance of these results are discussed. This is the first study comparing the transcriptomes of two thymuses from a single individual. Our finding supports that both thymuses are functionally equivalent and drive T–cell development. These results are an important first step in the understanding of the genetic processes that govern marsupial immunity. Furthermore, this dataset has been used to improve the immune gene annotations of the tammar genome assembly. Of the 603 immune genes I annotated, 209 were not detected by Ensembls tammar wallaby gene build.

T68: Characterization of Tasmanian devil MHC class I genes

Y. Cheng, H. Siddle, K. Belov

Faculty of Veterinary Science, The University of Sydney, NSW, Australia

The Tasmanian devil (*Sarcophilus harrisii*) is under threat of extinction due to a newly emerged and fast spreading contagious cancer: devil facial tumour disease (DFTD). DFTD cells are transferred between devil individuals as allografts[1] without inducing immune rejection[2]. Investigations on the major histocompatibility complex (MHC) of devils, which plays a central role in disease resistance and graft rejection, provide significant insights into this highly unusual disease. In this talk, I will focus on our strategy to characterize Tasmanian devil MHC classical class I genes, via isolation of cDNA transcripts which are matched to genomic sequence on BACs. I will discuss sequence variation, copy number variation and genomic organization of devil MHC genes. I will also discuss the challenges we have encountered and the strategies we used to solve these puzzles.

[1]Pearse, AM, Swift, K 2006. Allograft theory: transmission of devil facial-tumour disease. *Nature* **439**, 549.

[2]Siddle HV, Kreiss A, Eldridge MDB, *et al.* 2007. Transmission of a fatal clonal tumor by biting occurs due to depleted MHC diversity in a threatened carnivorous marsupial. *PNAS* **104**, 16221.

Disperal and Invasion

T69: Chasing invasive moths step 1: Mitochondrial population genetics of *Epiphyas postvittana*

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[1]*The New Zealand Institute for Plant and Food Research Limited, Auckland, New Zealand,* [2] *USDA-APHIS, Edinburg, TX, USA*

Invasive species are not only problematic for native wildlife and ecosystems but also for authorities and industries if they represent pests or pathogens of crops, animals and humans. Here we investigate the popula-

tion structure and potential invasion pathways of the light brown apple moth, *Epiphyas postvittana*, a horticultural pest native to Australia that has invaded New Zealand, Hawaii, Europe and more recently California. A 2,216 base pair region of the mitochondrial genome containing the CO I and II genes was sequenced from 681 individuals from across the moths global range. Measures of both nucleotide and haplotype diversity were highest in Australia and New Zealand, with evidence for structuring and haplotype sharing in both countries. Levels of nucleotide and haplotype diversity were higher in Californian populations, compared with the other recently invaded regions of Hawaii and Europe. Furthermore, there are likely to have been two introductions into California, one into northern California and another into Los Angeles.

T70: Wolbachia: an agent for biocontrol

E. McGraw
School of Biological Sciences, The University of Queensland, St. Lucia Qld

A strain of the endosymbiont, *Wolbachia pipientis* has been transferred into the mosquito, *Aedes aegypti* as part of a planned biocontrol strategy against dengue fever. The strain of *Wolbachia* shortens the host insect's lifespan. This would be useful in the field as only old mosquitoes are capable of transmitting pathogens to humans, such as dengue fever. This strain of *Wolbachia* also causes cytoplasmic incompatibility that could assist in the rapid spread of the infected mosquitoes into wild populations. In an attempt to more broadly understand the effects of the *Wolbachia* on the host, we examined mosquito feeding behaviour and the ability of vectored pathogens to coexist with the symbiont. We found a decreasing ability for the mosquitoes to feed as they aged and that the presence of *Wolbachia* in the mosquito limited the replication of dengue virus, Chikungunya virus and the malaria parasite. In combination with the life-shortening trait, these characteristics may enhance the ability of field released *Wolbachia* infected mosquitoes to prevent transmission of disease to humans. Recent population field cage trials are demonstrating the ability of *Ae. aegypti* infected with *Wolbachia* to spread and compete in a mixed population.

T71: Dispersal, connectivity and invasion in regulated rivers: insights from weed population processes

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The movement dynamics of riverine plants are difficult to predict and are not well understood, particularly in river systems impacted by heavy regulation. Results are presented of a study that identifies the patterns of dispersal and connectivity among invasive weed populations in the Ramsar–listed Barmah Forest and tributaries on the River Murray floodplain, Victoria, Australia. The prevailing flow regime, regulatory infrastructure and unique hydrogeomorphological characters can together influence the tempo and directionality of dispersal among river habitats. Clonal and seed propagules may both contribute to the movement of individuals, but occur at different spatial scales. We used demographic studies, molecular genotyping and network analyses as complementary research approaches to determine the implications of reproductive strategy and dispersal for spatial connectivity among streams, using *Sagittaria platyphylla* (Alismataceae) as a model, recently introduced aggressive weed species. Findings are discussed in the context of deciphering population structure and connectivity in river environments, and improving the detection and management of population processes in prominent riverine plant species in the Murray–Darling River Basin.

T72: Dispersal dynamics of the invasive willow, *Salix cinerea*, in southeastern Australia

T. Hopley[1,2], *A. Young*[1]
[1] *CSIRO Plant Industry, Canberra, ACT, Australia,* [2] *Australian National University, Research School of Biology, Acton, ACT, Australia*

Willows are aggressive exotic components of many river systems in southeastern Australia and they have the potential to expand their range. Current controls efforts for the most highly invasive species, *Salix cinerea*, are extensive, costly and not always successful due to rapid post removal reinfestation. An improved knowledge of the dispersal dynamics of this species will help to minimise future expansion and make current control efforts more effective. A survey of populations in the Ovens River catchment of southeastern Australia has been undertaken to determine seed and pollen movement within and between populations of *S. cinerea*. Preliminary paternity analysis using molecular markers show that up to 50% of seed on trees are sired from outside the home population. Genetic profiling of populations in surrounding rivers will allow us to identify the most likely pollen sources thus providing data on the scale of pollen movement. Parentage analysis to ascertain the origin of seedlings in these same populations will allow us to directly measure the scale of seed dispersal. The results from this study will provide information on patterns of willow seed and pollen movement and its relationship to landscape structure. These results will assist land managers responsible for controlling willows to develop more effective eradication strategies.

T73: Australians making themselves at home; the population genetics of some recent New Zealand invaders

G. Houliston[1], *P. Heenan*[1], *R. Jain*[2]
[1] *Landcare Research, Lincoln, New Zealand,* [2] *Landcare Research, Auckland, New Zealand*

Reconstruction of the introduction history of a plant emerging from the so–called "lag-phase" and becoming a serious threat outside their native biogeographical ranges is an important step towards understanding and designing strategies that prevent or manage invasions. New Zealand has a high number of weed species (700 new emerging weeds have been recorded

in the past 20 years), and an even higher number of naturalised plants (ca. 3500). Despite this, we have a poor understanding of the ecological and evolutionary processes between naturalisation and weediness. Using two Australian species that are emerging weeds in New Zealand (Alpine wattle, *Acacia pravissima*; Coastal Banksia, *Banksia integrifolia*), we will compare the population genetics in the native range with both the cultivated and naturalised populations in New Zealand. By comparing the amount of genetic variation in naturalised and native species, we may gain insights into what factors determine the success of new invaders.

T74: Gene flow unaffected by landscape context: levels of out–crossing maintained in fragmented populations of the bird–pollinated Emu bush (*Eremophila glabra ssp. glabra*), in south–eastern Australia

C. P. Elliott[1,3], A. Zwart[2], D. Lindenmayer[3], S. Cunningham[4], A. Young[1]

[1]*CSIRO Plant Industry, Canberra,* [2]*CSIRO Mathematics, Informatics and Statistics, Canberra,* [3]*Fenner School of Environment and Society, ANU, Canberra,* [4]*CSIRO Entomology, Canberra*

We investigated the effect of landscape context on gene flow on a common, bird–pollinated, autohexaploid shrub (*Eremophila glabra ssp. glabra*) to assess connectivity in fragmented landscapes, in south–eastern Australia. We contrasted three replicated landscape contexts (interior element; near element; far element) at different distances from a large vegetation remnant. We compared the frequency of selfing and both within (local) and between (foreign) population out–crossing through paternity exclusion analysis and examined the relationship between pollinator movement and gene flow by assessing the ratio of within versus between population matings. *Eremophila glabra ssp. glabra* was highly out–crossed (>90%) and we found no difference in the frequency of local or foreign gene flow of populations in different elements. We attribute this lack of difference to self–incompatibility and highly mobile pollinators. We also found no relationship between pollinator movement patterns and gene flow patterns. We demonstrate that gene flow of a common plant species, with a highly mobile pollinator was unaffected by the spatial context of fragmented remnants as evidenced by high levels of foreign out–crossing. Therefore, we conclude that current immigrant gene flow is crucial to populations and pollinators were key to maintaining genetic connectivity among populations in highly fragmented landscapes.

Keynote: Wednesday afternoon

T75: The Equine Genome

Claire M. Wade[1,2] *and The Equine Sequencing Consortium*

[1]*Faculty of Veterinary Sciences, The University of Sydney, NSW Australia,* [2]*Broad Institute of the Massachusetts Institute of Technology and Harvard, Cambridge, MA, USA*

The Equine Genome Sequencing consortium has produced a 6.8× high quality version 2 draft assembly (N50 contig size 112kb and N50 supercontig size of 46Mb) of the ∼2.68Gb genome of a Thoroughbred mare. Roughly 96% of the sequence has been ordered and oriented on the chromosomes ($2n = 64$) using existing linkage maps and FISH. In addition to the genome assembly, a SNP map (∼1.2 million SNPs) has been generated by identification of SNPs within the genome assembly and by generation of ∼100,000 whole genome shotgun reads from each of seven horse breeds; Akhal–Teke, Icelandic, Arabian, Andalusian, Quarter Horse, Thoroughbred and Standardbred. The breeds were chosen to represent a mixture of ancient and recent populations. The haplotype structure of the equine genome has been studied using 10 random 2 Mb regions that have been genotyped in 24 individual representatives from diverse horse breeds as well as 24 representatives from each of 11 horse breeds: Thoroughbred; Arabian; Quarter Horse; Icelandic; Hokkaido; Hanoverian; Andalusian; Belgian Draft; Norwegian Fjord; and French Trotters. These analyses were used to design the Illumina 60K horse genotyping arrays that are now in production. This talk will highlight the special features of the equine genome assembly and analysis and will discuss the results from the coat color mutation detection using hybrid capture technology in conjunction with Illumina Genome Analyzer (Solexa) sequencing.

The Ross Crozier Symposium

T76: Application of GWA data to address issues of genetic loading in complex human diseases

J. N Painter[1], H. Lee[2], S. McGregor[2], D. Nyholt[3], P. Visscher[2], G. W. Montgomery[1]

[1]*Molecular Epidemiology.* [2]*Queensland Statistical Genetics,* [3]*Neurogenetics Laboratories, Queensland Institute of Medical Research, Brisbane, Australia*

Endometriosis is a common gynaecological disease associated with severe pelvic pain and sub–fertility. We have conducted a genome–wide association (GWA) study using 540,082 SNPs in 3,194 surgically confirmed endometriosis cases and 7,060 controls from Australia and the UK. There is currently some debate as to whether different endometriosis stages represent disease progression or whether the milder form of the disease is an epiphenomenon. Prior to conducting our association analyses we applied novel statistical methods to estimate the proportion of variation explained by the GWA markers (which serves as a measure of heritability in a case:control population)[1] and perform polygenic predictive modelling[2]. Both of these methods showed significantly increased genetic loading among cases with moderate–severe (Stage B) disease. Based on these results we then conducted our association analyses on "all" and "stage b" cases and found the strongest association signals for the

stage b analysis. This approach has been successfully applied to other complex traits for which GWA data have been generated at QIMR. Examples of these will be presented to demonstrate the application of GWA data to issues of genetic loading underlying phenotypic variability in complex human diseases.

[1]Yang *et al.* Common SNPs explain a large proportion of heritability for human height. Nat. Genet. accepted.

[2]The International Schizophrenia Consortium. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* **460**, 748–752 (2009).

T77: Why do species differ in their rate of molecular evolution?

L. Bromham

Centre for Macroevolution and Macroecology, Research School of Biology, ANU, Canberra

Despite hopes that the processes of molecular evolution would be simple, clock–like and essentially universal, variation in the rate of molecular evolution is manifest at all levels of biological organization. Furthermore, it has become clear that rate variation has a systematic component: rate of molecular evolution can vary consistently with species body size, population dynamics, lifestyle and location. This suggests that the rate of molecular evolution should be considered part of life–history variation between species, which must be taken into account when interpreting DNA sequence differences between lineages. I will discuss the biological factors that can influence both the mutation rate and the substitution rate, creating consistent differences in rate of molecular evolution between lineages. In particular, I will present evidence that differences in mutation rate between species are linked to the rate of lineage diversification.

T78: No sex please: We're Cape bees

B. P. Oldroyd[1], *M. H. Allsopp*[2], *J. Lim*[1], *M. Beekman*[1]

[1]*Behaviour and Genetics of Social Insects Lab, School of Biological Sciences, University of Sydney, Sydney,* [2]*Plant Protection Research Institute, Agricultural Research Service, Stellenbosh, Republic of South Africa*

The honey bee population of South Africa is divided into two subspecies: an arrhenotokous population in the north (*A. m. scutellata*), and a thelytokous population in the south (*A. m. capensis*). A stable hybrid zone separates the two populations. However, on at least three occasions (two historical and one current) the *Scutellata* population has become infested by reproductive workers derived from the *Capensis* population. These parasitic workers lay eggs in host *Scutellata* colonies parthenogenetically, resulting in yet more parasites. Genetic analyses have shown that the current infestation derived from a single worker that lived more than 10 years ago. The longevity of this infestation is surprising because an asexual lineage is expected to show a decline in vigour over time due to increasing homozygosity and an increase in mutational load. The decline is expected to be particularly acute in honey bees, where homozygosity at the sex locus is lethal. To understand the mechanisms that may contribute to the longevity of this lineage we surveyed 51 colonies from throughout the zone of infestation. We genotyped putative parasites at two sets of tightly linked loci (Shaibi *et al.*, 2008), one set linked to the sex locus (Sex) , and one linked to a region thought to be involved in the regulation of theltoky (The). We confirm that there is indeed a single clonal lineage of parasites. The lineage shows minor variations arising from recombination events, but no mutations were observed. Within the clonal lineage the The loci show remarkably high levels of heterozygosity. This heterozygosity may be maintained by selection against homozygotes, or by a reduction in recombination frequency within the lineage. The relative merits of these alternative hypotheses will be discussed. Surprisingly, the Sex loci are invariably homozygous. Yet the individuals are unequivocally diploid females, and are heterozygous at the sex locus itself.

Shaibi T., Lattorff H.M.G. and Moritz R.F.A. 2008. A microsatellite DNA toolkit for studying population structure in *Apis mellifera*. *Molecular Ecology Resources* **8**:1034–1036.

T79: What can the bees teach us about sex?

F. Goudie[1], *B. P. Oldroyd*[1], *M. Beekman*[1], *M. H. Allsopp*[2].

[1]*Behaviour and Genetics of Social Insects Laboratory, School of Biological Sciences, University of Sydney, Sydney, NSW,* [2]*Honey Bee Research Section, Agricultural Research Council–Plant Protection Research Institute, Stellenbosch, Republic of South Africa*

The "two-fold cost of sex" makes the near ubiquity of sexual reproduction an enduring evolutionary mystery. The Cape honey bee, *Apis mellifera capensis*, is one of a small subset of organisms in which sexual reproduction is facultative, providing an opportunity to directly investigate the "Paradox of Sex". Cape queens produce daughters sexually, whereas workers produce clonal female offspring by thelytokous parthenogenesis. Thelytoky has enabled clonal parasitic lineages to emerge that endure for decades without sex. Thelytoky in the Cape honey bee is predicted to lead to a decline in heterozygosity and therefore fitness, particularly egg viability. However, surprisingly high rates of heterozygosity are observed in the clonal offspring of Cape workers, including a parasitic lineage. It is currently hypothesised that workers avoid loss of heterozygosity by reduced meiotic recombination. We have detected an increase in rates of heterozygosity between clonal eggs and pupae. This increase is evidence of selection against recombinant offspring, rather than reduced recombination. We therefore propose that Cape workers can endure a substantial reduction in egg viability, whereas queens cannot. Thus the costs and benefits of sex in the Cape honey bee vary between castes and are conditional rather than absolute.

T80: Confirmation of Three Genetic Loci Associated with Worker Sterility in Honey Bees (*Apis mellifera*)

A. Faiz, P. Oxley, B. Oldroyd
Behaviour and Genetics of Social Insects Laboratory, School of Biological Sciences, University of Sydney, NSW
One of the defining features of eusocial insect societies is reproductive division of labour, yet genes that limit reproduction in worker castes–so called "genes for altruism"–have not yet been identified. In honey bees (*Apis mellifera*), a selected "anarchist" line exists, in which up to 40% of workers have activated ovaries in contrast to 0.1% in wild–type strains. Four genetic loci (OvA1–4) linked to ovary activation have been previously identified in this line. We report the results of a second backcross experiment in which we characterise the mode of inheritance of anarchy and confirm the existence of the genetic loci previously identified. A total of 267 workers exhibiting both extremes of ovary activation were genotyped at 59 loci spanning chromosomes 1, 7, 13 and 15. Six genetic markers linked to OvA2–4 were found in which workers with active ovaries were significantly more likely to be homozygous for the anarchistic alleles. Based on functional annotation, we have identified three candidate genes linked to OvA2, one of which was found to be differentially expressed between anarchist and wild–type workers in a previous microarray study. We therefore confirm the presence of three loci involved in expression of the anarchy phenotype and will present the primary gene candidates for the regulation of worker sterility in the honey bee.

T81: Exposing wildlife traffickers using DNA: Australian Museum case studies in wildlife–forensics using DNA–identification

R. Johnson, R. Mason, A. King
Australian Museum DNA Laboratory, Sydney Australia
Illegal wildlife trafficking is both a multi–billion dollar industry and a worldwide problem that puts species survival and unique biodiversity such as Australia's at risk through potential introduction of pest species. Museums and Herbaria hold unique and extremely valuable natural history collections that provide unrivalled reference material for validation when unidentifiable fauna and/or flora are seized and DNA identification is required. Accurate species identification is often essential so penalties can be imposed under relevant Australian legislation. The Australian Museum has been involved with cases involving wildlife forensics for almost 10 years. For those seizures of animal parts unable to be identified using traditional taxonomy, such as exotic bird embryos and shark fins, DNA is critical for species identification so the full extent of the species involved can be revealed. Wildlife trafficking is not only cruel to the individual animals involved, but because the target species are often rare or endangered it also places the survival of that species at risk. There is an extremely large cost associated with these crimes through policing and enforcing border security measures as well as the risk to the unique biodiversity of Australia through potential introduction of exotic species. This presentation will give an overview of some wildlife forensics cases the Australian Museum has been involved with where species identification has been critical that have involved DNA–based identification.

T82: The evolution of multipartite mitochondrial genomes is associated with extreme mitochondrial gene rearrangement

T. Gibson[1], *D. Farrugia*[1], *J. Barrett*[1], *D. Chitwood*[2], *J. Rowe*[3], *S. Subbotin*[4], *M. Dowton*[1]
[1]*Centre for Biomedical Sciences, School of Biological Sciences, Wollongong University, Wollongong, NSW, 2522, Australia.* [2]*Nematology Laboratory, USDA, Beltsville MD, USA.* [3]*Nematode Interactions Unit, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom.* [4]*Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, CA, USA*
The heteroderid nematodes, *Globodera pallida* and *G. rostochiensis* are one of the few groups of animals that have a multipartite mitochondrial genome. In such genomes, mitochondrial genes are distributed on multiple circles. We sequenced multigenic fragments from the mitochondrial genomes of a range of heteroderid nematodes, in order to understand the evolution of multipartitism. Sequences were obtained from the the entire coding region of the mitochondrial genome of *Heterodera glycines*, while multigenic fragments from two other heteroderids, *Heterodera cardiolata* and *Punctodera chalcoensis*, were obtained. There was no evidence of multipartitism in *H. glycines*, nor in *H. cardiolata*. Genome organization was very similar between the *Heterodera* and *Punctodera* species, but very different to that described in *G. pallida* and *G. rostochiensis*. Outgroup comparison, employing the pratylenchid *Radopholus simulis*, revealed that *G. pallida* and *G. rostochiensis* have an extremely derived genome organization, while all other heteroderids have an organization similar to *Radopholus*. These data suggest that mulitpartitism has evolved relatively recently, during the divergence of the Punctoderinae, and may be restricted to the genus *Globodera*. Further, the evolution of multipartitism is associated with extreme genome reorganization.

T83: Integrating indirect selection into the Hamiltonian evolutionary theories of Aging: Formalization of sociality in genetic theory

S. Newman, S. Easteal
Predictive Medicine Group, Department of Genome Biology, JCSMR
Investigations into the evolutionary nature of aging are based upon theory that has always been, by their own author's admissions, inadequate (Hamilton, 1966, especially pp.12, 23, 37–41; Williams, 1957, p. 407). A wide disparity between prediction and evidence arises when aging theory is applied to social species. The source of this disparity is the implicit use of reproductive output as a surrogate for fitness. This theoretical disparity is discussed, and formulae in which both direct and indirect fitness are integrated into a cogent theory of aging is put forward. Unlike

other attempts to redress the inadequacy of the current evolutionary theory of aging (*e.g.* Lee 1993), this approach is based on quantifiable, real–world variables that are generally applicable to all species. Sources of data sufficient to provide empirical tests are provided, and a roadmap to statistically testing the theory is presented.

R.Lee. Rethinking the evolutionary theory of aging: Transfers, not births, shape senescence in social species. Proc Natl Acad Sci U S A, 100(16):9637–9642, 2003

W.D. Hamilton. The moulding of senescence by natural selection. J Theor Biol, 12(1):12–45, 1966

G.C. Williams. Pleiotropy, natural selection and the evolution of senescence. Evolution, 11(4):398–411, 1957.

T84: Using evolutionary genetics to determine the prevalence of phosphine resistance in Australian *Cryptolestes* species

W. T. Tay

CSIRO Entomology, Black Mountain Laboratories, Clunies Ross Street, ACT, Australia

Australia's ability to access premium grain export markets greatly relies on zero tolerance to live insects in exported grain commodities. Currently phosphine is by far the most widely–used fumigant to control stored grain pest insects, as it is considered to be residue–free, and is easy and cheap to apply. The development of resistance to phosphine in stored grain pest insects in Australia is therefore of concern as this has the ability to drastically affect Australia's grain exporting potential. In the flat grain beetle species *Cryptolestes ferrugineus*, weak and strong phosphine resistance have been reported since the late 1990s. More recently, high phosphine resistance was detected in northern NSW *C. ferrugineus* populations, although no information on the molecular evolution of this economically important pest beetle genus is available to–date. Here I report on preliminary evolutionary genetics of the flat grain beetle *Cryptolestes* genus present in Australia, and discuss the implications of the findings with respect to phosphine resistance status based on the mitochondrial DNA partial Cytochrome Oxidase I gene as genetic marker.

T85: Population and colony genetic structure of the eusocial ambrosia beetle *Austroplatypus incompertus*

S. M. Smith, A. J. Stow

Department of Biological Sciences, Macquarie University, NSW, Australia

The Ambrosia beetle, *Austroplatypus incompertus*, has two distinctive qualities. First, it is the only described beetle species out of approximately 300,000 known to be eusocial. Second, it is one of only a handful of species worldwide which bore into living, healthy trees. Inhabiting Eucalypt trees, their gallery and food source is relatively stable and although extensive, possesses a single small entrance that is easily defended. Due to this cryptic lifestyle, direct behavioral observations are unattainable and molecular approaches are required to learn more about this unique system. Trapping of beetles at four localities throughout NSW has yielded sufficient specimens to examine spatio–temporal molecular variation. Considerable disparity in mitochondrial sequence data signifies very strong divergence at a regional scale. This pattern will be further explored using a set of newly developed microsatellite markers. We will characterize, for the first time, the mating system, social structure (group composition) and population genetics of *A. incompertus* within *Eucalyptus pilularis* from the Eastern ranges of Australia. The relatedness data generated in this project will also increase our ability to pursue alternative hypotheses for the evolution of eusociality within Coleopterans.

Mammalian Genetics II

T86: Forward genetics in the mouse to discover gene functions relevant to human disease

M. Dobbie

Australian Phenomics Facility, ANU, Canberra

This presentation will outline the phenotype–driven approach to functional genomics and the way outcomes can contribute to better understanding of the genetic basis of human health and disease. With the sequencing of a large number of genomes, efforts are now being made to assign functions to every gene. A powerful, unbiased way to reveal gene function is to use an efficient, genome–wide mutagen to create collections of mutant organisms that can be screened for phenotypes of interest, an approach known as forward genetics. In the mouse, the most efficient mutagen is the chemical N–ethyl–N–nitrosourea (ENU), which also has the advantage of introducing point mutations, thereby mimicking the most common form of disease–causing allelic variants in humans. At the Australian Phenomics Facility (APF), in partnership with other Australian Phenomics Network (APN) members, we combine this mutagenic process with systematic phenotype screening to identify those mutant mice carrying informative mutations that are relevant for a wide variety of biological questions, covering normal and pathogenic processes.

T87: Major Histocompatibility Complex Class II–DZB diversity in the platypus (*Ornithorhynchus anatinus*)

M. Lillie[1], *R. E. Woodward*[2], *C. Sanderson*[1], *M. D. B. Eldridge*[2], *K. Belov*[1]

[1] *Faculty of Veterinary Science, University of Sydney, Sydney, NSW, Australia,* [2] *Australian Museum, Sydney, NSW, Australia*

The platypus (*Ornithorhynchus anatinus*) is a semi–aquatic monotreme found in waterways of eastern Australia, Tasmania, Kangaroo Island and King Island. Although the platypus is classified as least concern by the IUCN, its dependence on healthy water systems imposes an inherent sensitivity to habitat degradation and climate change. The major histocompatibility complex (MHC) is a highly polymorphic multigene family with a key role in the vertebrate immune response. As the interface between infectious pathogens and the

host immune system, high variability in the MHC confers wide pathogen recognition to an individual. At the population level, high MHC diversity equates to higher immunological competence of a population in the face of emergent disease, as there is an increased chance that resistance genes will be present in the gene pool. The MHC Class II–DZB–β1 locus was sequenced from 70 platypuses sampled across their distribution. Relatively high levels of allelic richness were observed, with balancing selection maintaining MHC diversity across populations. In contrast, the King Island platypuses were monomorphic at the DZB–β1 locus, and loss of genetic diversity at neutral microsatellites suggests predominance of genetic drift. As a result these platypuses may have compromised immunological fitness and disease resistance.

T88: Fine Scale Genetic Structuring and Inbreeding Avoidance in the Mountain Brushtail Possum

M. Blyton[1,2], *S. Banks*[1], *R. Peakall*[2]

[1] *The Fenner School of Environment and Society, ANU, Canberra,* [2] *Research School of Biology, ANU, Canberra*

Inbreeding depression can be a powerful factor in the evolution of mating patterns; driving selection for kin avoidance. Detailed observational studies combined with radio tracking have revealed the existence of complex social and sexual behaviours in animals. The advent of powerful genetic markers and individual–based statistical techniques has enabled further insights into these social behaviours and their influence on the spatial distribution of kin. Inbreeding avoidance is commonly detected by comparing the genetic similarity of mates to that expected under random mating. However, this approach ignores restricted access to mates and fine scale genetic structuring, which can lead to the hypothesis of kin avoidance in mate choice being rejected. Genetic analysis of the mountain brushtail possum, that takes advantage of an 18 year demographic study, has found evidence for inbreeding avoidance in female mate choice, when restricted mate access is considered. However, genetic similarity was only important in mate choice when females selected more distant mates, illustrating the complexity of interacting factors that determine mating patterns.

T89: Dispersal and population genetic structure of the grey–headed flying–fox, *Pteropus poliocephalus*

H. J. Baldwin[1], *P. Eby*[2], *J. T. Chan*[3], *A. J. Stow*[1]

[1] *Department of Biological Sciences, Macquarie University,* [2] *Department of Environment and Climate Change* [3] *Evolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences UNSW*

The grey–headed flying–fox (*Pteropus poliocephalus*) is a threatened species that has experienced a rapid population decline of approximately 30% in the last twenty years. This study investigates population structuring among permanent grey–headed flying–fox colonies. The genetic analyses conducted to date suggest that the grey–headed flying–fox breeds as a single panmictic population, but the strength of this conclusion is limited by the analyses used (allozymes and mitochondrial DNA), which are capable of showing only broad patterns. Here I use microsatellite data to address several key questions: *(1)* is the species a single unit or a metapopulation? *(2)* does natal philopatry occur? and *(3)* is dispersal sex–biased? Analyses of five colonies extending from Melbourne to Brisbane revealed high levels of dispersal and the absence of evidence for natal philopatry or sex–biased dispersal. This research has important implications for the management practices of grey–headed flying–foxes. This study supports managing the species as a single connected population, and therefore advocates amendment of the conservation status to vulnerable in all states to conform with the federal listing. It is recommended that culling and forced dispersals should be suspended as they are likely to be ineffective, and are incompatible with the continued survival of the species.

T90: Conservation genetics of blue whales (*Balaenoptera musculus*) in Australia and surrounding regions

C. R. M. Attard[1], *L. B. Beheregaray*[1,2], *K. C. S. Jenner*[3], *P. C. Gill*[4], *C. L. K. Burton*[5], *M–N. Jenner*[3], *M. G. Morrice*[4], *J. L. Bannister*[6], *R. G. LeDuc*[7], *L. M. Möller*[2]

[1] *Department of Biological Sciences, Macquarie University NSW,* [2] *School of Biological Sciences, Flinders University SA,* [3] *Centre for Whale Research, Fremantle WA,* [4] *Blue Whale Study, Narrawong, VIC,* [5] *Western Whale Research, Dunsborough WA,* [6] *The Western Australian Museum, Welshpool DC, WA,* [7] *Southwest Fisheries Science Center, La Jolla, CA, USA*

Whaling has dramatically reduced the abundance of endangered blue whales (*Balaenoptera musculus*) worldwide. In the Southern Hemisphere two subspecies are recognised. In Australia there are two known Australian feeding aggregations that occur seasonally at the Perth Canyon off the coast of Western Australia, and at the Bonney Upwelling off South Australia and Victoria. Blue whales are also known to travel through Geographe Bay in Western Australia, but the population and subspecific identity of these blue whales is unknown. We are using microsatellite markers, the mitochondrial DNA control region and intron markers to investigate the genetic diversity and structure, subspecies differentiation and historical demography of blue whales in Australia and surrounding regions. We have found that the two known Australian feeding aggregations are likely to constitute one genetic population, which is suspected to breed in Indonesia and/or the Solomon Islands, and have detected a genetic bottleneck in these Australian blue whales. We will also present preliminary findings about subspecies differentiation and historical demography. Our research will provide valuable information for national and international programs aimed at managing the recovery of these endangered whales.

T91: MHC–linked microsatellite diversity in the NSW feline population

K. Kirby, K. Morris, M. Lillie, V. Barrs, J. Beatty, K. Belov

Faculty of Veterinary Science, University of Sydney

The major histocompatibility complex (MHC) genes are primarily involved in immune function and are the most polymorphic genes in the vertebrate genome. The feline MHC has been shown to exhibit high levels of diversity. We have used six MHC–linked microsatellite markers to measure levels of genetic variation in 130 out–bred and 50 Burmese cats collected in NSW. The MHC–linked microsatellite loci in the out–breds have greater allelic richness (5.95–11.08) than the Burmese (3.96–7.96), but similar alleles are seen in both populations. Four of six and three of six loci in out–bred and Burmese respectively, deviate from Hardy–Weinberg Equilibrium. There is a highly significant heterozygous deficiency in both the out–bred (all six loci) and Burmese populations (three of six loci). Implications of these results will be discussed including that both NSW out–bred and Burmese cats had small founder populations or higher levels of inbreeding than the British and American domestic cat populations. Comparisons with genetic diversity at non–MHC–linked microsatellite markers will allow us to determine whether the unexpected results are indicative of a generalised heterozygosity deficiency across the genome or whether these results are restricted to the MHC region.

T92: The relationship of native Australian dingo to a domestic dogs and wolves

A. N. Wilton[1,2], *Z. C. Wu*[1], *M. K. K. Wong*[1], *B. Zangerl*[3], *B. M. von Holdt*[4], *J. R. Shearman*[1]

[1]*School of Biotechnology and Biomolecular Sciences, University of New South Wales,* [2] *Ramaciotti Centre for Gene Function Analysis, University of New South Wales,* [3]*School of Veterinary Medicine, University of Pennsylvania, PA, USA,* [4]*Dept Ecology and Evolutionary Biology, University of California Los Angeles, CA, USA*

The dingo is an Australian native animal that arrived on the island continent 4000 years ago. MtDNA and microsatellites show there is limited genetic variation in dingoes suggesting a small founding population. Dingoes are under threat of extinction from interbreeding with European domestic dogs and markers are needed to differentiate them. We typed 6 dingoes from different localities using the Affymetrix–Canine–SNP–Chip V2. When compared to the CanFam2 data of 900 dogs and 500 wolves (*Nature* **464**:898, 2010), the dingo is shown to be related to domestic dogs but it groups with ancient breeds as the genetically least variable, most distinct of the dog breeds. A comparison of dingoes at 48,000 SNP loci with 500 dogs of 15 breeds shows a particular excess of homozygosity in the dingo on CFA15 at 32–56 Mb, which contains the IGF1 gene, haplotypes for which are associated with size in dog breeds. The homozygosity could be a result from selection at other genes, from adaptation to the Australian environment, or a remnant of selection during the early stages of domestication.

T93: Finding foxes in Tasmania: faecal DNA analysis reveals widespread distribution of an elusive introduced predator

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The red fox (*Vulpes vulpes*) was recently introduced to Tasmania, an island refuge for many Australian species at risk of predation. Eradication of the fox population at this early stage is important for both conservation and agriculture in Tasmania but monitoring this elusive pest presents special problems. We use DNA analysis of faeces to identify fox traces and highlight areas of fox activity. Since 2007, we have screened around 7000 scats collected as part of a strategic survey across Tasmania and in response to fox sightings. Special attention is needed to maximise amplification success and to prevent contamination when working with trace DNA, especially given the large scale of this project and the unknown ages of the scats. Consequently we apply strict protocols at all stages of our work, from scat collection in the field to sample handling and analysis in our dedicated trace DNA facilities. Fox DNA has been identified from scats collected across Tasmania, demonstrating a widespread distribution of this top predator ranging from the central north, to many sites in the east and south east. Genotyping with microsatellites and a sex marker is now underway to identify individual foxes and assess their relatedness.

Epigenetics

T94: Statistical support for the period–10 dinucleotide encoding of nucleosome positioning in yeast and mouse

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Nucleosomes are the fundamental packaging unit of eukaryote DNA and serve a critical function in the epigenetic control of gene regulation. Consisting of ~146 bp of DNA wrapped around a histone octamer, nucleosomes affect the accessibility of DNA to the gene regulatory apparatus. A role for DNA sequence in encoding nucleosome locations has been conjectured for some time with a ~10 bp periodicity of certain dinucleotides being identified as a nucleosome positioning sequence. We refer to these period–10 nucleosome positioning dinucleotides as the NPS. The functional significance of NPS have been convincingly demonstrated experimentally in a number of independent studies. Direct evaluations of the association between NPS and individual nucleosome locations have not been done due in part to the lack of suitable methods for

identifying NPS. We developed a confirmatory period estimation technique that measures the strength of a given (*e.g.* putatively dominant) periodic component and determines its significance. We applied the resulting technique to the entire genomes of yeast and mouse. We found a striking NPS distribution in yeast and strong support for the period–10 occurrence of NPS in mouse. These results remove a critical barrier for evaluating the role of periodic elements in encoding nucleosome positioning.

T95: The phenotypic significance of CpG variation within protein coding genes

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The challenge in genome resequencing studies is how to distinguish phenotypically significant SNPs, such as those with disease association, from neutral variants. The CpG dinucleotide constitutes ~2% of all human dinucleotides but their variations contribute to ~40% of human SNPs putatively due to an elevated mutation propensity from 5–methyl–cytosine (5mC). We evaluated a prediction from population genetic theory that stronger purifying selection should operate on CpG–containing codons resulting from the interplay of great mutability and selective constraints. We contrasted evolutionary dynamics and the mode of natural selection between genomes that methylate and do not methylate their DNA, using primates and yeast respectively. By applying a new class of codon substitution models with CpG context-dependent parameters and properly adjusted background constraints, our results confirmed that CpG–containing codons are under greater purifying selection in primates and not in yeast. Finally, we present a method for predicting candidate phenotypically influential CpG positions in human genes and demonstrate that it successfully recapitulates known disease–causing variations.

T96: Silencing the X

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In most mammals females have two X chromosomes, whereas males have just one X and a testis determining Y. This leads to an imbalance of X gene dose between the sexes, for which a dosage compensation mechanism is required to restore. In placental mammals, balanced expression between the sexes of X genes is achieved by transcriptional silencing of one X chromosome in the somatic cells of females. This dramatic example of chromosome wide gene silencing, called X chromosome inactivation (XCI), is achieved by the inactive X (Xi) acquiring a characteristic set of histone modifications that alters it into transcriptionally silent facultative heterochromatin. Marsupial mammals also achieve dosage compensation by XCI; however, the repertoire of histone modifications recruited to the inactive X is somewhat different. On the marsupial Xi, histone modifications classically associated with the placental mammal Xi are enriched at much lower levels, or missing altogether. Surprisingly, the Xi in marsupials is characterised by modifications generally associated with pericentromeric heterochromatin. Marsupial inactive X chromatin might, therefore, represent an ancestral epigenetic system of dosage compensation that was hijacked from neighbouring constitutive heterochromatin.

T97: JMJ14, a JmjC domain protein, is required for RNA silencing and cell–to–cell movement of an RNA silencing signal in Arabidopsis

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RNA silencing is a sequence–specific RNA degradation process conserved in fungi, animals and plants that is associated with cell–to–cell movement of a mobile silencing signal. Here we describe a putative histone H3 lysine 4 trimethyl demethylase, JMJ14, that is required for mobile RNA silencing in an Arabidopsis transgene system. In addition to an effect on mobile silencing the *jmj14* mutants also had reduced CHH DNA methylation, increased abundance of endogenous transposon transcripts and they flowered earlier than wild type. We placed the activity of JMJ14 at a downstream point in RNA silencing pathways because the subcellular locations of upstream components RNA–dependent RNA polymerase (RDR2) and Argonaute (AGO4) were not perturbed in *jmj14* mutants. These results illustrate the potential for a link between RNA silencing and demethylation of histone H3 trimethylysine. We propose that JMJ14 acts downstream of the Argonaute effector complex to demethylate histone H3 lysine 4 trimethyl residues at the target of RNA silencing.

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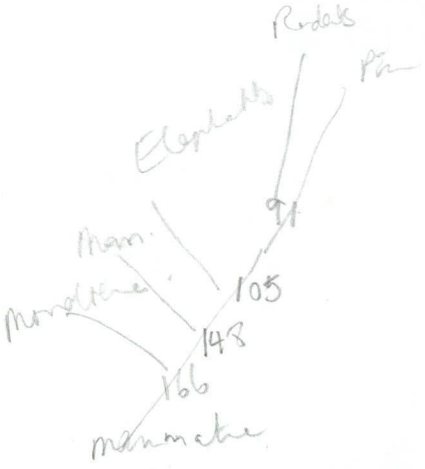

148 myr marsupials

166 myr Rodentia

CGX

AGA