C. MORAN

ISSN 1329-2420

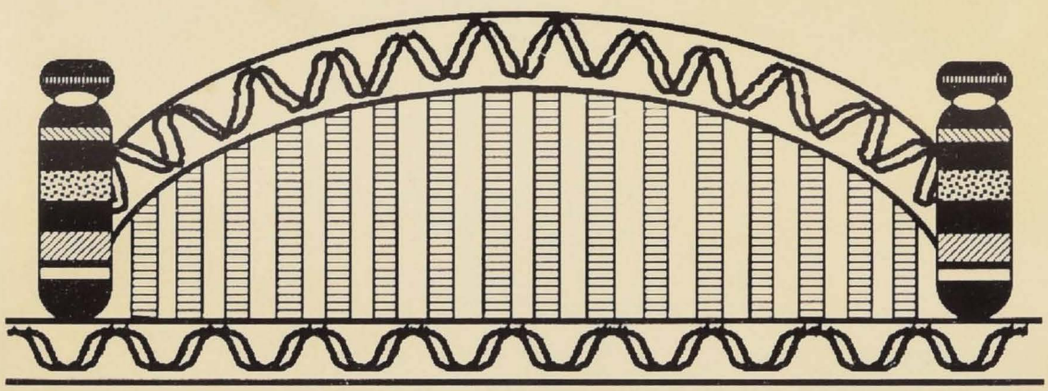
GSA

Genetics Society of
Australia, Inc

49th Annual Meeting of Genetics Society of Australia

and

Comparative Genomics: 7th Australasian Gene Mapping Workshop



Sydney 2002

Celebrating GSA's 50th Anniversary

Conference Abstracts

49th Annual Meeting of Genetics Society of Australia, Inc

9th July to 11th July, 2002

and

Comparative Genomics: 7th Australasian Gene Mapping Workshop

11th July and 12th July, 2002

at University of New South Wales

CONFERENCE ABSTRACTS

Genetics Society of Australia, Inc - Conference Abstracts
ISSN 1329-2420
Edited by Alan Wilton
2002 Sydney, NSW

Genetics Society of Australia, Inc
c/o School of Biotechnology and Biomolecular Sciences
University of New South Wales
Sydney NSW 2052, Australia

CONTENTS

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SPONSORS

As well as the usual trade display fee the following companies have provided addition support for the conferences for which we are very grateful.

Applied Biosystems

is the major sponsor for Comparative Genomics:
7th Australasian Gene Mapping Workshop
and the joint conference dinner

Co-operative Research Centre for Innovative Dairy Products

have sponsored a session at ***Comparative Genomics:***
7th Australasian Gene Mapping Workshop

GeneticXchange

has sponsored the lunch at the joint poster session of GSA meeting and the Comparative Genomics meeting on Thursday 11th.

Beckman Coulter

have sponsored the lunch for the GSA poster session on Wednesday, 10th

QIAGEN

have sponsored a student prize

Corbett Research

have sponsored a student prize

Genesearch

have sponsored a student prize

AGRF (Australian Genome Research Facility)

have sponsored a student prize

Conference Organising Committee

Committee: Alan Wilton (Convenor)
Ian Dawes
Bill Sherwin
Greg Peters
Don Colgan
Margaret Heslewood
Shaun Barclay

Program Committee: Alan Wilton
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Frank Nicholas
Greg Peters
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Assistance from: Ben Oldroyd
Emilie Cameron
Chris Cotsapas
Melissa Gunn
Jyoutsna Gupta
Michael Krützen
Mark Tanaka

GUEST SPEAKERS

OVERSEAS INVITED SPEAKERS

Genetic Society Meeting

Robert Wayne, UCLA, USA. Bob is interested in conservation genetics in mammals as well as looking at recent evolution using archeological specimens.

Lutz Frönicke, National Cancer Institute, Frederick, USA. Lutz is a cytogeneticist who is using cross species chromosome painting to look at evolution using chromosome rearrangements as markers. He is also looking at the distribution of chiasma in meiosis and mitosis.

Comparative Genomics Meeting

Daniel Pomp, University of Nebraska at Lincoln, USA. Daniel's work focuses on genomics of obesity in mice. He looks at comparative genomics of polygenic fatness in rodents and other animal models (pigs, cattle) with an emphasis on the relationship to human gene discovery. He focuses on the relationship between predisposition and physiological control of complex traits such as obesity, an area where he is integrating high-throughput methodologies in DNA, transcriptional, proteomic and metabolomic analysis on a large-scale population basis.

Lutz Frönicke, National Cancer Institute, Frederick, USA. Lutz is a cytogeneticist who is using cross species chromosome painting to look at evolution using chromosome rearrangements as markers. He is also looking at distribution of chiasma in meiosis and mitosis.

Bernard Dujon, Pasteur Institute, France. Bernard has looked at the genomes of several species of yeast and is looking at their evolutionary relationships.

Louise McKenzie, Jackson Laboratories, USA. Louise has moved on from her PhD work at Macquarie University on tammar wallabies to managing the comparative database on the mouse genome at the Jackson Laboratories.

John McEwan, AgResearch, New Zealand. John has worked in a number of sheep research areas over the past 20 years, for the past 8 mainly undertaking QTL experiments in sheep, concentrating on host resistance to internal parasites and carcass quality and meat traits. As well as this work, lately he has spent the majority of his time setting up bioinformatics at AgResearch to enable them to utilise several large EST sequencing projects. The primary reason has been to provide better tools for gene variant discovery from sheep, beef and deer QTLs.

LOCAL INVITED SPEAKERS

Genetic Society Meeting

David Martin, Victor Chang Cardiac Research Institute. Epigenetics and effect of transposable elements on human gene expression.

Greg Arndt, Johnson & Johnson Research. J&J had a long term interest in developing practical applications of active RNA compounds and this has extended to the study of RNA interference to study expression.

Bill Warren, James Cook Univ. Bill has recently moved from Peter MacCallum Cancer Institute to James Cook where he continues his work using RNAi to study gene expression and role of Drosophila *rad21* in regulating chromosome dynamics in mitosis.

Hatch Stokes, Macquarie University. Hatch's work on integrons has been very revealing about the role of mobile gene cassettes in bacterial evolution.

Phillip Bell, Macquarie University. Phillip has developed a speculative but interesting story on the possible origin of the nucleus.

Roger Reddel (Children's Medical Research Institute). Roger's interest is in the role of telomeres in the development of cancer.

Gavin Huttley, John Curtin School of Medical Research. Gavin is well known for his analysis of linkage disequilibrium in the human genome. He has extended this work in collaboration with Simon Easteal to look at evolution of interacting loci such as multiple positions in BRAC1.

Frank Nicholas, Sydney University. Back by popular demand. Frank and his wife have written a book on Charles Darwin in Australia. It follows the travels of Darwin showing the places he visited as they were then and as they look now. Frank was cut short when giving this talk at GSA in at Macquarie University about 10 years ago. Now he will have the chance to finish it.

Bruce Sheldon, formerly of CSIRO Animal Production. Bruce is one of the few people who are still with us and who attended the first meeting of the Genetics Society of Australia which was held in Sydney in 1952 . He will be our guest at the meeting and will give a brief talk on the first evening of the conference.

This meeting is 50th Anniversary of meetings of Genetics Society of Australia.

LOCAL INVITED SPEAKERS

Comparative Genomics Meeting

Mike Goddard, Victorian Institute of Animal Science. Mike's work is on QTL identification cattle. His talk is being sponsored by the CRC for Innovative Dairy Products.

Robert Henry, Centre for Plant Conservation Genetics, Southern Cross University. Professor Henry has set up a well recognised research group on plant genetics at Lismore and will talk at this meeting on comparisons of plant genomes.

Jenny Marshall Graves, Australian National University and University of Melbourne. Jenny is well known for her work on marsupial genetics and she will put the case for a kangaroo genome project.

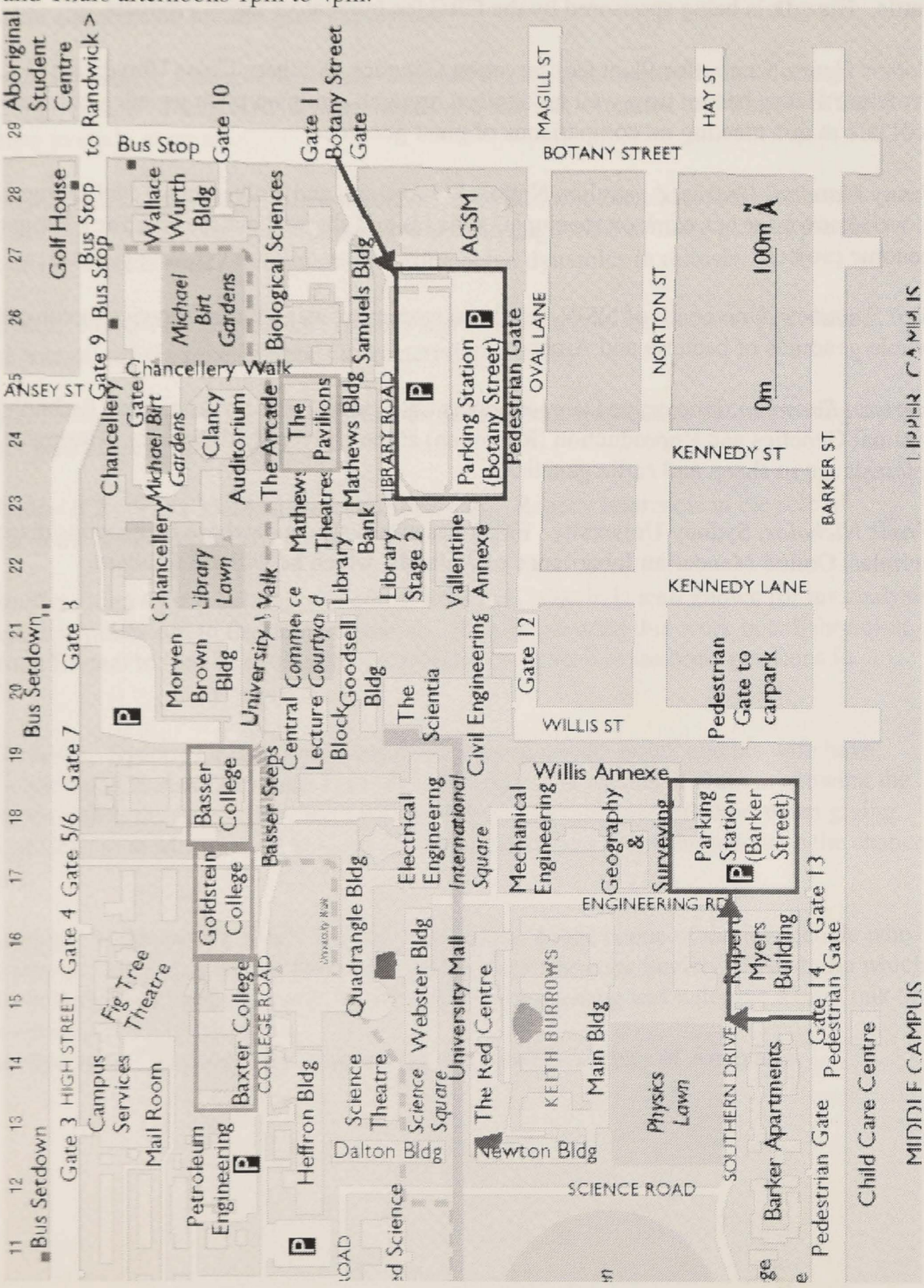
Neil Saunders, University of NSW. Neil is a researcher using bioinformatics to compare whole genomes of bacteria and Archaea.

Herman Raasdma, Innovative Dairy Products CRC and Centre for Advanced Technologies in Animal Genetics and Reproduction (Reprogen) at the University of Sydney. Herman's interests are in sheep and cattle genetics.

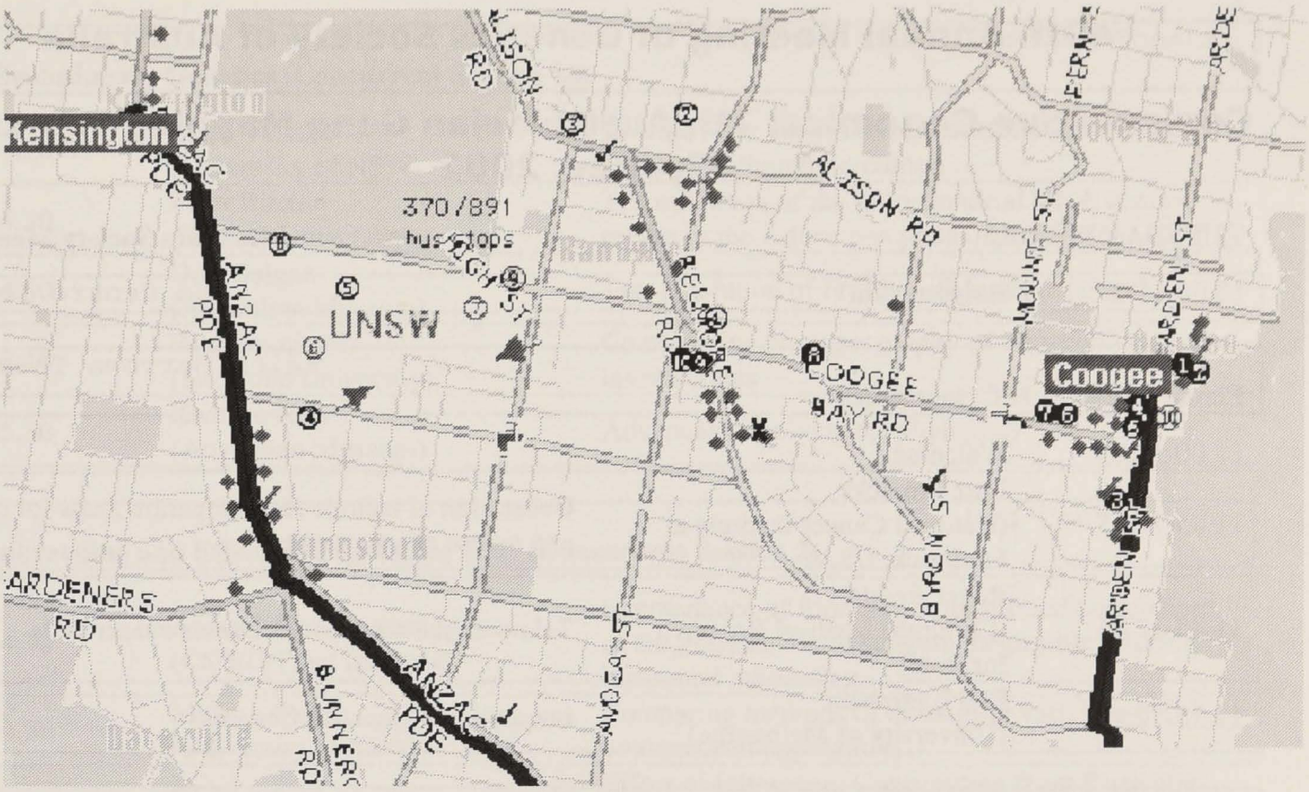
Frank Nicholas, Sydney University. Frank has established a database on genetics disorders in animlas, Online Mendelian Inheritance of Animals, which he will tell us about.

UNSW Campus Map

Lectures will be held at Keith Burrows Theatre, Red Centre and Webster Building. Posters and trade displays will be held in the Pavilions, upper campus, on Wed and Thurs afternoons 1pm to 4pm.



Map of surrounding suburbs of UNSW



Recommended eating areas
The Spot, Belmore Rd (south of High St)
Coogee, Coogee Bay Rd

- Restaurants
- ✓ Pubs
- ✓ Clubs, Coogee RSL, Souths Juniours Leagues (Anzac Pde), Labour Club (Alison Rd)

Legend

Accommodation

❶ The Beachhouse
❷ Coogee Sands Apartments
❸ Crown Plaza Hotel
❹ Surfside Backpacker (last resort)
❺ Park Agency Apartments
❻ Beachside Budget Accomm
❼ Wizard of Oz Backpackers
❽ The Agean Backpackers
❾ Royal Hotel
❿ High Cross Park Lodge
⓫ Randwick Lodge
⓬ Avonmore on the Park
⓭ Alison Lodge
⓮ Barker Street Lodge
⓯ Kensington Colleges

Other

⓰ Keith Burrows Theatre (lectures)
⓱ The Pavillions (Posters and Trade)

⓲ 370 bus stop
⓳ 370, 400 and 891 bus stops
⓴ Busses to city and UNSW (370)

X Ritz Movie Theatre, The Spot

UNSW parking

Program

49th Annual Meeting of Genetics Society of Australia
&
Comparative Genomics: 7th Australasian Gene Mapping Workshop
Sydney, 2002

TUESDAY 9th July — **Genetics Society Meeting**

10.00-11.00	**Registration**	Red Centre Foyer
		Keith Burrows Theatre
Plenary Session 1. Cytogenetics		
11.00-11.05	Welcome	
11.05-11.45	Lutz Frönicke (National Cancer Institute at Frederick, USA)	Generation of meiotic recombination maps for cat and mouse chromosomes
11.45-12.25	Roger Reddel (Childrens Medical Research Institute)	Telomere maintenance in human cancer cells
12.25-12.40	Phil Batterham (University of Melbourne)	International Genetics Congress
12.40-1.40	**LUNCH fend for yourselves**	*Committee Members Only - GSA Committee Meeting - Old Main Building 228*
		Keith Burrows Theatre
Plenary Session 2. Epigenetics and Mobile elements		
1.40-2.20	David Martin (Victor Chang Cardiac Res Inst)	Phenotypic variation in mammals as an epigenetic effect of retrotransposon activity
2.20-2.40	Emma Whitelaw (University of Sydney)	Metastable Epialleles
2.40-3.20	Hatch Stokes (Macquarie University)	The floating genome: The role of mobile gene cassettes in bacterial evolution
3.20-3.50	**TEA**	Red Centre Foyer
3.50-5.30	**Concurrent Sessions**	See next page for details
1A Evolutionary Genetics (Concurrent Session 1)		Keith Burrows Theatre
1B Inbreeding and Effective Population Size (Concurrent Session 2)		Red Centre G001
1C Mating Behaviour and Breeding (Concurrent Session 3)		Webster B
5.30-6.15	**Drinks and nibblies**	Red Centre Foyer
		Keith Burrows Theatre
EVENING SESSION - open to the public		
6.15-6.30	Bruce Sheldon Attended 1st meeting 1952	50th Anniversary of GSA meetings
6.30-7.10	Ben Oldroyd (University of Sydney)	**M.J.D. White Address -** Evolution of worker sterility in social insects
7.10-7.50	Frank Nicholas (University of Sydney)	Charles Darwin in Australia

Dinner somewhere in the Spot for those interested - meet at Royal Hotel near top of High St

Keith Burrows Theatre

1A Evolutionary Genetics (Concurrent Session 1)

3.50-4.10	Mark Tanaka (University of NSW)	IS wide shut: copy number control of IS6110 in Mycobacterium tuberculosis
4.10-4.30	Peter Ritchie (Massey University)	The evolution of the mitochondrial DNA control region in the Adélie penguins of Antarctica
4.30-4.50	Don Colgan (Australian Museum)	The Evolution of Fruitbats (Megachiroptera)
4.50-5.10	Paul Sunnucks (La Trobe University)	Comparative phylogeography of saproxylic invertebrates
5.10-5.30	Kathryn Hall (Australian Museum)	Advances in polychaete phylogeny

Red Centre G001

1B Inbreeding and Effective Population Size (Concurrent Session 2)

3.50-4.10	Andrew Graham Young (CSIRO Plant Industry)	Maintenance of fitness in mate-limited populations of the grassland herb suggests negligible effects of biparental inbreeding
4.10-4.30	Melissa Gunn (University of NSW)	Comparing methods of measuring variation in bottlenecked populations
4.30-4.50	W. Y. Nicola Man (University of Sydney)	Effect of Inbreeding Contribution from Particular Ancestors: An Analysis of the First Three Lactation Milk Yields
4.50-5.10	Christoph Vorburger (La Trobe University)	Homozygous and heterozygous fitness effects of clonally transmitted genomes in waterfrogs
5.10-5.30	Neil Murray (La Trobe University)	Estimating Effective Population Size: A Comparison of Demographic and Pedigree-based methods in the Helmeted Honeyeater

Webster B

1C Mating Behaviour and Breeding (Concurrent Session 3)

3.50-4.10	David Runciman (La Trobe University)	Attractive traits and adaptive sex allocation in Zebra Finches revisited: what was all the hurley-burley about?
4.10-4.30	Michael Krützen (University of NSW)	Alliance formation is a strong determinant of male mating success in a population of wild bottlenose dolphins in Shark Bay,
4.30-4.50	Alyson Ashe (University of Sydney)	Gene expression in anarchistic honey bees
4.50-5.10	Adam Stow (Macquarie University)	Family group structure in wild populations of Cunningham's skink: revealed by genetic determination of parentage and site fidelity.
5.10-5.30	John James (Sydney Univesity)	A little BLUP

Plenary Session 3. RNA interference / eukaryotic origins — Keith Burrows Theatre

8.30-9.10	Greg Arndt (Johnson and Johnson Research)	RNA interference to study expression
9.10-9.50	Bill Warren (James Cook University)	dsRNA-mediated genetic interference studies of sister-chromatid cohesion in *Drosophila*
9.50-10.30	Philip Bell (Macquarie University)	Viral eukaryogenesis: was the ancestor of the nucleus a complex DNA virus?

10.30-11.00 **TEA** — Red Centre Foyer

11.00-1.00 **Concurrent Sessions** — See next page for details

2A Evolutionary Genetics (Concurrent Session 4) — Keith Burrows Theatre

2B Conservation Genetics (Concurrent Session 5) — Red Centre G001

2C Population Structure (Concurrent Session 6) — Webster B

1.00-4.00 **LUNCH** *Lunch is sponsored by* ***Beckman Coulter***
POSTERS AND TRADE — The Pavilions

4.00-5.00 **Concurrent Sessions** — See next 2 pages for details

3A Evolutionary Genetics (Concurrent Session 4 cont) — Keith Burrows Theatre

3B Conservation Genetics (Concurrent Session 5 cont) — Red Centre G001

Plenary Session 4. Conservation Genetics / Evolutionary History — Keith Burrows Theatre

5.00-5.40	Bob Wayne (University of California, Los Angeles)	The use of molecular techniques for conservation of endangered species
5.40-6.20	Gavin Huttley (JCSMR, ANU)	The effect of linkage disequilibrium among epistatically interacting genes on the power of association studies

Joint Mixer / Comparative Genomics registration 6:30 to 8:30 pm — Kensington Colleges Goldstein College

Keith Burrows Theatre

2A Evolutionary Genetics (Concurrent Session 4)

11.00-11.20	Michelle Guzik (James Cook University)	Evidence for concerted evolution in an intron-less copy of octopus Elongation Factor-1a
11.20-11.40	Kira Bulazel (Macquarie University and University of Connecticut)	Identification and Distribution of Sex Chromosome Centromere Repeats in Macropodidae
11.40-12.00	Rene Vaillancourt (University of Tasmania)	Sharing of nuclear and cpDNA variation across eucalypt species
12.00-12.20	Brenda McDonald (James Cook University)	Molecular Evolution in the Paenungulates: Evidence from the Mitochondrial DNA
12.20-12.40	Darren Crayn (Royal Botanic Gardens, Sydney)	Molecular phylogeny indicates multiple origins of Crassulacean acid metabolism in the Neotropical plant family Bromeliaceae
12.40-1.00	Chester Sands (La Trobe University)	Molecular phylogenetics of Southern Ocean cephalopods

Red Centre G001

2B Conservation Genetics (Concurrent Session 5)

11.00-11.20	Jane Hughes (Griffith University)	Translocation causes extinction in a local population of the freshwater shrimp *Paratya australiensis*
11.20-11.40	Alison Shapcott (Univ Sunshine Coast)	Comparative conservation genetics of four *Graptophyllum* (Acanthaceae) species from Queensland
11.40-12.00	Teena Browning (Macquarie University)	Conservation genetics of the "Critically Endangered" Victorian brush-tailed rock-wallaby, *Petrogale penicillata*
12.00-12.20	Anthony Stuart Gilchrist (University of Sydney)	A whodunit in the Top End: what are the new fruit fly pests in Northwest Australia
12.20-12.40	Shaun Barclay (University of NSW)	Genetic management of endangered species: does it work?
12.40-1.00		

Webster B

2C Population Structure (Concurrent Session 6)

12.00-12.20	Alexander Anderson (James Cook University)	Population genetic structure in a rainforest bird, the Grey-headed Robin: traces of Pleistocene climate change
12.20-12.40	Yvonne Parsons (La Trobe University)	Cryptic speciation in Australian *Drosophila serrata*
12.40-1.00	Sean MacEachern (La Trobe University)	The effects of habitat fragmentation on the wood-dwelling cockroach *Panesthia australis*
12.00-12.20	Ryan Garrick (La Trobe University)	Genetic variation within and between two Victorian populations of *Thelymitra circumsepta* (Orchidaceae)
12.20-12.40	Andrew Baker (Griffith University)	Patterns of genetic variation in the fresh-water shrimp *P. australiensis*: evidence for restricted dispersal and cryptic species
12.40-1.00	Juanita Renwick (Queensland University of Technology)	Phylogeography of southeast Queensland populations of the Wallum froglet, *Crinia tinnula*

3A Evolutionary Genetics (Concurrent Session 4 cont)		Keith Burrows Theatre
4.00-4.20	Stuart Barker (University of New England)	Flower breeding Scaptodrosophila - is the oldest genus of the Drosophilidae really different?
4.20-4.40	Steve Donnellan (South Australian Museum)	Molecular evidence for the origin of parthenogenesis in the Australian skink lizard Menetia
4.40-5.00	Richard Newcomb (HortResearch, NZ)	Effects of selection on variation in and around the insecticide resistance locus, *Rop-1*, in the sheep blowfly, *L. cuprina*

3B Conservation Genetics (Concurrent Session 5 cont)		Red Centre G001
4.00-4.20	Maurizio Rossetto (Royal Botanic Gardens Sydney)	The consequences of rainforest fragmentation on Elaeocarpus species
4.20-4.40	Jodi Neal (University of New England)	Viability of Fragmented *Macadamia integrifolia* Populations
4.40-5.00	Richard Frankham (Macquarie University)	Inbreeding and Extinction: Effects of Rate of Inbreeding

Start at 8-40.

THURSDAY 11th July JOINT SESSION GSA and Comparative Genomics
Applied Biosystems sponsor of Comparative Genomics: 7th Australasian Gene Mapping Workshop

8.30-8.35	Welcome Keith Burrows Theatre	Comparative Genomics: 7th Australasian Gene Mapping Workshop

Plenary Session 5. Comparative Animal Genomics - joint session (sponsored by CRC for Innovative Dairy Products)		Keith Burrows Theatre
8.35-9.15	Mike Goddard (Victorian Inst Animal Sciences)	QTL mapping in dairy cattle
9.15-9.55	Daniel Pomp (Univ of Nebraska - Lincoln, USA)	Polygene Discovery for Body Weight Regulation in Animal Models and Relationship to Human Gene Discovery
9.55-10.35	Herman Raadsma (University of Sydney)	Comparative QTL mapping in sheep and applications in cattle
10.35-11.00	**TEA**	Red Centre Foyer
Plenary Session 5. Comparative Plant Genomics joint session		Keith Burrows Theatre
11.00-11.40	Robert Henry (Southern Cross University)	Comparison of Plant Genomes by Analysis of Gene Sequences, SSR and SNP
11.45-1.05	**Concurrent Sessions**	See next page for details
4A Genomics and Mapping (Concurent Session 7 - GMW)		Keith Burrows Theatre
4B Evolutionary and Developmental Genetics (Concurrent Session 8 - GSA)		Red Centre G001
4C Population Structure (Concurrent Session 9 - GSA)		Webster B
1.05-4.00	**LUNCH & POSTERS AND TRADE**	*Lunch is sponsored by* ***GeneticXchange*** The Pavilions Student Prizes awarded 3:30 pm
4.00-4.40	**Concurrent Sessions**	See next page for details
5A Genomics and Mapping (Concurent Session 7 cont - GMW/GSA)		Keith Burrows Theatre
5B Evolutionary and Developmental Genetics (Concurrent Session 8 cont GSA)		Red Centre G001
Plenary Session 6. Technological Solutions - Joint session		Keith Burrows Theatre
4.40-5.20	Richard Harrison (Applied Biosystems)	Genomic analysis for all creatures great and small
5.20-6.00	GSA Annual General Meeting	Keith Burrows Theatre
7.00 for 7.30 to midnight	**Joint Conference Dinner** Dancing to the Major Groove *Dinner sponsored by* ***Applied Biosystems***	Crowne Plaza - Coogee

Keith Burrows Theatre

4A Genomics and Mapping (Concurent Session 7 - GMW)

11.45-12.05	Rachel Rusholme (HortResearch, NZ)	Mapping the genes controlling resistance to turnip mosaic virus in *Brassica*
12.05-12.25	Michael Devey (CSIRO)	QTL associations for density and diameter in *Pinus radiata* and the potential for marker-aided selection
12.25-12.45	Tony Brown (CSIRO Plant Industry)	Polyploid evolution in perennial *Glycine*
12.45-1.05	A. Becerra Lopez-Lavalle (CSIRO Plant Industry)	The use of alien chromosome addition aneuploid lines facilitates genetic linkage mapping of the wild *Gossypium* species

Red Centre G001

4B Evolutionary and Developmental Genetics (Concurrent Session 8 - GSA)

11.45-12.05	Michael Bogwitz (Centre for Environmental Stress and Adaptation)	Fine scale mapping of a *D. melanogaster* variant indicates Cyp6g1 overexpression is involved resistance to multiple insecticides
12.05-12.25	Janine Deakin (Australian National University)	Does X Chromosome Inactivation Exist in Monotremes?
12.25-12.45	Melanie Norgate (University of Melbourne)	Approaches to studying copper homeostasis in *Drosophila melanogaster*
12.45-1.05	Victoria Jane Metcalf (University of Canterbury)	Expansion and Duplication of the Serum Albumin Gene in Tuatara

Webster B

4C Population Structure (Concurrent Session 9 - GSA)

11.45-12.05	Michael N Dawson (University of New South Wales)	Geographic variation in jellyfishes: *Aurelia* and *Mastigias* (Cnidaria, Scyphozoa)
12.05-12.25	David Hurwood (Queensland University of Technology)	Phylogeny and Historical Biogeography of the Freshwater Fish Genus, *Mogurnda* (Pisces: Eleotridae), in Australia
12.25-12.45	Michelle Waycott (James Cook University)	Adaptive radiation and dispersal in the seagrass genus *Halophila*
12.45-1.05	Melanie Lancaster (La Trobe University)	Hybridisation among three species of fur seal (Arctocephalus spp.) occurring in sympatry on Macquarie Island

Keith Burrows Theatre

5A Genomics and Mapping (Concurent Session 7 cont - GMW/GSA)

4.00-4.20	Ian Hughes (University of Queensland)	Use of Linkage Disequilibrium Mapping in Domestic Dog Breeds
4.20-4.40	Neil Gemmell (University of Canterbury)	Mitochondrial mutations may drive Y chromosome evolution

Red Centre G001

5B Evolutionary and Developmental Genetics (Concurrent Session 8 cont - GSA)

4.00-4.20	David Loebel (Children's Medical Research Institute)	Downstream Targets of Twist Activity in Mouse Embryonic Development
4.20-4.40	Peter William Hunt (CSIRO Plant Industry)	Expression and functions of hemoglobin genes in plants.

FRIDAY 12th July **Comparative Genomics: 7th Australasian Gene Mapping Workshop**
Applied Biosystems sponsor of Comparative Genomics: 7th Australasian Gene Mapping Workshop

		Keith Burrows Theatre
Plenary Session 7. Comparative Genomics		
8.45-9.25	Neil Saunders (University of NSW)	Comparative Microbial Genomics
9.25-10.05	Bernard Dujon (Institut Pasteur, France)	Yeast Comparisons
10.05-10.45	John McEwan (AgResearch, New Zealand)	Use of EST sequence and comparative genomics to aid QTL gene discovery in farmed ruminants: a view from the trenches
10.45-11.15	**TEA**	Red Centre Foyer
11.15-12.55	**Concurrent Sessions**	See next page for details
6A Gene and QTL mapping (Concurrent Session 10)		Keith Burrows Theatre
6B Mapping and Animal Breeding (Concurrent Session 9A)		Red Centre G001

* Red Centre G001 not available 1-2

1.00-2.00	**LUNCH**	**Fend for yourselves**

		Keith Burrows Theatre
Plenary Session 8. Comparative Genomics		
2.00-2.40	Lutz Frönicke (National Cancer Institute at Frederick, USA)	New Insights into Mammalian Genome Evolution by Molecular Cytogenetics
2.40-3.20	Jenny Graves (Australian National University)	Toward a Kangaroo Genome Project
3.20-3.40	**TEA**	Red Centre Foyer

Plenary Session 9. Comparative Genomics (cont)		Keith Burrows Theatre
3.40-4.20	Louise McKenzie (The Jackson Laboratory)	The Mouse Genome Database: A Resource for Comparative Genomics
4.20-5.00	Frank Nicholas (University of Sydney)	Online Mendelian Inheritance in Animals (OMIA)
5.00	**Conference close**	

Keith Burrows Theatre

6A Gene and QTL mapping (Concurrent Session 10)

11.15-11.35	Ian Franklin (Macquarie University)	Identifying QTL for wool traits in sheep
11.35-11.55	Cynthia Bottema (University of Adelaide)	Comparative QTL mapping in mice for livestock traits
11.55-12.15	Kyall Zenger (Macquarie University)	The First Comprehensive Genetic Linkage map of a Marsupial - the Tammar Wallaby (*M. eugenii*)
12.15-12.35	Frank Gruetzner (Australian National University)	400 Million Years of Conserved Synteny of Human Xp and Xq Genes on Three Pufferfish Chromosomes
12.35-12.55	Kathy Belov (Australian Museum)	Immunoglobulin genes of marsupials and monotremes

Red Centre G001

6B Disease gene mapping (Concurrent Session 11)

11.15-11.35	Scott Melville (University of New South Wales)	Neuronal Ceroid Lipofuscinosis in Border Collie Dogs
11.35-11.55	Merridee Ann Wouters (Victor Chang Cardiac Research Institute)	Bioinformatics approaches to candidate gene selection in dilated cardiomyopathy (DCM)
11.55-12.15	Guanglan Guo (Victor Chang Cardiac Research Institute)	Evaluation of the LMNA gene in families with dilated cardiomyopathy and conduction-system disease
12.15-12.35	Renee Frances Badenhop (Garvan Institute of Medical Research)	Genetic refinement and physical mapping of a 2.3Mb probable disease region defining a bipolar affective disorder susceptibility locus on chromosome 4q35
12.35-12.55	John Edwards (Oxford University)	The sib-similarity problem

Poster Presentations:
49th Annual Meeting of Genetics Society of Australia
and
Comparative Genomics: 7th Australasian Gene Mappping Workshop

ABSTRACTS

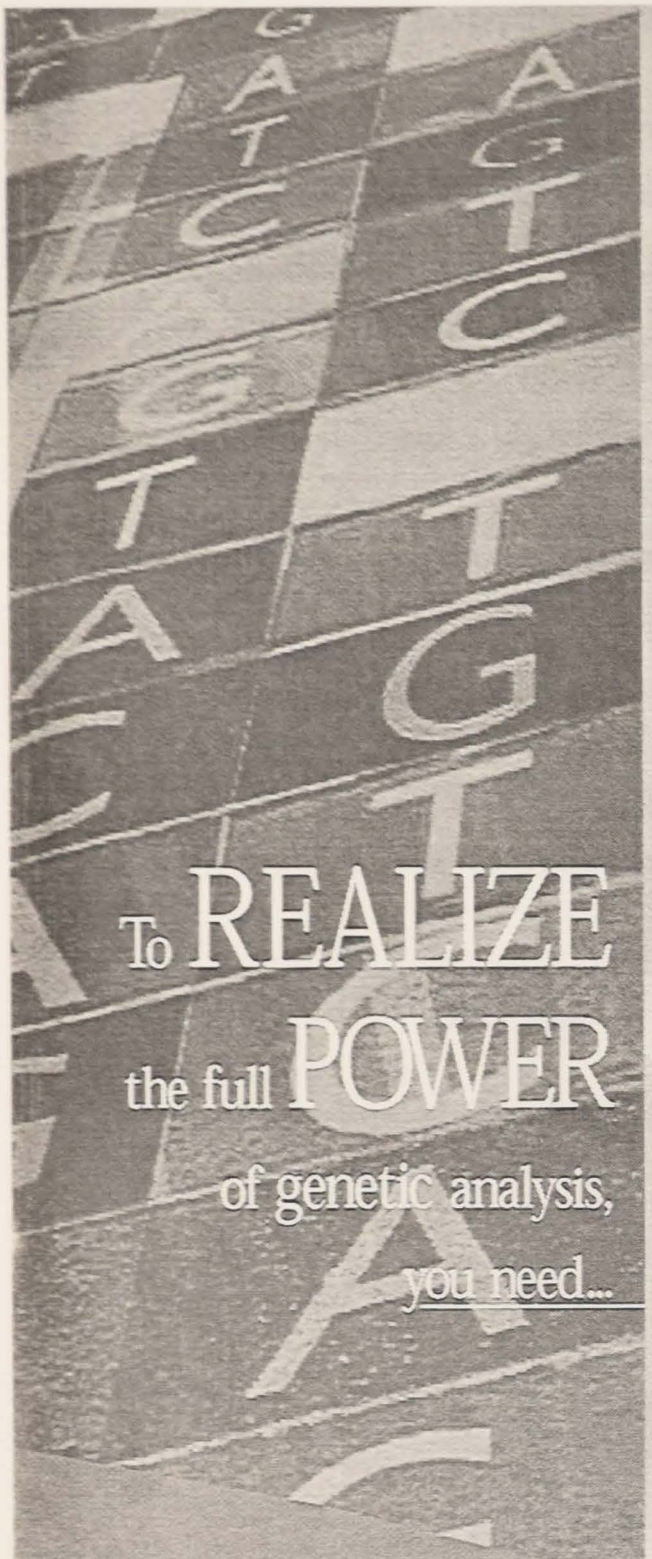
in alphabetical order of presenter

for

49th Annual Meeting of Genetics Society of Australia

and

Comparative Genomics: 7th Australasian Gene Mappping Workshop



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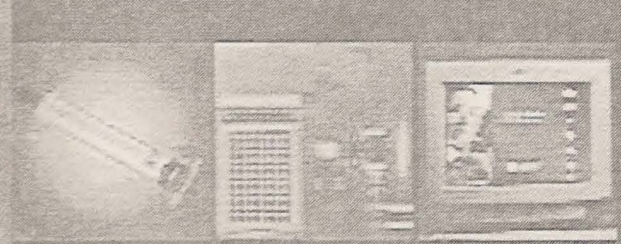

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Refining the comparative map for pig chromosome 10

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We are interested in quantitative trait loci (QTL) on pig chromosome 10 (SSC10). In order to identify positional candidate genes, we require a more refined comparative map of SSC10 and orthologous segments of human chromosomes (HSA9, HSA10 and HSA1) than that based on ZOO-FISH, which provides a low resolution view of the evolutionary breaks occurring in pig and human chromosomal evolution. Recognition of conserved blocks of genes enables us to infer the location of uncharacterised genes in the pig. ZOO-FISH has revealed that most of the long arm, SSC10q12–q27, corresponds to HSA10p. However SSC10p, corresponding to an interstitial region, HSA1q4.1-4.2, and SSC10cen-q21, corresponding to an interstitial region, HSA9p1.3-1.2, are not as precisely defined. Therefore we are mining the relevant chromosomal regions of the public human genome sequence to find genes unmapped in the pig with which we BLAST porcine EST databases. We then use the porcine sequences to design primers that are used to map the genes using a French somatic cell hybrid panel. So far, we have physically mapped twelve genes from human chromosomes 9 and 10. Eight, ACO1, ATP5C1, BMI1, DNAJA1, GDI2, NUDT2, PHYH and VIM, were assigned to SSC10 as predicted. However four loci, ADFP, HARC, TESK1 and VLDR, initially expected to be on SSC10, were assigned to SSC1. By mapping more genes, we will refine the identification of the evolutionary breakpoints between these species, further improving our ability to mine the human genome sequence for positional candidate loci for economically important genes.

Expression of circadian genes in the brain cells of Queensland fruit fly

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The Queensland fruit flies *Bactrocera tryoni* and *Bactrocera neohumeralis* are two sibling Tephritid species. The two species occur sympatrically with the distribution of *B. neohumeralis* contained entirely within the wider distribution of *B. tryoni*. A definite form of mating isolation, based on the specific time of mating, persists between the species: *B. neohumeralis* mates in the middle of the day and *B. tryoni* mates at dusk when light intensity drops to a low level. This phenomenon indicates that genetic mechanisms of circadian regulation and light response differentiate the two species. Previously, we have isolated the homologue of the *period* gene and the 3′ end of the *cryptochrome* gene from both *B. neohumeralis* and *B. tryoni*, showing that the amino acid sequences are identical between the two species. Since the brain is the source of control for circadian behaviours, we examined the location and circadian expression of two circadian gene products within the brain of the two Bactrocera species. Immunohistochemical staining with anti-PER antibody and anti-PDF (pigment dispersing factor) on fly brain sections reveal cells in specific brain regions in both fly species, and show variation of PER expression during different times of the day.

Population genetic structure in a rainforest bird, the Grey-headed Robin: traces of Pleistocene climate change.

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In the Wet Tropics World Heritage area of North Queensland, paleobotanical data show that the last glacial maximum (18000 ya) was marked by contraction of rainforest into small isolated upland refugia, located on the Atherton and Carbine/Windsor tablelands. Rainforest in the Spec Uplands may have been lost altogether. Based on community structure, mitochondrial DNA phylogeny and bioclimatic modeling, it is hypothesised that during this period, Grey-headed Robins (*Heteromyias albispecularis*) were restricted to refugia in the Atherton Uplands, locally extinct in the Spec Uplands, and only expanded to their current distribution in the late Holocene (7000 ya). A plausible alternative to this hypothesis is the persistence of both Spec and Atherton Uplands refugial populations that were reconnected by Holocene rainforest expansion. Genetic variation in the Wet Tropics populations of *H. albispecularis* was assessed in 200 individuals at seven polymorphic microsatellite loci. Population structure estimated using fixation indices shows low but significant differentiation across the study region. Tests for isolation by distance indicate that subpopulations may not be at equilibrium. In a sedentary habitat specialist like *H. albispecularis*, this suggests recent population expansion consistent with the recolonisation hypothesis. A north-south decline in genetic diversity across the region is further consistent with bottlenecks and founder effects occurring during range expansion from a central refugium. However, distance from the putative refuge and size of sampled rainforest areas are strongly correlated, so that the effects of size and distance from the putative refuge are confounded in this analysis. Unique alleles were detected only in the Atherton/Kirrama and Carbine Upland subpopulations, suggesting strongly that refuges for *H. albispecularis* were limited to these areas during the last glacial maximum. A significant nestedness of allele assemblage structure is also interpreted with caution as support for the recolonisation hypothesis. Spatial patterns of differentiation assessed using principle components analysis showed a north-south trend of decreasing ordination distance separating subpopulations, as the mean distance between sample sites is also largest in the Atherton tableland, geographic and ordination distances are confounded in the analysis,. A maximum likelihood tree connecting *H. albispecularis* subpopulations shows a star-like topology consistent with history of range expansion from a refugium in the central Wet Tropics, and a single deep split consistent with the older barrier isolating the Carbine Uplands. While tests for the heterozygosity effects of expansion show non-significant heterozygote excess, power limitations of these tests could be addressed with further screening. Morphological variation was limited, but showed some difference between subregions, and appeared explicable in terms of environmental gradients. It is concluded that Grey-headed Robins recolonised the Spec uplands from refugia in the Atherton Uplands during the Holocene rainforest expansion.

The effect of heart fatty acid binding protein (HFABP) gene variants on intramuscular fat (IMF) and back fat depth in commercial pig in Australia.

Payam Arasta[1], Yizhou Chen[1], R Kerr[2], Chris Moran[1].

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Improving meat quality is a very important objective in pig breeding. The level of intramuscular fat (IMF) is an important determinant of flavour and perceived quality of meat. Selection for reduced back fat levels in pigs has caused an undesirable correlated decrease in intramuscular fat levels. A Dutch research group has claimed that variants of the heart fatty acid binding protein (HFABP) gene are responsible for variation in intramuscular fat levels and have patented a genotyping test, potentially useful for improving IMF levels. We have studied the effect of these genetic variants at the porcine HFABP gene on IMF content and back fat thickness using 348 pigs from eight sire families bred at Bunge Meat Industries. Two primer pairs were used to amplify fragments of the gene and the PCR products were digested with either HaeIII or HinfI. Analyses of the data showed no significant effect of either of these polymorphisms of HFABP on IMF or back fat depth in agreement with preliminary studies in Australia and other recently reported studies in Europe.

RNAi and Gene Expression

Greg M. Arndt

Johnson and Johnson Research Pty Ltd, One Central Avenue, Australian Technology Park, Sydney, Australia Tel 61 2 8396-5837.

Double-stranded RNA (dsRNA) specific for a target mRNA has been shown to be a powerful and specific inhibitor of gene expression in a wide variety of eukaryotic organisms, and is now commonly referred to as RNA interference (RNAi). Until recently, the application of dsRNA as a genetic tool for controlling specific genes was restricted to non-mammalian cell types. This was overcome by studies showing that *in vitro*-generated long dsRNA could mediate gene silencing in mouse oocytes and pre-implantation embryos, that small 21-mer dsRNAs (called small interfering RNAs) could suppress gene expression in conventional mammalian culture cells, and that gene-expressed small hairpin RNAs could be used to silence genes in a stable format. The use of dsRNA to control the expression of specific genes is fast-becoming the method of choice for probing gene function in mammalian cells. In this talk, I will discuss the present understanding of the mechanism of RNAi from both biochemical and genetic experimentation. This will include the identification of genetic host factors that enhance the efficacy of RNAi. In addition, I will summarise efforts by ourselves and others to use RNAi to validate specific novel target genes for drug development and on a genome-wide level in mammalian cells.

Gene expression in anarchistic honey bees

Alyson Ashe[1].

[1] *School of Biological Sciences, University of Sydney, NSW 2006.*

In normal honey bee (*Apis mellifera*) colonies, the queen is the sole reproductive female. Workers usually refrain from reproduction, and will reproduce only once a colony has become queenless. In some colonies, however, workers reproduce despite the presence of a fertile queen. These 'anarchistic' bees have mutations allowing them to circumvent the normal control mechanisms preventing worker reproduction. I compared workers with and without activated ovaries from an anarchistic backcross colony to try and find differences in expression of various genes that might be associated with the regulation of worker reproduction. I compared these levels of expression with those found in young bees, older foragers, virgin queens, laying queens, and queens that I forced to stop laying by caging them. I found many differences in gene expression among the various castes and laying statuses studied. Most notably, the gene 'Alien' shows a pattern of expression that suggests that it plays a role in the regulation of ovary activation.

Genetic Refinement and Physical Mapping of a 2.3Mb Probable Disease Region Defining a Bipolar Affective Disorder Susceptibility Locus on Chromosome 4q35.

Renee F Badenhop[1,2], Melissa J Moses[1], Anna Scimone[1], Linda J Adams[1], John BJ Kwok[1], Anne-Marie Jones[3], Gail Davison[2], Mary R Evans[2,4], Kenneth C Kirkby[4], Jane E Hewitt[3,5], Jennifer A Donald[6], Philip B Mitchell[2] and Peter R Schofield[1]

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A susceptibility locus for bipolar affective disorder has been mapped to chromosome 4q35 in a large multigenerational pedigree. We have expanded this analysis to include 55 pedigrees (674 individuals, 214 affecteds). The evidence for linkage to 4q35 was strengthened in this larger cohort, with a maximum two-point LOD score of 3.2 for marker D4S1652. Several other markers spanning a 50cM region from D4S3047 (which lies 32cM centromeric to D4S1652) to the telomere gave LOD scores greater than 1.5. To further refine this region, haplotype analysis was carried out in 16 of the 55 pedigrees that showed evidence of linkage. As there is no evidence for an ancestral haplotype, nor a one-to-one correspondence between the disease and putative disease haplotype, we undertook an analysis based on pedigree-specific, identical-by-descent allele-sharing in order to define a probable disease region. This analysis indicated that the percentage sharing of alleles, identical-by-descent, in affecteds of all linked pedigrees increases from 60% at the centromeric markers to 75% for markers at the telomere. Maximal allele sharing occurred between markers D4S3051 and 4qTEL13 with this 24cM region defining a probable disease region. We have constructed a physical map of the 4q35 interval consisting of a YAC contig and BAC clones. Based on this map the probable disease region between D4S3051 and 4qTEL13 corresponds to only 2.3Mb. This region is very gene poor with only 3 known genes indicated from the YAC/BAC map. The small number of genes will facilitate systematic screening for variations associated with bipolar disorder.

Patterns of genetic variation in the freshwater shrimp *Paratya australiensis:* evidence for restricted dispersal and cryptic species

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Previous studies of genetic variation in *Paratya australiensis* (the Glass shrimp) have suggested that dispersal may be very limited among sites within different parts of a catchment. In this study we aimed to use levels of genetic differentiation within and among subcatchments to predict the likely rates of recolonisation following disturbance. Using part of the COI mitochondrial gene, we show that earlier results are supported and that dispersal appears to be very limited, even within a single subcatchment. This has implications for the utility of stream rehabilitation programs given that species may be unable to reach restored sites. The data also suggest that there is more than one species in eastern Australia and that we are dealing with a species complex.

Genetic management of endangered species: Does it work?

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As more and more species face extinction through human-induced causes, conservation programs are using captive management to provide stock for reintroduction, but the genetic success of these programs is rarely monitored. We are conducting experiments based on the captive breeding and successful reintroduction of the Greater stick-nest rat (*Leporillus conditor*), an endangered native rodent of Australia. By taking advantage of an extensive tissue collection and documentation of breeding and reintroductions, this study provides a rare opportunity to check on genetic changes during sampling, captivity, reintroduction, and establishment and maintenance of new populations.

Using nuclear and mitochondrial markers we show that the reintroduced populations have lost genetic variation compared to source populations, including some populations which appear to lack representation from one source, despite the presence of source animals in the reintroduction. Although gene diversity has been reduced relative to source populations, we show gene diversity in reintroduced populations is higher than predicted by empirical models. This has implications in the management of all endangered species, suggesting that reduction in genetic variation may not be as severe as expected.

Flower breeding Scaptodrosophila – Is the oldest genus of the Drosophilidae really different?

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[3] *Department of Genetics, La Trobe University, Bundoora VIC.*

Scaptodrosophila species have been largely neglected in genetic studies, primarily because they generally are difficult to maintain in the laboratory. One species that breeds in native Hibiscus flowers in Australia – *S. hibisci* – was chosen as a likely model system for evolutionary/population genetic studies, and 20 microsatellite loci were developed. Family studies done to confirm Mendelian inheritance of these loci showed a number of unexpected features – five are X-linked, 13 had non-amplifying (null) alleles segregating, and there was male recombination. Null alleles were detected at three additional loci in population analyses. In addition, two of the loci that appeared to be autosomal showed aberrant segregation, most simply explained by fusion of an autosome to the Y chromosome to form a neo-Y.

Scaptodrosophila hibisci is distributed along the coastal areas of NSW and Queensland, and a sibling species – *S. aclinata* – has been found in the Northern Territory. Nine populations of *S. hibisci* and five of *S. aclinata* have been assayed for the microsatellite loci. The number of alleles and expected heterozygosities were much less in *S. aclinata*.

Over all loci, populations within each species showed significant genotypic differentiation. For *S. hibisci*, spatially close (less than 2 km) populations breeding on different *Hibiscus* species showed similar levels of differentiation and genetic distances as populations from the same *Hibiscus* species, but at locations 1500 km apart – indicating both geographical and host plant differentiation.

The use of alien chromosome addition aneuploid lines facilitates genetic linkage mapping of the wild *Gossypium* species

Augusto Becerra Lopez Lavalle, Curt Brubaker

Centre for Plant Biodiversity Research; CSIRO Plant Industry; GP0 Box 1600; Canberra, ACT 2601,

Primary germplasm pools represent the most readily accessible source of new alleles for crop improvement, but when the most effective alleles are not available in the primary germplasm pool, breeders must confront the difficulties associated with introgressing genes from the secondary and tertiary germplasm pools, in cotton, by using synthetic polyploids as introgression bridges. Successful introgression using bridging species occurs when homoeologous recombination is frequent enough that the target genomic region has been introgressed before the donor chromosome is lost as each generation of progeny is recurrently backcrossed to the recipient genome. To track the fidelity and frequency of donor chromosome transmission in a *G. hirsutum* X *G. australe* (G genome) and *G. hirsutum* x *G. sturtianum* (C genome) hexaploid bridging families, 690 *G. australe* and 756 *G. sturtianum* chromosome-specific markers were used to screen first and second generation aneuploid alien chromosome addition lines, each of which contained the full complement of *G. hirsutum* chromosomes plus several *G. australe and G. sturtianum chromosomes.* In the *G. hirsutum* x *G. australe* and *G. hirsutum* x *G. sturtianum* families roughly half the chromosomes are lost at each meiosis. In both families, although the average number of chromosomes per individual decreased by roughly half per meiosis, the frequency of with which individual chromosomes were transmitted varied considerably, with one chromosome in both families being inherited nearly all the time, while others were quite rare. The genetic analysis showed that 690 *G. australe* chromosome-specific markers identified 17 distinct linkage groups and, similarly, 756 *G. sturtianum* chromosome-specific markers identified 19 distinct linkage groups. Identifying more than 13 linkage groups suggests that at least some *G. australe* and *G. sturtianum* chromosome restructuring has occurred. For the *G. hirsutum* x *G. australe* family the location and extent of these events could be determined by comparison to a *G. australe* x *G. nelsonii* F_2 map. Comparison of the two data sets also further resolved the genetic linkage map suggesting that when the homologous recombinations is low, first generation aneuploids are a useful adjunct to genetic linkage mapping.

Viral Eukaryogenesis

Philip Bell.

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It is generally accepted that all cellular life falls into either the archaeal, bacterial or eukaryal domains. However, the exact evolutionary relationships between these three domains remains unclear. In one school of thought, the archaeal and the bacterial domains diverged first from some ancient prokaryotic ancestor, and the eukaryal domain emerged later. Analysis of the complete genomes of members from each of the domains has revealed a complex and unexpected pattern of gene phylogeny. In general, the information processing genes of eukaryotes are more closely related to the archeal versions of these genes than the bacterial versions. By contrast, the metabolic genes of eukaryotes appear more closely related to bacterial versions of these genes than they are to archaeal versions. I have proposed a radical new theory that provides a rationale for the observed gene distribution, whilst simultaneously providing an explanation for the origin of the nucleus, and the radical changes in genetic architecture that differentiate the eukaryotes from the two prokaryotic domains (Bell, J. Mol. Evol. 53, 251-256 (2001). In the model, the nucleus is derived from a nucleocytoplasmic large DNA virus that established a persistent presence in the cytoplasm of a mycoplasmal methanogen. It is proposed that several characteristic features of the eukaryotic nucleus derive from its viral ancestry. These include, mRNA capping, linear chromosomes, and the separation of transcription from translation. In the viral eukaryogenesis model, phagocytosis and other membrane fusion based processes are derived from viral membrane fusion processes and evolved in concert with the nucleus. The co-evolution of phagocytosis and the nucleus rendered much of the host archaeal genome redundant, since the proto-eukaryote could obtain raw materials and energy by engulfing bacterial prey. Transfer of the functionally useful genes to the viral genome followed by loss of the redundant archaeal genome generated an organism similar to eukaryotic amoeba. The evolution of phagocytosis allowed the eukaryotes to be the first predators, sending them on a different evolutionary trajectory to the more ancient prokaryotes.

Immunoglobulin genes of marsupials and monotremes

Katherine Belov

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The cloning and characterisation of the heavy chain and light chain immunoglobulin genes from marsupials and monotremes has established that the immunoglobulin repertoire of non-eutherian mammals does not differ markedly from that of eutherian mammals, but is very different to that of birds. The four major "mammalian" heavy chain classes (IgG, IgM, IgA and IgE) are present in marsupials and monotremes, as are both kappa and lambda light chains. The presence of IgG, IgE, a three domain IgA and hinge regions in both marsupials and monotremes pinpoints the timing of the "second big bang" of immunoglobulin evolution to between 310 million years ago (after the separation of the mammalian lineage from other amniotes) and 170 million years ago (prior to the separation of extant mammals).

Phylogenetic analysis of immunoglobulin constant regions has provided strong support for the Theria hypothesis, while IgM data suggests that the monotreme lineage separated from therians 170 million years ago, the marsupial and eutherian lineages separated approximately 130 million years ago and that the extant marsupials separated approximately 65 million years ago.

Knowledge of the immunoglobulin gene sequences has also allowed the production of specific immunological reagents and the study of gene expression throughout marsupial pouch young development.

QTL mapping for growth rate in mice

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A large F2 resource pedigree (1128 mice) has been bred from two inbred lines (Inbred Quackenbush Swiss Line 5 (IQS5) and CBA) differing enormously in platelet count [IQS5 is higher by 5-6 phenotypic standard deviations than CBA] and body weight [IQS5 is higher by 7-8 phenotypic standard deviations than CBA]. Quantitative trait loci (QTL) responsible for these differences are being investigated using genome-wide and chromosome-targeted scans respectively. Using an IQS5 and C57BL/6 resource, Kirkpatrick *et al.* (1998) previously identified QTL on chromosomes 4 and 11 affecting six-week weight. The aim of the present study is to investigate these growth QTL in a different resource pedigree. Twelve microsatellite markers covering chromosomes 4 and 11 are being genotyped in a total of 228 mice (10% from the lower and upper extremes for body weight after adjusting for sex and collection date effects). Genotyping is almost complete and preliminary analysis supports the presence of QTL for growth rate on both chromosomes.

Fine scale genetic mapping analysis of a *Drosophila melanogaster* variant from the field indicates that *Cyp6g1* overexpression is involved in the mechanism conferring resistance to multiple insecticide classes.

Michael R. Bogwitz[1], Trent Perry[1], Tom G. Wilson[2], René Feyereisen[3], David G. Heckel[1], and Philip Batterham[1].

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Lufenuron and nitenpyram are potent insecticides used primarily in the control of the household cat flea *Ctenocephalides felis*. Nitenpyram is a neonicotinoid, a new class of insecticides also used to control sucking pests on agricultural crops. Lufenuron is an insect growth regulator that has not seen significant field usage. Previous studies into field resistance in US and Australian natural populations of *Drosophila melanogaster* have identified widespread resistance in this non-pest species [Wilson, T. G., and Cryan, J. R., *In* T. Brown [ed.] Molecular Genetics and Ecology of Pesticide Resistance. American Chemical Society Symposium, Series 645, 141-148, 1996].

One strain (WC2) was identified at having >100 fold resistance to Lufenuron. Resistance was mapped in this strain to the 48F region of chromosome 2 which contains a cluster of three P450s (*Cyp6g1, Cyp6g2, and Cyp6t3*). Daborn *et al.* (2001) showed that in some strains, resistance to DDT and the neonicitinoid imidacloprid is associated with overexpression of *Cyp6g1* [Daborn, P., Boundy, S., Yen, J., Pittendrigh, B., and ffrench-Constant, R., Molecular Genetics and Genomics 266, 556-563, 2001]. Expression analysis using WC2 on a P450 microarray chip revealed massive specific overexpression of *Cyp6g1*.

We undertook fine-scale mapping of Nitenpyram resistance and Lufenuron resistance using molecular markers. Our data indicates that both resistances map within this cluster. Molecular analysis of the WC2 *Cyp6g1* gene has identified an insertion of a partial transposable element (Accord) 292bp upstream of the transcription start site. This insertion contains a putative consensus promoter sequence, that may be responsible for the observed overexpression.

Comparative QTL mapping in mice for livestock traits

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Time taken to reach market weight is still one of the primary determinants of profitability in livestock production. Thus, while larger animals are likely to consume more feed, selection for growth continues to be important. Consequently, finding the genes controlling growth in livestock is of considerable interest. The usual approach to identify potential candidate genes is to map the trait and verify the quantitative trait loci (QTL) in the livestock genome. Unfortunately, QTL mapping in cattle is hampered by 1) long generation times, 2) small numbers of progeny per dam, 3) the lengthy period required to measure lifetime performance, and 4) the expense of large animal experimentation. An alternative is to map growth in a model species such as mouse. Accordingly, traits including growth were mapped in mouse lines selected for high and low feed intake. QTL for growth were identified in both mice and cattle and the corresponding chromosomal regions aligned comparatively. Notably, of the 4 QTL mapped for average daily gain in mice and the 5 QTL mapped in cattle, 3 were in common. Of the 3 QTL mapped for weaning weight in mice, one corresponded to a cattle QTL for weaning weight and another corresponded to a cattle QTL for average daily gain. Some of the QTL also corresponded to growth QTL previously reported in mice, cattle, pigs and humans. The results suggest that comparative QTL mapping for traits such as body composition and feed efficiency in mouse will be an expedient approach to help identify potential candidate genes for cattle.

Polyploid Evolution in *Glycine*

A.H.D. Brown[1], R.G. Palmer[1,2], J.L. Doyle[3], J. Rauscher[3] and J.J. Doyle[3]

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Strategies for the conservation and use of the wild genetic resources of crops should rest on knowledge of the evolutionary processes and relationships among their related species. The soybean genus *Glycine* has two subgenera, subg. *Soja* (which includes the crop species *G. max*) and subg. *Glycine*. The latter subgenus consists of 20 named perennial species and has diversified extensively throughout Australia. Two of these species (*G. tomentella* and *G. tabacina*) are widespread complexes containing diploid and polyploid cytotypes. Comparison of these allopolyploid complexes, their diversity, origins and distribution provides hypotheses on the success they have had in spreading to a diversity of habitats. Recent DNA sequence information for the single-copy nuclear histone H3D locus has confirmed and greatly extended earlier evidence from hybrid cytogenetic analyses, isozyme polymorphisms, 5S-RNA, chloroplast RFLPs and 18S-26S ribosomal gene (nrDNA) ITS sequences concerning the multiple origins of these polyploid complexes. The *G. tabacina* complex includes two distinct reproductively isolated allotetraploid races (here denoted AB' and BB'). The sources of the three H3-D B-alleles found in tetraploids so far, are two diploid B-species (*G. tabacina* (2X) and *G. latifolia)*. The H3D sequence identity argues that for these lineages, both polyploidy and its subsequent spread to the western Pacific have happened within 30,000 years. The diploid B-species donors are interfertile, and their BB' *G. tabacina* tetraploid derivatives have recombined to generate a diverse complex. This inter-fertility differs from the *G. tomentella* polyploid complex. Diploid *G. tomentella* is polymorphic and polyphyletic with several clades well defined in gene trees using ITS and histone H3-D sequences. Artificial hybrids between these diploid clades are sterile. Polyploid *G. tomentella* races combine genomes of the diploid races and some other *Glycine* taxa. Each distinct combination has proved to be reproductively isolated. Some races have evidence of more than one origin providing scope for lineage recombination. Most H3-D alleles in the polyploids are identical or closely related to alleles in diploids, suggesting recency of origin and spread. One further twist is that one race in each of the tetraploid species complexes (*G. tabacina* race AB', and *G. tomentella* race T2) share the same putative donor (*G. tomentella* D4 for both), linking the two in a higher order complex.

Linkage analysis of resistance to Bacillus thuringiensis in cotton bollworm *helicoverpa armigera*

Sarah Brown

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Conservation genetics of the "Critically Endangered" Victorian brush-tailed rock-wallaby, *Petrogale penicillata*.

Teena L. Browning[1], David A. Taggart[2], Mark D.B. Eldridge[1]

[1]*Biological Sciences Macquarie University*, [2]*Department of Zoology, University of Melbourne,*

The brush-tailed rock-wallaby, *Petrogale penicillata*, has a broad distribution throughout south-eastern Australia. The only rock-wallaby to occur in Victoria, *P. penicillata* was once widespread and abundant. Severe declines have occurred since European settlement and populations are now increasingly patchy and isolated. In 1995, only remnant populations existed in Victoria, with a small cluster in East Gippsland and in the Grampians further to the west. Previous data has suggested that the Victorian populations of *P. penicillata* are genetically distinct and may constitute a separate ESU from other *P. penicillata* populations. This may have significant implications for conservation and management plans. Analyses of karyotypes, microsatellites and mitochondrial control region sequence have been examined to assess genetic variability. Each remnant population was found to be distinct, possessing unique microsatellite alleles and control region haplotypes. The distribution of genetic diversity was found to be mostly between, rather than within populations. All of the control region haplotypes present in the Victorian populations were found to be closely related (average 1.3% sequence divergence). In contrast, there is considerable divergence between the Victorian haplotypes and those found in *P. penicillata* elsewhere in the species range (average 7.7% sequence divergence). Victorian *P. penicillata* haplotypes form a distinct and well supported monophyletic group that excludes haplotypes from other *P. penicillata*. It is likely that Victoria's brush-tail rock-wallaby populations constitute a separate "Critically Endangered" ESU . Although further analysis of nuclear genes will be required to confirm this.

Identification and Phylogenetic Analysis of Sex Chromosome Centromere Repeats in Macropodidae

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The family Macropodidae, one of the largest in the Marsupial taxa, has 45 species ranging in diploid chromosome number from 2n= 10/11 to 22. Several species of wallabies have unusually lengthened centromeres that are up to half the length of the chromosome and contain condensed heterochromatin. Microdissection and microcloning (Kao F, Yu J. 1991) of the centromere of the X chromosome from a *M. rufogriseus* x *W. bicolor* hybrid male yielded sequences of traditional and non traditional satellites, and retroelements. The presence of these sequences are restricted to only a subset of the Macropodidae and their copy number varies widely within the family, having the highest copy number in those species displaying abnormally lengthened centromeres. Southern hybridization has been used to assess presence, quantity and methylation status across the family. Fluorescence *in situ* hybridization (FISH) has been used to localize sequences to their specific chromosomal locations in *M. rufogriseus*, one of the species bearing lengthened centromeres.

Do Stingless bees live in a police state?

Emilie C Cameron, KE Palmer and Ben P Oldroyd

School of Biological Sciences, University of Sydney, NSW Australia, 2006 .

Stingless bees live in societies consisting of a singly mated queen and hundreds of workers. Microsatellite analysis has revelled that the majority of drones produced by a colony are sons of the queen. Workers are physiologically able to produce sons themselves, so what is keeping them from reproducing?

Worker policing is any action by a worker that reduces the reproductive output of other workers. In multiply mated species of social insects, such as honey bees, workers are related by 0.25. In this situation policing is expected, and has been demonstrated. Workers in singly mated species however, have a greater relatedness (0.75) and are more likely to tolerate other workers laying eggs. *Austroplebia australis* is a singly mated species of stingless bee native to Australia. When queen-laid and worker-laid eggs were transferred into a queen-right discriminator colony there was no difference in the treatment of each egg type. This suggests an absence of worker policing in this species.

The Evolution of Fruitbats (Megachiroptera)

Don J. Colgan and P. Da Costa

Evolutionary Biology Unit, The Australian Museum, 6 College Street, Sydney 2010

The morphological assessment by Andersen in 1912 remained the best hypothesis of megachiropteran evolution for nearly a hundred years. In the 1990's studies of RFLPs, DNA-DNA hybridisation and mitochondrial DNA sequences indicated the requirement for substantial revisions of this hypothesis, although the studies disagreed as to what changes are needed. This investigation was undertaken to increase the number of studied species and to add the first data from a nuclear gene sequence. We studied 12S ribosomal sequences (aligned length of 405 positions) from seventy-five Megachiroptera (50 species in 20 genera) and two microchiropteran outgroups. We studied c-mos sequences (aligned length of 488 bases) from 56 Megachiroptera (42 species in 19 genera) and used three eutherians from GenBank as outgroups.

The results confirm that the nectar-feeding sub-family Macroglossinae is not monophyletic with the long tongued phenotype arising at least twice. The existence of a major clade including a monophyletic endemic African component and biogeographically neighbouring genera such as Rousettus and Eonycteris is supported. The phylogenetic position of one African genus, Eidolon, remains uncertain. A cynopterine section (excluding Nyctimene and Myonycteris) is supported, albeit weakly, as a monophyletic group. Pteropus and the related, possibly polyphyletic genus Pteralopex are unexpectedly basal compared to previous molecular studies.

The best estimate of the root of the Megachiroptera is between Nyctimene, the only studied insectivorous genus, plus Notopteris the only long-tailed fruitbat, and the other genera . Several alternative rootings are, however, not rejected by the data, suggesting a rapid early radiation. Generic distributions indicate that this may have occurred in Melanesia and molecular dating on the basis of 12S rDNA suggests that this may have been during the late Oligocene.

Genomic Organisation of a Cellulase Gene in Redclaw Crayfish (*Cherax quadricarinatus*) that Encodes a Functional Endo-β-1,4-glucanase

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The most abundant organic compound produced by plants is cellulose, however it was believed for a long time that most animals simply do not produce the necessary enzymes required for its degradation. Here, the genomic organisation of an endogenous cellulase gene in redclaw crayfish (*Cherax quadricarinatus*) is presented, consolidated from a previous cDNA sequence. The translated amino acid sequence from the N-terminal portion of the gene was found to be identical to the N-terminus from one of two cellulase enzymes purified from redclaw gastric fluid. The N-terminal sequences for the two cellulases are identical for 7 from 11 amino acid residues, and are likely to be products of separate genes. In addition, both enzymes [Cellulase 1 – 48kDa; Cellulase 2 – 50kDa] were found to be active against cellulose substrates, however Cellulase 2 was also active against xylan substrates. Cellulase enzymes may be used by the organism to obtain energy from cellulose substrates or as a tool to access other nutrients within plant cells. By breaking down plant fibre within the gut, other crayfish digestive enzymes may have improved access to appropriate substrates. Applications of these studies may result in the development of improved artificial diets for crustacean aquaculture species, incorporating low-cost feed components sourced from plant material. Furthermore, those species that do possess endogenous cellulase genes may be amenable to approaches designed to increase *in vivo* activity.

Molecular Phylogeny Indicates at Least Three Origins of Crassulacean Acid Metabolism in Bromeliaceae

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The Bromeliaceae, one of the largest families of plants with a primarily Neotropical distribution, are often celebrated as an extraordinary example of adaptive radiation. Explosive speciation in bromeliads, particularly in epiphytic niches, appears to have been linked to several key innovations, including water- and nutrient-impounding 'tanks' (phytotelmata), the absorptive epidermal trichome, and the water-conserving crassulacean acid metabolism (CAM) mode of photosynthesis. Although various scenarios have been proposed for the origins of CAM and the epiphytic habit in bromeliads, these have remained purely speculative in the absence of a well-supported phylogeny for the family. To help reconstruct the evolutionary origins of these traits, we have analyzed sequence data for 51 taxa of bromeliads using the plastid loci *matK* and the *rps16* intron by successive weights parsimony analysis. Photosynthetic pathway was also determined for 1870 species in the family by carbon-isotope analysis. This study confirmed the monophyly of two of the traditionally circumscribed subfamilies, Tillandsioideae and Bromelioideae, whereas Pitcairnioideae is clearly paraphyletic. Optimization of character-states onto the strict consensus tree indicated that both CAM and the epiphytic habit evolved a minimum of three times in the family. The phylogeny revealed that: (i) the most recent common ancestor of Bromeliaceae was a terrestrial C_3 mesophyte, possibly similar to present-day pitcairnioids of moist, nutrient-poor habitats; (ii) the ancestral tillandsioid was an epiphytic C_3 mesophyte, with CAM being a later development in more xeric lineages; and (iii) the ancestral bromelioid was a terrestrial CAM xerophyte, with epiphytism developing as certain lineages spread into forest habitats, and some taxa reverting to C_3 photosynthesis in less xeric habitats.

Identification of a juvenile hormone esterase gene from *Drosophila melanogaster* and evidence for a gene duplication.

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Juvenile hormone esterase (JHE, EC 3.1.1.1) has an important role in insect metamorphosis, hydrolysing juvenile hormone (JH) prior to pupation. The enzyme has previously been purified from whole *Drosophila melanogaster* prepupae. The purified enzyme was used to produce a peptide mass fingerprint, using MALDI-TOF mass spectrometry to determine the masses of the tryptic digest peptides. Only one predicted gene product from the *D.melanogaster* genome matched the JHE tryptic fingerprint with high confidence. The predicted JHE sequence includes features conserved among all active members of the serine carboxylesterase multigene family as well as features peculiar to JHEs from other species. A possible duplication of this predicted JHE has been identified upstream of this gene. Two additional genes were also identified as being related through sequence analysis of the *D. melanogaster* genome. The cloning and preliminary characterisation of these genes is discussed here.

Geographic variation in jellyfishes: *Aurelia* and *Mastigias* (Cnidaria, Scyphozoa)

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We present data describing ecological and molecular variation in two jellyfishes. The moon jellyfish, *Aurelia*, has a circumglobal distribution, limited morphological variation, and physiological commonalities across its range. Yet DNA sequence data indicate ancient divergences (~15-25% in Cytochrome oxidase c subunit I [COI]; ~10-40% in Internal Transcribed Spacer One [ITS1]) among at least eleven species. In contrast, the golden jellyfish, *Mastigias*, described from Palau, exhibits morphological, behavioural, and physiological differences among populations only a few kilometres apart. DNA sequence data describing *Mastigias* vary very little (~1% in COI; ~2% in ITS1). Despite their superficial differences, results for both species are consistent with limited dispersal and local adaptation. The results have implications for both estimates of biodiversity in the Scyphozoa—for example, species diversity has already increased by approximately 5% (from ~200 to ~210) simply as a result of one molecular study of one "species"—and patterns and rates of evolution in marine taxa.

Does X-inactivation Exist in Monotremes?

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Dosage compensation for X-borne genes between XX females and XY males is achieved in mammals via inactivation of one X chromosome in female somatic cells. X chromosome inactivation has been characterized in two of the three extant mammalian groups. In eutherian mammals, X inactivation is random, stable and complete. It appears to be under the control of an X inactivation centre known as *XIST*. A more simplistic form of X inactivation is evident in marsupials. Marsupials diverged from eutherians 130-150 million years ago. X inactivation in these mammals is characterized by being paternal, tissue specific and incomplete. Since X inactivation occurs in both of these distantly related mammalian groups, a basic mechanism for X inactivation is likely to have been in place in a common ancestor prior to their divergence. Monotremes diverged from eutherians 170 million years ago and may assist in unravelling the evolution of X chromosome inactivation mechanisms. Of particular interest is the evolution of the *XIST* gene. Genes flanking *XIST* in eutherians (*MNK, CDX4, XPCT*) have been cloned in the platypus (*Ornithorhynchus anatinus*) to determine the chromosomal location of this region in monotremes. Additionally, it is anticipated that the cloning of these genes will now assist in the discovery of the *XIST* gene (or its progenitor) in the platypus by chromosome walking. Three genes representative of different regions of the platypus X chromosome, one from the pseudoautosomal region of Xp (UBE1) and two from Xq (*AR, G6PD*) have also been cloned. Expression studies on these genes indicate that there is X chromosome inactivation in monotremes.

QTL Associations for Density and Diameter in *Pinus radiata* and the Potential for Marker-Aided Selection

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We have shown that marker-aided selection (MAS) should be possible in radiata pine (*Pinus radiata* Donn. ex D. Don). Quantitative trait loci (QTL) detection experiments were carried out for juvenile wood density (JWD) and diameter at breast height (DBH) using a large full-sib family. The QTL detection populations for JWD and DBH consisted of 1379 and 4435 trees, respectively. Selective genotyping was used in both experiments to improve efficiency and reduce costs. Following phenotypic measurements, the 50 highest and 50 lowest trees for JWD and the 100 highest and 100 lowest trees for DBH were selected for marker genotyping. RFLP and microsatellite markers were selected from an existing linkage map to be evenly spaced throughout the genome at an average distance of 20 cM. Analyses of variance indicated that 27 markers were significantly ($P < 0.05$) associated with JWD and 13 markers were significantly associated with DBH. An independent set of 400 progeny from the same family were used to validate these results, and with a somewhat lower level of significance (P values ranged from 0.001 to 0.1), 19 markers were validated for JWD and three were validated for DBH. Based on map location, this would correspond to at least nine QTL positions for JWD and three for DBH. The percent variance accounted for by the markers ranged from 0.59 to 3.57%, suggesting a genomic architecture of many small effect genes. All but three of the markers associated with JWD did not show a detectable association with DBH suggesting that it should be possible to improve density without adversely affecting diameter. Marker-based selection was used in the validation population to estimate gain. Depending on the number of markers used for selection, the average expected gain in JWD for the top 20 trees ranged from 9.5 to 17.5% and the observed gain was 4.5%. Two unrelated "bridging" families were chosen as candidates for MAS, and six microsatellite markers showing an association with either trait were tested in these families. Of these, four markers showed a consistent association with JWD in one or both of the bridging families. Based on these results, a pilot scale MAS study has been initiated.

Molecular evidence for the origin of parthenogenesis in the Australian skink lizard *Menetia*

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A detailed survey of more than 300 skinks of the genus *Menetia* from across Australia with molecular genetic approaches, karyotyping and gonad based sex identification has revealed a complex of sexual and asexual taxa within the *Menetia greyii* species complex.

The main points are:

1) Based on analysis of allozyme profiles, mitochondrial *ND4* and nuclear intron nucleotide sequences, *Menetia* comprises a number of sexual lineages with the two nominate species *amaura* and *greyii* and some populations of *maini* forming a single lineage, the "*greyii* complex", well embedded within the genus. Within the "*greyii* complex" at least 10 sexual taxa have been identified. The other nominate sexual species and some other populations of *maini* form ever increasingly distant sister lineages to the "*greyii* complex". The greatest diversity of sexual lineages appears to be in western Australia with other diverse areas being northern and eastern central Australia, although this reflects to some degree the availability of samples.

2) Four asexual lineages have been identified on the basis of their all-female nature, triploid karyotypes, the presence of triploid allozyme expression patterns, triploid microsatellite allele profiles at some loci, and ancestry of their mitochondrial DNA's. Further incomplete data suggests presence of at least one other asexual lineage in north-western Australia.

3) All of the asexual lineages occur within the "*greyii* complex".

4) Phylogenetic analysis of the intron sequence data from the asexuals indicates that some lineages have a "trihybrid" origin, ie their alleles are related to extant alleles from three divergent sexual lineages, while others appear to have a single allele indicating a more restricted origin.

5) Three of the four asexual lineages have wide distributions across WA and SA, while the fourth is restricted to north-western Australia.

6) There appears to be two centres of geographic origin of the maternal ancestors of the asexuals, one on the west coast of WA and the other in north-eastern SA.

7) The mtDNA and intron data so far provide a variety of perspectives on the specific ancestors of the asexuals. For one lineage the intron data do not show the maternal contribution at all, suggesting the occurrence of gene conversion.

Comparative Yeast Genomics

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Following the sequencing of its genome, the first eukaryotic genome entirely deciphered back in 1996, the baker's yeast *Saccharomyces cerevisiae* already a very important model system for modern Biology, has played a key role in the development of post-sequencing Genomics and, at present, over 65 % of its genes are now functionally characterized. At the same time, the yeast genome itself, although relatively simple, has been reinterpreted and annotated using novel pieces of information such as transcript mapping, gene fusion experiments, or comparative analysis. As for all other eukaryotes (and nearly all genomes sequenced so far), the yeast genome shows a significant, though limited, degree of internal redundancy resulting from ancestral duplications. Families of two, three or four genes are prominent in number, but larger gene families also exist, raising specific problems for functional studies.

Given the relative genomic sizes and complexities among eukaryotes, Hemiascomycetes, the group to which *S. cerevisiae* belongs, appear as most appropriate for comparative sequencing studies. Three years ago, with the Génolevures consortium[1], I have started the genomic exploration of thirteen such yeast species selected for their phylogenetic positions and sequenced at low coverage. Analysis of the sequences were made by comparisons to *S. cerevisiae* and to general sequence databases. Beside the fact that over 20 000 novel yeast genes were identified (all are publicly available). The important aspect of this program was that, for the first time, eukaryotic genome evolution could be explored at a large scale (ca. 20-40 % of the genes of each yeast species were compared) and on a large number of species belonging to a unique phylogenetic group. The relative importance of phenomena such as sequence divergence, gene loss or acquisition, chromosome rearrangements, sequence duplication, gene family expansion or contraction, could be estimated, leading us to a dynamic view of genome evolution in which segmental duplications and gene effacement play an important role. Direct evidences in favor of such mechanisms have now been obtained.

Comparative genomics of Hemiascomycetous yeasts has now been extended to the complete sequencing of the pathogenic yeast *Candida glabrata* (whole genome assembly terminated), *Yarrowia lipolytica*, *Debaryomyces hansenii*, two alkane-utilising yeasts distantly related to *S. cerevisiae*, and *Kluyveromyces lactis*. Preliminary results on the *C. glabrata* genome will be presented.

1 The Génolevures Consortium is composed of the laboratories of J-L. Souciet, M. Aigle, M. Bolotin-Fukuhara, B. Dujon, C. Gaillardin and M. Wésolowski-Louvel, in collaboration with the CNS-Génoscope (Jean Weissenbach). It has been recently extended to the laboratory of A. Nicolas and is working in collaboration with the Génopole (C. Bouchier, L. Frangeul) of Institut Pasteur.

Levels of extra-territory paternity in the white-backed Australian magpie, *Gymnorhina tibicen tyrannica*.

Kate Durrant, Jane Hughes

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The Australian magpie is a strongly territorial passerine that displays a wide variety of social strategies across its range and races. Black-backed birds from the north-east of the country live in pairs in large territories, while western birds live in large family groups of up to twenty birds in large territories and most young do not disperse. White-backed birds from south-eastern Australia live in small groups averaging three adults in small territories, and there is a well defined non-breeding, non-territorial flock system into which young birds disperse. Hybrids between the black and white-backed races, living in central Victoria, live in large multiple adult groups and also have a flock system. Levels of ETP's in the western population are extremely high (up to 95%) and it is thought that this is an inbreeding avoidance strategy in this region where there is no flock and young do not disperse. In order to test this, levels of ETP's and dispersal were investigated in a population with a flock system. A sample population of the white-backed race of the Australian magpie was banded at a field site near Rowsley in south-western Victoria. These birds have been observed over two breeding seasons, August to October 2000-2001, and 34 territorial family groups have been identified. At the time of capture, all birds were blood sampled. Nine microsatellite loci were employed to determine parentage of chicks born to these territorial groups over the two breeding seasons. The levels of extra territorial paternity were 17% in 2000 and 27% in the 2001 breeding season. These levels were found to be substantially lower than levels of ETP's detected in populations of western and hybrid magpies. Dispersal data from the white-backed population indicates that dispersal is male-biased and it is thought that many of these young male birds will join a flock. The white-backed population data offers support to the theory that where dispersal is limited, ETP levels will be higher, possibly to avoid inbreeding. Where dispersal to a flock is possible, as in the white-backs, levels of ETP's will be lower.

The sib-similarity problem

John H Edwards

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The major investment in trawling the human genome for influential loci has been by typing affected sib-pairs with parents (ASPs). Over a hundred major studies have been published with limited success (Altmueller et al. Am. J. Hum. Genet, 2001. 69: 936-950). No substantial studies of normal sib pairs have been undertaken, making this family of surveys one of the largest undertaken in the absence of controls. Penrose, who introduced the procedure (Ann. Eugen., 1935. 6: 133-138), used ascertainment for sibships with one or more affected sibs, not two or more as in ASPs, greatly simplifying the collection of data and providing controls. In mammals there are substantial losses between conception and birth, of the order of 50% in our species, only about a third being large enough for miscarriage to be noticed. In addition, the hundreds of sperm that embrace the ovum, many arriving within seconds of the first, provide ideal opportunities for preferential, or even obligatory, fertilisation between gametes that differ at alleles at one or more loci: a cost-free method of conserving genetic variability widely exploited in plants and unlikely to be absent in vertebrates. Unfortunately, both in humans and the farmyard, raw data from which genetic maps are constructed is rarely published, and data on sib-similarity that distinguishes the parental contributions is rarely available. This is mainly due to assuming that the usual methods for ordering loci, while reliable with small numbers of loci, can be automated and extended to large numbers using various short-cuts, approximations and assumptions, including 'average-sex'. With multiallelic markers, paternal and maternal gametes can provide independent evidence on order and position. Many associations found in sib-pair analyses tend to be similar in diverse disorders: these are presumably due to events between fertilisation and birth when the proportion of losses are many times greater than the prevalence of any common disorder. The obvious causes of excess sib-similarity include embryonic lethals and selective fertilisation. The former would be expected to lead to similar paternal and maternal excess sib-similarity, relative to random association, and the latter to purely paternal gametic effects, in the absence of imprinting, X-linked effects, or strongly homologous X-linked and autosomal loci. The general pattern is easily seen on visual displays based on simple counts from informative meioses. These are displayed in medvet.angis.org.au, with the original papers in sib-pair analyses of Penrose.

An extension of AceDB to the Oxford Grid and OMIA

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The Oxford Grid [Edwards JH, Ann. Hum. Genet. 55, 17-31 (1991)] provides a simple way of comparing linkage data from either the averaged genome of two species or the paternal and maternal gametic maps from the same species. We demonstrate the power of AceDB (www.acedb.org), a genome database system developed for *C. elegans* but applicable to other species, to display data from the human and dog genetic maps with the constraints imposed by in-situ mapping using data from the human and dog genomes. Chromosome mapping of the dog has been hampered by the high chromosome number (2n=78) and the difficulty in distinguishing the seventeen smallest chromosomes. Two groups [Breen et al., Genomics 61, 145-155 (1999); Yang et al., Genomics 62, 189-202 (1999)] have resolved this independently; we use the Yang nomenclature here. The integration of the Oxford Grid within the ACEDB software, and its recent extensions, allows the positioning of points defining estimated position, and their colouring under defined categories. This gives instant access on pointing to OMIA, OMIM and, shortly, LDB and to external related DNA and protein databases. The inferred positions of pairs can be overlaid by transparent rectangles showing their inferred constraints on in-situ mapping. ANGIS, the "Australian National Genomic Information Service" has, within its server, OMIA, a mirror of OMIM, and shortly, LDB, allowing very rapid cross referencing. See www.medvet.angis.org.au for further details.

Absence of association of androgen receptor trinucleotide expansion and poor semen quality

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Genetic mutation, in genes essential to normal fertility is suspected to be the cause of poor semen quality in 70% of men considered idiopathically infertile [Yong *et al.*, J. Endocrinol. Invest. 23,573-577 (2000)]. The androgen receptor (AR) triggers the initiation and maintenance of sperm production in response to stimulation by male sex androgen, testosterone and dihydrotestosterone. Expansion of a polymorphic trinucleotide (CAG) repeat encoded within exon 1, the transcription-activating domain of the human androgen receptor gene is hypothesised to be associated with an increased risk of poor semen quality. We aimed to investigate the possible relationship between semen quality and AR-CAG repeat number in a New Zealand Caucasian sample population. Variation in CAG-repeat number was analysed in a total of 171 men with poor semen quality and 94 men with normal semen quality. Men with poor semen quality had similar AR-CAG repeat number to men with normal semen quality (21.57 ± 0.24 vs 20.97 ± 0.28, $p = 0.052$). These results argued against the hypothesis that higher CAG repeats are associated with infertility in men.

High Throughput Mouse Mapping Microsatellite Marker Sets

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With the increasing demand for mouse mapping of specific mouse strain crosses, the Australian Genome Research Facility (AGRF) in collaboration with the Genetics and Bioinformatics group at the Walter and Eliza Hall Institute have developed, and are continually improving, a strain specific mouse microsatellite marker database.

The markers in this database have all been chosen according to an assessment of their usability in a high throughput system. All marker information regarding actual size for each microsatellite for each listed strain has been qualified through the system. Each marker pair has been fluorescently labelled and is stored.

The importance of such a tool is that the AGRF will now be able to quickly process genotyping runs on specific mouse strain crosses using well defined and characterised markers. This eliminates the time consuming and costly exercise of ordering and testing markers on specific crosses before panels of markers can be assembled for full genome screens. The database is continually being improved with additional strains being tested.

The availability of a quick, high throughput mouse genotyping service such as the one developed here has been necessary to develop and will be a valuable tool for future mouse mapping.

Clinal distribution of inversions in *Drosophila serrata*

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Drosophila *serrata* is an Australian endemic species of the montium group with a continuous range extending from Papua New Guinea in the north, to Wollongong NSW in the south. A habitat generalist, it exists across a very long cline ranging from tropical rainforest to temperate urban environments. Our study has shown a cline in inversion polymorphism on three of the four main chromosomal arms of D. *serrata,* with southern serrata free of polymorphism, and high levels of polymorphism in the north, around Cairns. There are two overlapping inversions (relative to the southern sequence) on the 3R chromosomal arm, one very common, and one relatively rare, which might indicate the presence of a cluster of stress related genes in this region. Inverted sequences in a population are thought to contain suites of co-adapted genes, and future studies on the fitness characteristics of D. *serrata* with these inversions, and the response of the inversions in lab stocks to selection pressures, might elucidate the function of the polymorphisms in an ecological context.

Inbreeding and Extinction: Effects of Rate of Inbreeding

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Deleterious alleles may be removed (purged) by natural selection in populations undergoing inbreeding. However, there is controversy regarding the effectiveness of selection in reducing the risk of extinction due to inbreeding, especially in relation to rate of inbreeding. We evaluated the effects of rate of inbreeding on reducing extinction risk, in populations of *Drosophila melanogaster* maintained using full-sib mating (160 replicates), or effective population sizes of 10 (80) or 20 (80). Extinction rates in the populations maintained using full-sib mating occurred at lower levels of inbreeding than in the larger populations, which did not differ significantly. Inbreeding coefficients at 50% extinction were 0.62, 0.79 and 0.77 for the full-sib, N_e = 10 and N_e = 20 treatments, respectively. Populations of effective size 20, that remained extant after 60 generations, showed inbreeding depression, progeny production being only 45% of that in outbred controls. Purging may slow the rate of extinction slightly, but it cannot be relied upon to eliminate the deleterious effects of inbreeding.

Identifying QTL for wool traits in sheep

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CSIRO generated, in the mid 1990s, a sheep mapping reference/ resource flock with the aim to improve the sheep linkage map and to identify QTL for a range of traits of commercial significance in wool production. In addition to 27 parents and grandparents, there are approximately 400 hundred second generation individuals, all of which have been typed for DNA markers, mostly microsatellites, that provide a fairly even coverage of the genome. The progeny comprise both full-sib and half sib families (170 and 230 respectively). The second generation progeny have been measured, over three years of production, for wool production and various components of this trait, such as fibre diameter, wool follicle density, staple length and strength, *etc.* In addition, phenotypes for a number of other traits -- such parasite resistance, reproductive performance, body size and composition – have been collected.

In parallel, our group has generated several thousand skin ESTs, collected data on their probable function, and screened over half of these for *in-situ* expression patterns

In this talk, I discuss the preliminary analysis of the QTL analysis for wool traits, and of the EST data, and the implications of this work for the genetic improvement of Merino sheep.

New Insights into Mammalian Genome Evolution by Molecular Cytogenetics

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Over the last decade comparative gene mapping and comparative molecular cytogenetics have demonstrated that the genome organization in placental mammals is generally highly conserved. Interspecies chromosome painting revealed that large tracts of the human genome have been conserved in syntenic groups for about 90 million years of Placentalia evolution. For every mammalian order analyzed, species have been identified with highly or fairly conserved karyotypes. Only a minority of taxa displays extensive genome reshuffling. The general rarity of chromosome rearrangements in the history of mammals should allow the reconstruction of the ancestral Placentalia genome as well as the identification of landmarks of its evolution.

For this purpose we extended our comparative chromosome painting studies in two ways: I) we analyzed the karyotypes of two species belonging to old mammalian orders ii) we enhanced the traditional analysis by using probe sets derived from several species for multidirectional painting experiments which yield more detailed insights into chromosome evolution.

i) According to recent molecular DNA sequence analysis the Rodentia and particularly the Afrotheria constitute some of the oldest placental orders. Therefore their analysis should be of special value for identifying traits of the ancestral mammalian genome. We have generated chromosome specific libraries for the African Elephant and the Eastern Grey Squirrel by chromosome flowsorting and DOP-PCR and analyzed their genomes by forward and reverse chromosome painting.

ii) We report on comparative chromosome painting studies with human, pig, and sheep paint probes in a baleen whale, the hippopotamus, and cattle. These studies allow us to identify the characteristic traits of the ancestral cetartiodactyl karyotype and to resolve some phylogenetic questions.

Generation of meiotic recombination maps for Cat and Mouse chromosomes

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The recent years have witnessed a rapid increase in the knowledge of the molecular mechanisms of meiotic recombination. Many of the proteins involved in meiosis have been identified and analyzed. The immunostaining of one of these proteins (MLH1) has greatly facilitated the analysis of meiotic recombination in mammals. Here we used multicolor chromosome painting in combination with these immunostaining techniques to analyze pachytene spermatocytes of the mouse and the cat.

The new approach allowed for the generation of a detailed cytological recombination map for the male mouse genome. We show that SC length has a major influence on crossover frequency and distribution. Furthermore the cytologically defined regions of increased recombinatorial activity do not necessarily coincide with molecularly identified hotspots. The results indicate that recombination is regulated to a large amount on a cytological level. We demonstrate that the re-probing of the SC spreads with genetically characterized BACs, is a robust strategy for integrating the recombination data with meiotic and mitotic chromosome structure, as well as genetic linkage and physical contig maps.

The new method permits the comparative analyses of meiotic recombination between different species and subspecies without the need for breeding experiments. Thus it promises valuable new insights into the regulation of meiotic recombination.

Characterisation of the genetic control of vegetative propagation in *Eucalyptus*

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E. grandis, *E. globulus* and *E. urophylla* are all of major importance in the commercial plantation industry. *E. globulus* has some of the best wood properties for pulp production but has very irregular adventitious rooting behaviour, hampering improvement programs dependent on the vegetative propagation of superior genotypes. On the other hand, *E. grandis* and especially *E. urophylla* are considered to have good rooting ability. Both of these species are used in hybrid breeding programs to incorporate good rooting into other desirable genetic backgrounds.

Studies have been carried out in the Molecular Breeding Laboratory (CSIRO FFP) on the genetic control of root setting in stem cuttings of *Eucalyptus nitens*. A genetic map was constructed with 96 RFLPs and 12 microsatellites, using a test pedigree (n=326). Seven putative QTL sites for rooting of stem cuttings were initially identified. Loci that appeared to be linked to QTL in the test pedigree were assayed in a validation pedigree (n=210), with one parent in common with the testing family. Two QTL were confirmed in the validation pedigree.

This study documents aspects of the previous study, and describes the process of further validation by identifying QTL influencing the rooting ability in other eucalypt species. An *E. urograndis/E. globulus* hybrid cross (n=117) was utilised in the construction of a second molecular map incorporating 37 RFLP and 41 microsatellite markers, and in the assessment of rootability over three settings. Results are presented. Identification of genetic markers reliably linked to good rooting in more than one *Eucalyptus* species would suggest the proximity of genes of importance to rooting and should aid in incorporating the characteristic into the *E. globulus* background in future breeding cycles.

Genetic variation within and between two Victorian populations of *Thelymitra circumsepta* (Orchidaceae)

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The Naked Sun-orchid (*Thelymitra circumsepta* Fitzg.) is a vulnerable species in Victoria. Although restricted to wet, open sites, populations are found across a variety of habitats ranging from near coastal to alpine areas, suggesting that some genetic variation is likely to exist. In a previous study, Sydes (1994) found a lack of genetic variation in all individuals positively identified as *T. circumsepta* from six Victorian populations, which included Brisbane Ranges National Park (BRNP), based on protein electrophoresis. However, this pilot study revealed the presence of genetic variation at two enzyme loci across two Victorian populations: BRNP and Royal Botanic Gardens Cranbourne (RBGC). Furthermore, *T. circumsepta* is known to reproduce via autopollination (i.e. non-random mating) and cross-pollination via biotic vectors is thought to be almost impossible due to constraints imposed by column structure and infrequent flower opening. Yet, this study indicated that some out-crossing (i.e. random mating) had occurred within both RBGC and BRNP populations on the basis that the frequency distributions of genotypes at each polymorphic locus were consistent with Hardy-Weinberg expectations, and alleles at these loci exhibited linkage equilibrium.

Mitochondrial mutations may drive Y chromosome evolution

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The human Y chromosome contains very low levels of nucleotide variation. It has been variously hypothesized that this invariance reflects historic reductions in the human male population, a very recent common ancestry, a slow rate of molecular evolution, an inability to evolve adaptively, or frequent selective sweeps acting on genes borne on the Y chromosome. I propose an alternative theory in which human Y chromosome evolution is driven, at least in part, by mutations in the maternally inherited mitochondrial genome that impair male fertility and ultimately lead to a reduction in the effective population size (N_e) and consequently the variability of the Y chromosome.

Defining eradication units: Population genetics of the brown rat (*Rattus norvegicus*) on South Georgia Island.

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Human aided dispersal of rat species has result in numerous conservation crises, particularly on oceanic islands. Here rats have profoundly altered the insular ecosystem and often are responsible for species extinctions (e.g. Lord Howe Is. and Big South Cape Is. New Zealand). Over the last decade, innovations in rat eradication techniques have allowed conservators to eliminate rats from ever-increasing sized areas – the largest attempted thus far is Campbell Is. (11,300 ha). Rat eradication programs necessarily require sound knowledge of the target population; particularly when eradicating fragments of a population. Clearly, targeting a population fragment that is exchanging migrants with other fragments will result in a costly failure. Molecular genetics can provide invaluable information on gene flow and population differentiation enabling the identification of populations where eradication might be achievable.

Here we assess a glacially isolated population of brown rats (*Rattus norvegicus*) on South Georgia Is earmarked for eradication. This population (Greene Peninsula) is demarcated by glacial activity, precipitous peaks, permanent ice and sub Antarctic waters: all hypothesised barriers to rat dispersal. Comparing this population (sample n=40) with an adjacent population (sample n=40), we found levels of genetic variation at 18 microsatellite loci indicative of rare or absent gene flow (e.g. F_{ST} = 0.12). Using assignment tests, we assigned all (n=80) but one individual to its population of origin. Our results show that the Greene Peninsula population is a distinct unit that, given due care, could be successfully eradicated. We also show that rat dispersal on South Georgia Is requires land bridges and that glaciation and permanent snow are sound indicators of distinct populations. Importantly, the observed level of population differentiation indicates that eradication failures could be distinguished as either missed individuals or reintroductions via quarantine breaches.

Indicators of infratribal relationships within Abildgaardieae (Cyperaceae) from the chloroplast DNA *trn*L intron and *trn*L-*trn*F spacer

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A phylogenetic study, using sequences of the chloroplast DNA region *trn*L intron and *trn*L-*trn*F spacer, was done with members of the tribe Abildgaardieae, and close allies, in the family Cyperaceae (sedges). The genus *Arthrostylis*, which is currently classified in the tribe Arthrostylideae, was found to be nested within Abildgaardieae. The species of the genus *Bulbostylis* make a separate well-supported clade, which is a sister group to all the other members of the Abildgaardieae plus the genus *Arthrostylis*. We propose either that the genus *Bulbostylis* be excluded from Abildgaardieae, or else Abildgaardieae should be expanded to include (at least some members of) Arthrostylideae. Controversy between parsimony and maximum likelihood methods are also discussed.

The use of morphology, anatomy, chromosome number, and isozymes, in the phylogenetic assessment of the genus *Medicago* L. in Iran

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The existence of 15 species of *Medicago* L. in Iran, has made this country one of the richest gene pool of the genus. *Flora Iranica*, using leaf and fruit morphology together with the colour of flower, has treated this genus for Iran. Although fruit characters appear to be useful taxonomic characters, in some cases they can not separate particular species from each other (for instance *M. polymorpha*, *M. minima*, and *M. rigidula*). Likewise, leaf morphology and flower colour.

This study mainly involves the assessment of morphological, anatomical, cytological, and isozyme characters of the genus *Medicago* in Iran. The material of this work belong to 115 populations and 14 species distributed all around Iran.

Morphological studies were based on leaf, seed, fruit, and flowers using biometrical and statistical approaches.

Anatomical studies were conducted on the same material as above. The characters used included the structure of stem, root, and leaf, length of epidermal trichomes, and form and structure of epidermal cells and stomata.

Chromosome numbers were counted from mitotic root tips using colchicine pre-treatment and squash method. *Medicago* has mostly been reported to have diploid, tetraploid, and rarely hexaploid features, with the base numbers n = 7 & 8. The results of this study confirmed the literature.

Isozyme studies showed a distinct split in the genus to two groups, which does not match with the sections already suggested in the literature.

This survey has resulted in four new species records for Iran. We have suggested a new taxonomic key for the genus regarding the results of this study (particularly the morphological results). Several new chromosome counts are reported. As the final result, our study has shed light on the evolutionary relationships between the species of *Medicago* distributed in the *Flora Iranica* region.

A whodunit in the Top End: what are the new fruit fly pests in Northwest Australia?

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Until recently, northwest Australia was thought to be relatively free of serious fruit fly pests. However, during 2000, infestations of fruit fly were discovered in major commercial horticultural regions of Western Australia and the Northern Territory. This new horticultural problem represents a serious threat to the economic development of these areas and is of great concern to the relevant government agencies. However, it was unclear exactly which species of fruit fly is responsible. It was assumed initially that *Bactrocera aquilonis,* a previously benign species endemic to the region, may have altered its host preferences and begun infesting horticultural crops. But in recent years it appears that the Queensland fruit fly, *B. tryoni,* has become established in Darwin, and possibly in other areas of the northwest. Since the two species are extremely difficult to distinguish, some confusion has arisen as to which fruit flies are inflicting damage to crops. The situation is further complicated by the possibility that hybrids between the two species may also be present. We report the results of our initial microsatellite-based investigation of this problem and discuss the direction of future research as indicated by our results.

QTL Mapping in Dairy Cattle

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Dairy cattle are well suited to the traditional design used for QTL mapping of large half-sib families. However the power of such designs is still low for genes of modest effect that are typical of quantitative traits. More powerful designs can be achieved by - using half sib families whose members are progeny tested bulls, selectively genotyping only extreme animals from large half-sib families, analysing multiple traits and use of all relationships in more complex pedigrees. Collectively these experiments show that dozens of genes affecting milk production traits are segregating and provide some ability to estimate the distribution of their effects and their gene frequencies. We have attempted to estimate these distributions accounting for the bias introduced by measurement error and publication of only statistically significant effects. The effects range from occasional genes of large effect (gene substitution effect a > 1 genetic standard deviation) to several genes large enough to be detected (a > 0.4 genetic standard deviations) and many genes of smaller effect. That is, the distribution is leptokurtotic. The allele frequency at these genes also varies widely but is approximately as expected from a neutral model (ie more genes with extreme gene frequencies than frequencies near 0.5).

A major deficiency with most results to date is that the QTL cannot be positioned accurately - in most cases to 95% confidence interval is > 30 cM. This has implications for finding the gene and for utilising it in marker assisted selection. One approach to mapping the position of QTL more accurately is linkage disequilibrium (LD) mapping. LD is expected between genes close together as a result of finite population size. However it is a very variable phenomenon and a single marker close to a QTL can still be in linkage equilibrium with it. LD between a haplotype of markers and a QTL is more consistent and we have developed a new multi-locus measure of LD. This new measure can be used to map QTL by LD and an example of this will be given.

The Association of Single Nucleotide Substitutions in the Mitochondrial *ND1* and *ND2* Genes with Poor Semen Parameters in Man

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Mitochondria are the powerhouses of the cell, generating adenosine triphosphate (ATP) through oxidative phosphorylation. Mitochondrial mutations are known to be associated with a number of diseases in tissues that require a large amount of energy for normal function [Wallace, Science 283, 1482-1488 (1999)]. Most of these diseases involve single nucleotide substitutions in the mitochondrial genome [Kao et al., Biol. Reprod. 52, 729-736 (1995); St John et al., J. Androl. 21, 189-199 (2000)]. Single nucleotide polymorphisms (SNPs) in 2308 bp of the mitochondrial genome, encompassing the coding regions of *ND1* and *ND2*, were characterised by single stranded conformation polymorphism (SSCP) analysis and confirmed by DNA sequencing. From preliminary results the most common substitution occurred at nucleotide (nt) 3480 within the *ND1* gene. This substitution was observed in 6.25% of men with poor semen quality compared to 1.37% of men with normal parameters. Seven SNPs were identified within the *ND2* coding region, none of which occurred in men with normal semen parameters. Four of these SNPs changed the amino acid of the resulting polypeptide. Hydrophobicity plots and amino acid secondary structure predictions suggested that slight changes occur in the ND2 protein as a result of these amino acid changes. We propose these findings of high incidence of SNPs in 2308 bp of the mitochondrial genome in men with poor semen parameters to be continuing evidence that single nucleotide substitutions in the mitochondrial genome may play a role in determining male infertility.

Origins of Australian and New Zealand feral pigs

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European sailors and settlers introduced pigs into New Zealand and Australia in the 18th and 19th centuries, many of which became feral, but few records were kept of these introductions. Mitochondrial DNA (mtDNA) sequence may help distinguish between different origins for feral pig populations, as distinct European and Asian clades can be recognised on mitochondrial sequence. The D-loop mtDNA of four Australian and four New Zealand feral pigs was cloned, sequenced and compared with other breeds using a phylogenetic framework of European and Asian published and unpublished (Ganzhongnan Spotted, Guan Xiang, Tibet, Guizhou Xiang, Tamworth, Large Black and Wessex Saddle Back) domestic pig and Wild Boar sequences. Phylogenetic analyses were performed using HKY85 pairwise distances, Neighbor-Joining tree and the maximum-likelihood methods. Auckland Island (NZ), Warrumbungle Ranges (NSW) and Cooktown (Qld) feral sequences clustered with European domestic pig breeds, while Kune Kune (NZ) and feral sequences from Oberon (NSW) and Kowanyama (Qld) clustered with Asian pig sequences. The Asian mitochondrial DNA in Australian and New Zealand feral pigs may have arrived directly or via Asian introgression into European breeds in Europe in the 18th century. Further studies are being made on Australian feral pigs to further characterise this reservoir of porcine biodiversity.

Analysis of peccary microsatellites using porcine primers

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There are many examples of microsatellite primers designed from one species being used to amplify products from another. Interspecific amplification has been demonstrated among cattle, sheep, goats and deer, among chickens, quail and turkeys and among domestic pigs and wild Suiformes. Here we report on 61 porcine microsatellite primer pairs tested for amplification of microsatellite products from seven Colombian Collared peccaries (*Tayassu tajacu*), with 47 (74%) yielding products. Fluorescent genotyping of the PCR products revealed that 10 (16%) were polymorphic. All observed peccary alleles fall within the range of allele sizes found in Australian commercial pigs. The high success rate with porcine primers on peccaries implies a relatively low level of nucleotide divergence between Suidae and Tayassuidae. The amplification efficiency agrees reasonably well with our previous study (16/18; 89%), although the polymorphism level here is lower than in the previous study (11/16; 69%) By contrast, the only other reported study on peccaries (Lowden, pers. comm) found an amplification success rate of only 29% (9/31) using porcine primers. Perhaps surprisingly, porcine microsatellite primers are useful tools for population and phylogenetic studies of peccaries. Additional Collared peccary specimens and populations are being evaluated with these microsatellites looking for geographical patterning of the genetic variation in North and South America.

Toward a Kangaroo Genome Project

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It makes excellent sense to include a marsupial species among mammal and other vertebrate species lined up for serious genomic study. In comparisons between the more closely related eutherian species (eg human and mouse ~70 MY), it is hard to distinguish signal from background noise, whereas in comparisons between vertebrate classes (eg human and chicken ~310 MY) signal may be lost. Marsupials diverged from eutherians about 130 MYA, so sequence has diverged sufficiently for stringent detection of homologies that can reveal coding regions and regulatory signals.

Even more importantly, marsupials are mammals, and share many mammal-specific developmental pathways and regulatory systems with humans. For instance, X chromosome inactivation occurs in marsupials as well as eutherians, but is different at the molecular level. Comparisons of chromatin regulatory elements from marsupials, human and mouse will help us determine how X inactivation evolved, and how it works. Similarly, we are exploring the marsupial homologues of genes involved with testis determination in humans and mice, in the expectation that comparative sequencing and expression studies will help to sort out gene functions and interactions.

Our group has therefore undertaken to gather resources, develop expertise and foster Australian and international collaborations for a serious onslaught on thegenome of the model Australian marsupial *Macropus eugenii* (the tammar wallaby). Australia has, regrettably, made no real contribution to international genome projects – this might be our chance to contribute something uniquely valuable and uniquely Australian.

Construction and analysis of a cDNA library from larval midguts of cotton bollworm *Helicoverpa armigera*

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A cDNA library from midguts of fifth-instar larvae of *H. armigera* was constructed in the lambda-ZAP vector. A subset of the library was subjected to mass excision of the pBluescript phagemid and propagated as a phagemid library. Clones were gridded onto high-density filters and subjected to 5'end sequencing. 647 high quality ESTs were grouped into 89 contigs and 191 singletons. This dataset was analysed for similarity to Drosophila, *Bombyx mori* and Honeybee ESTs. Results of these analyses will be described.

400 Million Years of Conserved Synteny of Human Xp and Xq Genes on Three Pufferfish Chromosomes

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The freshwater pufferfish *Tetraodon nigroviridis* has become highly attractive as a compact reference vertebrate genome for gene finding and validation. To establish its syntenic relationships with other key vertebrate genomes, we have mapped genes, which are more or less evenly spaced on the human chromosomes 9 and X, on *Tetraodon* chromosomes using fluorescence in situ hybridization (FISH). PufferFISH revealed that the human X is an orthologous mosaic of three *Tetraodon* chromosomes. More than 350 million years ago, an ancestral vertebrate autosome shared orthologous Xp and Xq genes with *Tetraodon* chromosomes 1, 2 and 7. The shuffled order of Xp and Xq orthologs on their syntenic *Tetraodon* chromosomes can be explained by the prevalence of evolutionary inversions. The TNI 2 orthologous genes are clustered in human Xp11-21 and represent a recent addition to the eutherian X sex chromosome. By comparison with the X, the human chromosome 9 and the avian Z sex chromosome show a much lower degree of synteny conservation in the pufferfish. We propose that a special selection process during vertebrate evolution has shaped a highly conserved array(s) of X linked genes long before the X was used as a mammalian sex chromosome and many X chromosomal genes were recruited for reproduction and/or the development of cognitive abilities.

Comparing methods of measuring variation in bottlenecked populations

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Microsatellite markers are a popular method of determining the level of variation in an endangered species' genome. The assumption is made that microsatellites, which are neutral markers, behave in the same manner as quantitative traits, which often directly affect survival, and are therefore subject to selection. If this assumption were proved incorrect, then the use of neutral markers in conservation monitoring would have to be re-evaluated. We are conducting bottleneck experiments using *Drosophila melanogaster* to test the assumption that variation in quantitative traits under balancing selection declines at the same rate as variation in microsatellite markers, during a population bottleneck. We have initiated a number of bottlenecked populations of varying effective population size (Ne) and generation time. We are collecting data to compare levels of variation for 8 microsatellites with variation in egg numbers and sternopleural bristle numbers. Preliminary results using sternopleural bristles from 10 intense bottleneck replicates (Ne=2) and three large, non-bottlenecked populations indicate that variation is not always lost at the same rate as predicted by neutral theory (Ne x generations). Variation in microsatellites may not be the most appropriate measure of variation to monitor in endangered species.

Evaluation of the *LMNA* gene in families with dilated cardiomyopathy and conduction-system disease

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Dilated cardiomyopathy (DCM) is a heart muscle disorder characterised by dilation and contractile dysfunction of the left and/or right ventricles. DCM may result from a diverse variety of factors that impair cardiomyocyte function, such as coronary artery disease, viral infections or systemic diseases, but may also occur as a primary muscle disease. Recent molecular genetic studies suggest that at least one third of cases of primary DCM may result from an inherited gene defect. Familial DCM is clinically variable, and may present as an isolated disorder, or in association with conduction-system disease, skeletal myopathy, or sensorineural deafness. Linkage studies in families with DCM have shown that this disorder is genetically heterogeneous. For example, 12 chromosomal loci have been identified to date for isolated autosomal dominant DCM. Mutations in the *LMNA* gene, encoding the nuclear lamina proteins, lamins A and C, have recently been found to cause autosomal dominant DCM with conduction-system disease (DCM-CD). The relative prevalence of *LMNA* gene mutations in families with the DCM-CD phenotype is unknown. We screened the coding region of the *LMNA* gene by DNA sequencing in probands of 12 families with DCM-CD. Five families had a "typical" phenotype, characterised by early onset of conduction abnormalities with later development of DCM. Seven families had "atypical" DCM-CD, in which conduction-system disease occurred concurrently with, or subsequent to, DCM. One missense mutation, R571S (1711C>A) was identified in one family with the "typical" DCM-CD phenotype; no *LMNA* mutations were found in the families with "atypical" DCM-CD. In addition, three different polymorphisms were identified (A287A, 4/12 families; N446N, 4/12 families; H566H, 6/12 families). These data suggest that DCM-CD is genetically heterogeneous, and that the relative importance of *LMNA* gene mutations as a cause of this phenotype may be less than predicted previously. Further studies in a larger patient population are required to determine whether mutation screening of the *LMNA* gene should be restricted to families with the "typical" DCM-CD phenotype.

Evidence for concerted evolution in an intron-less copy of octopus Elongation Factor-1a

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Amplification and partial sequencing of EF-1a in species of the sub-family Octopodinae (Mollusca: Cephalopoda) has revealed two distinct loci; a ~700 base pair (bp) fragment which contains two introns (intron +), and a ~400 bp fragment which lacks introns altogether (intron-). Characteristically, protein-encoding genes contain introns and their absence is likely to be indicative of a non-functional, processed pseudogene. Alignment of both copies (by excluding introns from the intron+ sequences) reveals high similarity between loci within a species. Under phylogenetic analysis similarity between copies within a species is greater than between copies of the same locus among species. This is an unexpected result, as mutational constraints on non-functional pseudogenes are typically relaxed due to a lack of selection pressure and sequences are therefore prone to accumulating mutations. We suggest that a single duplication event of processed EF-1a occurred in a common ancestor resulting in insertion of the processed pseudogene into another region of the genome. Over time the second copy has is likely to have been "corrected" via concerted evolution and similarity to the true gene is maintained.

Phylogeographic trends in Australian populations of the bridled tern

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Australia's seabirds are amongst the few relatively intact populations of seabirds in the Asia-Pacific region Yet, even within the protected area of the Great Barrier Reef World Heritage Area (GBRWHA) one or more nesting populations of several species are demonstrated to be in decline and seabirds are recognised as in need of conservation management. The bridled tern, *Sterna anaethetus*, nests on many islands and cays of the GBRWHA. This project aims to determine the genetic structure of Australian populations of bridled terns. This data will then be used to assess if this seabird species is forming one or more ESU and thus shed light on the conservation significance of breeding populations in the GBRWHA. Approximately 90 individuals were sampled from locations spanning much of the length of the GBRWHA and the reefs and islands off the Western Australian coast between Penguin Island to the south of Perth and Bridled Island in the Lowendal Group on NW Shelf. These locations represent approximately one half to two thirds of the species' Australian range. Areas not sampled stretch from the NW shelf of Western Australia through the Northern Territory coast to northern tip of the GBRWHA. We developed sequencing primers that amplify two 500 bp fragments of the control region of mtDNA. Initial screening of sequences demonstrates that the 5' end of the mtDNA control region is well conserved among samples from across all populations sampled. The 490 base pair region sequenced contained approximately 8 substitutions, many only in one individual. The adjacent region, 500 bp toward the centre of the mtDNA control region, was considerably more variable. Samples from eastern Australian localities clearly contained two different haplotype lineages, both occuring within a southern locality, Frigate Cay in the Swains Reefs. At least 5% of sites were different in this DNA region. These results suggest the use of mtDNA control region will be useful in determining phylogeographic trends in this species of seabird. This group of organisms has proved particularly problematic for these analyses due to a lack of workable generic PCR primers. Considerable effort was required to develop the amplification primers used in this study. We believe it may be necessary to develop species specific primers in this genus and we are currently undertaking studies on four other terns locally common in the GBRWHA.

Advances in Polychaete Phylogeny

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Polychaetes and their relatives are ubiquitous invertebrates in marine environments, dwelling in a range of habitats including the soft sediments of intertidal mudflats, calcareous self-built tubes in coral reefs, deep sea hydrothermal vents and as ecto-commensals on starfishes. They constitute a vastly diverse assemblage (approx. 15,000 spp. described) and while polychaete families and most genera occur world wide, species typically have restricted distributions. Despite the abundance of taxonomic research, the molecular systematics of polychaetes has been largely ignored by contemporary phylogeneticists. Historically, polychaetes have been associated with earthworms and leeches (which comprise the clade "Clitellata") within the Phylum Annelida. Recent phylogenetic studies [Rouse & Fauchald, Zoo. Scr. 26, 139-204 (1997); Brown et al., Aust. J. Zool. 47, 499-516 (1999)], however, suggest that the "Annelida" is an unnatural assemblage. Further, the relationships of enigmatic groups such as Echiura, Pogonophora and Sipuncula to the polychaetes is unclear. Some authors contend that paraphyly of the Polychaeta is a consequence of recognition of these groups as independent phyla. Here, we expand the current molecular data for the Polychaetes with the addition of complete 18S rDNA, 28S rDNA (D1 and D9-10 expansion regions), U2 snRNA and histone H3 DNA sequences for more than 20 taxa from a diversity of polychaete families. We present the preliminary results from parsimony and likelihood analysis of this enhanced data set and compare our results with established morphological concepts of the assemblage.

Genomic Analysis for All Creatures Great and Small

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The recent rapid advances in genomics have largely been dependent on the development of some major advances in analysis technologies. The invention of industrial scale DNA sequencing and the Informatics tools to assemble, annotate and visualise genomes has created a requirement for tools to analyse genome diversity and variation on a similar scale through re-sequencing and microsatellite/SNP mapping. Because vast amounts of genome information are available on-line to researchers all around the globe, there has been a similar requirement to develop technologies that democratise access to the laboratory tools to generate new genome information for both model and economically important organisms. Experience gained in facilitating both public and private genome projects has allowed Applied Biosystems to release major new technology advances aimed at significantly reducing the cost and effort of sequencing and re-sequencing projects with concurrent improvements in SNP discovery and microsatellite analysis. In addition, following the successful development of a panel of 200,000 individual validated Human SNP targets available as pre-formatted assays, Applied Biosystems is able to offer custom SNP targets in the same robust assay format, removing the need for projects to perform extensive assay workup processes. This is particularly advantageous for projects involving organisms which are not the subject of major international collaborations and hence do not have large standing databases of validated targets.

Comparison of Plant Genomes by Analysis of Gene Sequences, SSR and SNP

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Plant genomes have often been compared by analysis of the sequences of specific loci. High throughput genomic analysis now allows large numbers of individual loci to be considered. Comparison of the genomes of individuals within species is often based on the analysis of simple sequences repeat (SSR) loci or single nucleotide polymorphism (SNP) loci. Molecular analysis has resulted in significant recent advances in the understanding of relations between different groups of higher plants and relationships within flowering plants. For example the older division of flowering plants into monocotyledons and dicotyledons is now being replaced by a more complex understanding. Recent chloroplast genome analysis indicates frequent transfer of chloroplast genes between species. SSR sequences within genes (EST) are often conserved between closely related plant species unlike SSR sequences in other parts of the genome. Rare examples of widely conserved SSRs have been found. Analysis of the distribution of SNP within genes allows targeting of SNP discovery to regions (eg. introns, 3'UTR, 5'UTR, promoters) with suitable levels of polymorphism. High throughput SNP analysis offers an important new tool for comparison of plant genomes.

A phylogenetic analysis of the *Chamelaucium* alliance (Myrtaceae).

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Sequence data from the *mat*K and *ndh*F genes and the *atp*B–*rbc*L intergenic spacer was analysed for over 90 representatives of the *Chamelaucium* alliance sensu Briggs and Johnson (1979). There is strong support for the monophyly of the alliance, but there is no support for their concept of suballiances based on fruit type: indehiscent fruit have arisen in multiple lineages. There is, however, strong support for *Calytrix*, *Homalocalyx* and *Ochrosperma* being the first lineages to diverge within the alliance.

A large number of genera are not monophyletic in this analysis: *Astartea*, *Babingtonia*, *Baeckea* sensu lato, *Balaustion*, *Darwinia, Hypocalymma, Malleostemon, Micromyrtus, Rinzia*, *Scholtzia,* and *Thryptomene.* This illustrates the high level of homoplasy in morphological characters used to define genera within the alliance. The monophyly of *Baeckea* sensu stricto, *Euryomyrtus*, *Ochrosperma* and *Triplarina*, however, are strongly supported. Several well-supported groups that may warrant generic status have been identified.

Re-evaluating the Cambrian explosion hypothesis

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The Cambrian explosion hypothesis states that modern metazoan lineages arose during a period of rapid diversification at the base of the Cambrian. A wide range of evidence, including the well known Ediacaran fauna and fossils from the Burgess Shale, supports this hypothesis. An opposing point of view that has gained support in the last decade argues for an extension of this radiation deep into the Precambrian, and is based primarily on phylogenetic studies of nuclear and mitochondrial genes and gene products. Support for this hypothesis was recently strengthened with the discovery in south Western Australia of 1.2 billion-year-old trace-like fossils that could be evidence of vermiform organisms, possibly crown-group metazoans (*Science* **296**, 1112-1115 [2002]). The difference between these two hypotheses may be due to a paucity of reliable fossil data or incorrect interpretation of the available fossil and molecular data. We have re-examined the molecular data with the aim of determining whether interpretations based on these data were incorrect. Several of the phylogenetic data sets were found to violate the assumption of stationarity, which is unfortunate because it may lead to a bias in the estimates of phylogeny and sequence divergence. Interestingly, our results may also explain why the estimated time of divergence for the modern metazoan lineages differ by as much as 1 billion years (*Proc. Natl Acad. Sci. U.S.A.* **95**, 606-611 [1998]; *Proc. Natl Acad. Sci. U.S.A.* **95**, 12386-12389 [1998]).

Sex Ratio In Wild Orange-Bellied Parrot *Neophema chrysogaster*

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The Orange-bellied Parrot *Neophema chrysogaster* is a critically endangered Australian bird. It has a single breeding population containing less than 200 adults in the wild, has steadily declined in abundance, and its range has contracted markedly since the 1920s. All wild individuals now breed as a single population in south-western Tasmania (Melaleuca) and overwinter on the Victorian and South Australian coast.

Since 1995 the sex ratios of the wild population have been observed to deviate from unity. There has appeared to be an excess of adult males in the banded population at Melaleuca. To explore whether this apparent excess was arising before fledging or subsequently, DNA based sexing was used. DNA was extracted from blood samples taken from newly fledged birds over the years 1992 to 2001.

It was found that the sex ratios of the wild population showed an excess of males (up to 62%) for three of the seven breeding seasons investigated, although these were not individually significant. Taken overall however the population has been producing approximately 55% males.

Whilst the basis of any sex ratio inequality is unknown, such demographic factors are important in the fate of small populations. Further investigations are required to clarify their source and aid their management. In the meantime, Population Viability Analysis of Orange-bellied Parrots needs to incorporate sex ratio inequality to make management predictions more accurate.

Use of Linkage Disequilibrium Mapping in Domestic Dog Breeds

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The demographic history of domestic dog breeds suggests that linkage disequilibrium (LD) mapping could be effectively utilised for initial, low density, genome wide scans. That is, breeds are genetic isolates founded by a small number of individuals less than 100 generations ago and where population expansion has been reasonably rapid. LD mapping is essentially a case-control comparison. This offers advantages over traditional linkage analysis as applied to canine populations as there is less requirement for pedigree details or tracking down parents in informative matings. Here, we demonstrate, using Canine Copper Toxicosis in the Bedlington terrier as the model, that LD mapping could be reasonably expected to be a useful strategy in low resolution genome wide scans in pure bred dogs. Using a case-control approach, significant LD was demonstrated over distances up to 33.3cM. Very recently, the Copper toxicosis in Bedlington terrier gene (*CT-BT*) was tentatively identified as the *MURR1* gene and isolated to canine chromosome 10q26 [van de Sluis et al., Hum. Molec. Genet. 11, 165-173 (2002)]. LD mapping confirmed this location and was able to do so in a population that was refractory to traditional linkage analysis.

Translocation causes extinction in a local population of the freshwater shrimp *Paratya australiensis*

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Here we report results of a translocation experiment where freshwater shrimps were translocated between pools from two different subcatchments within the same drainage system, the Brisbane River. Although not known at the time, it is now evident that populations in these two subcatchments represented lineages that were 6% divergent at the COI locus. After only 7 years (about 7 generations), at one of the sites, the resident genotype is now extinct. Evidence from nuclear (allozymes) and mitochondrial (COI) genes suggests that this extreme result could be explained by a mating preference by all females (resident and translocated) for translocated males, combined with non-viability of crosses between resident females and translocated males. The implications of this result will be

EXPRESSION AND FUNCTIONS OF HEMOGLOBIN GENES IN PLANTS.

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Arabidopsis thaliana has three hemoglobin genes we have named *GLB1*, *GLB2* and *GLB3*[1]. By homology to other hemoglobins with known 3D structure, the proteins encoded by *GLB1* and *GLB2* are "3-on-3" hemoglobins with the standard globin fold, but *GLB3* is a member of the "2-on-2" hemoglobin family[2,3], which have a shorter central helical domain.

We have analysed the biochemical properties and expression patterns of these hemoglobins, and are beginning to understand their functions *in planta*.

GLB1 and GLB2 are high affinity oxygen binding proteins whereas GLB3 has a lower affinity for oxygen.

The three *GLB* genes are differentially regulated by developmental, cell-specific and exogenous signals. *GLB1* is transcribed in response to hypoxia and over-expression of this gene imparts improved survival of plants to acute hypoxic stress. *GLB2* does not respond to hypoxia but can be induced in apical meristem and roots by cytokinin treatment. *GLB2* is not expressed in uninduced seedlings but is expressed constitutively in certain tissues of mature, flowering plants. *GLB3* is expressed in phloem cells only, raising the intriguing possibility that GLB3 may be a circulating oxygen transport protein in plants.

1. Hunt, P. W. *et al* (2001) *Plant Mol. Biol.* **47**(5): p 677-692
2. Pesce, A., *et al* (2000) *EMBO J.* **19,** 2424-34.
3. Watts, R. A. *et al* (2001) *Proc. Nat. Acad. Sci. (USA)* **98**(18): p 10119-10124

Phylogeny and Historical Biogeography of the Freshwater Fish Genus, *Mogurnda* (Pisces: Eleotridae) in Australia

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There are six species of *Mogurnda* recognised in Australia, three of which have been recently described (Allen and Jenkins 1999). Two of the species, *M. adspersa* and *M. mogurnda* have wide distributions in eastern and northern Australia respectively, while the other species (*M. clivicola*, *M. larapintae*, *M. oligolepis* and *M. thermophila*) have more highly restricted ranges in central and northwestern Australia. In this study we used mitochondrial 16S and ATPase 6 and 8 genes to determine the evolutionary relationship between species. The results support a species radiation in the early Quaternary. Particular attention was paid to populations on the Atherton Tableland in northeastern Queensland where the reported species ranges are largely parapatric. The population of *M. adspersa* in the upper Tully River was as divergent from other tableland populations as from *M. mogurnda* sampled from northern drainages suggesting a need for taxonomic revision in this area.

The effect of linkage disequilibrium among epistatically interacting genes on the power of association studies

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In 21st century Human Genetics there has been a shift away from total dependence on extended pedigrees to exploiting population samples for localisation of disease genes. Our capacity to utilise population samples with anonymous genetic markers arises from the existence of linkage disequilibrium (the non random association of alleles at different loci). The distribution of linkage disequilibrium in the human genome is extremely complex (Huttley et al 1998). At the very least this complexity implies that a nonuniform marker density will be necessary in order to achieve uniform power for association studies. More importantly, however, have been recent theoretical and empirical results demonstrating that ignoring epistatic interactions between genetic variance invalidates the conventional marker-at-a-time approach employed for genome scans. I will present theoretical results illustrating the consequences of failure to account for epistatic directions in disease association studies and present an example of this effect to the breast and ovarian cancer susceptibility gene BRCA1.

A little BLUP

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In animal breeding it is now common practice to estimate genetic progress by Best Linear Unbiased Prediction (BLUP) using an animal model, and it is becoming common for the analysis of laboratory selection experiments to be analysed in the same way, or by Restricted Maximum Likelihood (REML) using an animal model. It is well known that selection response estimated by BLUP is given by a combination of predicted and observed values. The relative weights given to predicted and observed responses are in general very difficult to compute. Here a simple model with a single generation of selection from a base population and single pair mating to produce the next (discrete) generation is examined.

In the absence of a control population one must estimate separate environmental terms for each generation and the BLUP estimate of selection response is exactly equal to the predicted value. With a control population only one mean need be estimated, and the BLUP measure of selection response is a combination of the predicted gain Sh^2 and the observed gain $R = \mu_O - \mu_P$, where S is the selection differential, h^2 is the assumed heritability, μ_P and μ_O are the mean phenotypes, adjusted for the control, of the parental and offspring generations.

If p is the proportion selected, $k = (1 - h^2)/h^2$, and f is the family size, then the relative weights for R and Sh^2 are given by

$1 + k[1 + f(1 - p)]$ and $k(k + 1)(2 + fp)$.

Observed gain thus has less weight when heritability is low.

Observed gain has more weight when families are large.

Observed gain has more weight when selection is intense.

As an example, when a quarter of both males and females are selected, numbers are equal in parental and offspring generations, and the heritability is 0.25, the BLUP estimate of response is

$$0.1509\ R + 0.8491\ Sh^2 .$$

Thus if the actual heritability is much different from the assumed value there may be considerable bias.

Molecular Analysis of x*prF*: a Gene Involved in the Response to Starvation

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It has been shown that hexokinases have an important regulatory role in addition to their catalytic role (phosphorylation) in the metabolism of 6-carbon sugars. Specific hexokinases in plants, fungi, and mammals are thought to be responsible for triggering glucose repression of gene expression, though the mechanism is unknown. The carbon starvation response is another form of carbon regulation, of which little is known. The *xprF* gene, which encodes an unusual hexokinase or hexokinase-like protein, plays a role in the response to carbon starvation in *Aspergillus nidulans*.

Sequence comparisons show that, unlike other fungal hexokinases, XprF has an equivalent degree of similarity (16-19%) to mammalian, plant and fungal hexokinases. Most of the residues thought to be of importance in the ATP- and sugar-binding domains of hexokinases, are conserved in XprF. However, there exist regions at the N- and C- termini of the putative gene product that are not found in other hexokinases. The *xprF* gene is also unique in that it is involved in the utilisation of certain nitrogen sources.

The *xprF1* mutation has been shown to be a nonsense mutation, which is predicted to result in a truncated protein containing 255 (out of 615) amino acids. In this study, an *xprF*Δ mutant was created. The phenotype of the knockout mutant was similar to the *xprF1* mutant in terms of regulation of extracellular proteases and utilisation of nitrogen sources. Like the *xprF1* mutation, the knockout exhibits partial dominance. This showed that the partial dominance exhibited by the *xprF1* mutation is not due to a dominant-negative effect of the truncated protein.

Using site-directed mutagenesis, we have shown that a number of unusual, as well as conserved, features of the *xprF* gene product are important for the maintenance of its functional integrity. Deletion of the unique sequences at the N- and C- termini showed that both domains were required for XprF function. It has also been shown that a number of highly conserved residues in the ATP-binding domains are not required for its regulatory function. Mutations in a putative nuclear localisation domain also affected gene function. The *xprF* gene will be expressed in *E. coli* in order to purify the protein and subsequently test for hexokinase activity. Nuclear localisation experiments, using *xprF*-gfp fusions, are also underway.

A consensus QTL map for production traits in dairy cattle based on public domain information.

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With the availability of dense genetic maps, whole genome scans have been undertaken in many elite dairy populations to identify QTL with a major effect on milk production. Based on published information, we have created a consensus QTL map for major traits in dairy cattle. Across studies results suggest that QTL affecting milk production are predominantly mapped on chromosomes 1, 3, 6, 9, 14, 20 and 23 while others chromosomes (4, 5, 10, 18, 19 and 26) show lower support for QTL. Consistent findings across studies identified a QTL for milk yield mapped near the centre of chromosome 6 close to the microsatellite marker BM143. A second QTL for the same trait has been located on the same chromosome in the region of the casein complex. BTA20 and BTA1 are also consistent for the presence of QTL for milk yield. There is strong evidence for the presence of QTL for protein percent on BTA3, BTA6 and BTA20. QTL for fat percent have been described on BTA3 and telomeric end of BTA14. The latter is the only QTL mapped todate to a definitive gene (acylCoA: diacylglycerol acyltransferase) through a positional cloning approach. Fewer studies have described QTL for dairy form, type traits, herd life, milking speed, somatic cell count and health traits. There are many difficulties in building of a consensus QTL map. Differences in the position and magnitude of the QTL effects among studies and among different families within studies makes global comparison difficult. Nevertheless, the combined QTL map for dairy cattle clearly indicates that some chromosomal regions contain genes with a significant influence on traits of interest to the dairy industry. Identification of such genes will remain a significant challenge in the foreseeable future given the paucity of information on candidate genes. The building of comparative maps remains a high priority to facilitate gene discovery in cattle.

Looks aren't everything: Freshwater crayfish (*Euastacus sp.*) systematics in the Sydney Basin & the morphospecies problem.

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The notion of using biodiversity as a measurement to determine an ecosystem's 'value' guides much of current environmental and development legislation at all levels of government. However using counts of morphologically identified species to determine biodiversity does not always accurately reflect the diversity of a given system. This problem is clearly visualised when examining widespread ambiguous cryptic species. One such example is the freshwater crayfish genus *Euastacus*. Researchers have often commented on the 'extreme morphometric variation across the *Euastacus* species. To determine the extent of this observed variation in and between populations, we have sequenced and analysed data for the 28S, 16S and CO1 genes from 54 specimens of *Euastacus* comprising 5 species from both lowland and highland populations within the Sydney Basin.

Morphological versus molecular: proposing a model for the development of molecular phylogenies of the extinct hominids

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For two centuries morphological analysis has dominated the study of the evolution of humankind. This is despite evidence that a reliance on morphological data is both a flawed and inadequate method from which to construct phylogenies. Such inadequacies include factors concerning taphonomy, environmental influences, and unintentional bias. Molecular data is suggested as an alternative and a complementary method from which to approach the phylogenies of ancient hominids and other primates. A feasibility study was conducted using selected regions of DNA from the 28S ribosomal subunit to determine if they could be used to construct the phylogenies of extinct hominids. The study found that variable region 3, 4 and 5 of 28S ribosomal DNA can be effectively applied to primate systematics and has great potential for ancient DNA studies of the hominids.

Alliance formation is a strong determinant of male mating success in a population of wild bottlenose dolphins (*Tursiops aduncus*) in Shark Bay, Western Australia

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Sexually mature male bottlenose dolphins in Shark Bay form several levels of alliances. Pairs or trios cooperate as first-order alliances to coerce females in reproductive condition. Teams of two first-order alliances sometimes cooperate as second-order alliances to attack other first-order alliances or defend against attacks. Some males choose an entirely different strategy and join forces in a large super-alliance to attack first- and second-order alliances, while other males do not seem to have alliance partners. This is the first comprehensive study in wild bottlenose dolphins to test the hypothesis that alliance membership and reproductive success are positively correlated. Using 11 microsatellite loci, we successfully assigned nine paternities to six out of 107 sexually mature males. Five out of the six successful males were members of an alliance, indicating that males with alliance partners are more successful in fathering offspring than males without partners. Compared to non-allied males, the chance of obtaining a paternity is significantly higher for members of first-order alliances but not significant for members of a super-alliance. Furthermore, reproductive success is significantly skewed among first-order alliances members.

Hybridisation among three sympatric species of fur seals (*Arctocephalus* spp.) at Macquarie Island

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Human impacts on natural populations include habitat disturbance and local extinctions, which can lead to secondary contacts between formerly isolated species. Macquarie Island is a large subantarctic island that suffered extensive seal harvesting in the nineteenth century and subsequent extirpation of the original seal population. Recent recolonisation (1955) of the island by three species of fur seal has been slow and complex. The two main breeding species on the island are the Antarctic fur seal, *Arctocephalus gazella*, and subantarctic fur seal, *A. tropicalis*, although matings have also occurred with New Zealand fur seals, *A. forsteri*. The extent of hybridisation among the three species and the potential for reduced fitness in hybrids is under investigation in a long-term monitoring program of the population. Species composition of the 1998/1999 population was determined and reproductive fitness between hybrid and non-hybrid males was compared. Mitochondrial DNA and single-locus nuclear DNA markers (microsatellites) were used for species assignment and subsequent paternity analysis. Genetic analysis revealed Antarctic fur seals to be the predominant species in pups, adult males and females, while subantarctic fur seals accounted for only 4% of the population. No pure New Zealand fur seals were detected in the breeding population, although most hybrids resulted from crosses of Antarctic and subantarctic females with New Zealand males. Observed matings were found to be poor predictors of paternity, and although reproductive success in hybrid and non-hybrid males was not significantly different, extra-territory matings were common, with almost half of all pups born in the 1998/1999 breeding season likely to have been sired by extra-territory males. The findings of this project suggest extensive hybridisation and some degree of hybrid fertility in the 1998/1999 population. There is also strong evidence for alternative mating strategies and some potential for positive assortative mating and female-mate choice.

Alternative mechanisms for the production of recombination by *P* elements in *Drosophila melanogaster*

Xiumei Liang, John Sved

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P element mobility is believed to be initiated by association of the left-hand and right-hand element ends, followed by excision, insertion and repair. We have shown that the left-hand and right-hand ends that associate are sometimes from different elements rather than from the same element, leading to formation of a 'hybrid element' and ultimately to chromosomal rearrangements. This mis-pairing occurs at exaggerated levels in an artificial genotype containing end-deleted elements, one of which contains only a left-hand end and the other only a right-hand end.

We have examined many recombinant progeny resulting from such left/right element combinations. A large number of these can be explained by a model, Hybrid Element Insertion or HEI, in which the hybrid element ends excise from their original location and insert elsewhere in the genome. Such recombinant progeny usually contain insertions or deletions adjacent to the original element site. However we have found that around 50% of the predicted HEI recombinants are chromosomally unaltered. A mechanism for such exact recombinants can be suggested in terms of dissociation of the hybrid element ends, followed by degrading and repair. A prediction from such a repair process is that gene conversion tracts should be produced at significant levels, in contrast to the HEI process which should not produce such tracts.

We have produced a genotype in which the left- and right-hand elements are flanked by closely linked RFLP markers. Use of such markers has shown that a significant proportion of recombinant progeny contain complex tracts surrounding the recombination point, as predicted from the repair process. We conclude, therefore, that the 'transpososome' structure, which is presumed to control both the excision and insertion processes, is not totally stable, sometimes leading to repair rather than insertion events involving P element ends. It is not known whether this instability might extend to normal P element excision and insertion, since abnormal tensions might accompany the formation of a hybrid element, leading to disassociation of the transpososome.

Analysis of genetic diversity in populations of the Australian lungfish, *Neoceratodus fosteri* (Dipnoi).

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The lungfish *Neoceratodus forsteri* Krefft 1870 is one of five extant representatives of the ancient air-breathing Dipnoan lineage. Fossils indicate that the distribution of *N. forsteri* reached the centre of the Australian continent prior to the Pleistocene. However, it is now restricted to the south-east corner of Queensland where it occurs naturally in the Burnett and Mary Rivers. Translocated populations exist in nearby rivers, some originating in the nineteenth century. Although legally protected, the Australian lungfish is potentially threatened as its range coincides with some areas that are extensively utilised for agriculture. Altered flow regimes for irrigation purposes could lead to the destruction of existing population structure, enhancing drift and causing loss of genetic variation. Frentui, Ovenden and Street (2001, Conserv. Genetics, 2: 63-67) non-lethally sampled 278 individuals representing two spatially distinct endemic populations, as well as one population thought to be derived from an anthropogenic translocation in the 1890's (Brisbane River). Two of 24 allozyme loci resolved from muscle tissue were polymorphic. Mitochondrial DNA nucleotide sequence diversity estimated across 2,235 base pairs in each of 40 individuals ranged between 0.000423 and 0.001470 per river. The observed low intraspecific genetic variation was attributed to population bottlenecks, possibly induced by Pleistocene aridity. Limited genetic differentiation was detected among rivers using nuclear and mitochondrial markers suggesting that admixture may have occurred between the endemic Mary and Burnett populations during periods of low sea level when the drainages may have converged before reaching the ocean. Genetic data was consistent with the explanation that lungfish were introduced to the Brisbane River from the Mary River. As anthropogenic demands on lungfish habitat are expanding, we are increasing our efforts to find variable genetic loci to determine the conservation status of populations for effective management. Currently we are developing methodology for microsatellite and AFLP loci.

Downstream Targets of *Twist* Activity in Mouse Embryonic Development

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The mouse *Twist* gene encodes a basic helix-loop-helix (bHLH) transcription factor required for normal morphogenesis of the limb and craniofacial structures.

To identify potential downstream targets of *Twist* regulation we have analysed the expression of candidate genes, chosen for their similarity in expression and mutant phenotype. We have found that *Alx3* and *Alx4,* which encode *Aristaless*-related transcription factors, are down-regulated in *Twist*$^{-/-}$ limb buds. Examination of flanking genomic sequences revealed the presence of several potential *Twist* binding sites upstream of *Alx3* and *Alx4*.

In another approach, we have compared the transcriptome of wild-type and *Twist*$^{-/-}$ forelimb buds by suppression subtractive hybridisation. In addition to known genes involved in various cellular processes, we have identified several putative novel genes that are enriched in wild-type limb bud cDNA. Differential expression of these clones in the forelimb bud of wild type and *Twist*$^{-/-}$ embryos was validated by whole mount *in situ* hybridisation. These genes are also differentially expressed in craniofacial tissues that normally express *Twist,* consistent with direct regulation by *Twist*. Two of the putative novel genes are more highly expressed in the forelimbs than hindlimbs, suggesting that they may be involved in mediating the differential effects of loss of *Twist* function on forelimb and hindlimb development. Further characterisation of these novel clones, by examination of cDNA and genomic sequences and analysis of their expression during development, is in progress.

Phylogenetic relationships of the hydrophid sea snakes.

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Evolutionary relationships among the hydrophid sea snakes remain the subject of considerable debate. The phylogenetic relationships among the three major groups of marine hydrophids, the *Hydrophis* group, the *Aipysurus-Emydocephalus* group, and the 'primitive'group that comprises 3 monospecific genera, *Ephalophis greyi, Hydrelaps darwiniensis* and *Parahydrophis mertoni*, have been well studied, however resolution is still weak. In addition, relationships within these groups, particularly among the 34 species in the genus *Hydrophis* and allied genera, are poorly understood. Most previous studies have used morphological characters to infer evolutionary relationships. We investigate the evolutionary relationships within the marine hydrophids using mitochondrial DNA sequences of the cytochrome *b* and 16S rRNA genes. Preliminary results from cytochrome *b* (1030bp) show that 1). The *Hydrophis* group and *H. darwiniensis*, representing the 'primitive' group, formed a clade; 2). The *Aipysurus-Emydocephalus* group formed a sister taxon to this clade; 3). *Hydrelaps darwiniensis*, formed a sister taxon to the *Hydrophis* group, and 4). Relationships among the 8 species of the *Hydrophis* group may be resolved using this locus. *Pelamis platurus*, the only pelagic marine hydrophid species, fell within the *Hydrophis* group, however *P. platurus* has diverged considerably from the remaining species of the *Hydrophis* group that primarily live in shallower coastal waters.

Population genetic structure and conservation of the olive seasnake, *Aipysurus laevis*, in the southern Great Barrier Reef

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Aipysurus laevis has an extremely aggregated distribution. Presence/absence data from the 1970's and 1980's show that *A. laevis* were extremely abundant on 22 reefs and absent from 17 reefs in the Swain Reefs complex (Heatwole 1975; Burns 1984; Burns & Heatwole 1998). Twenty of these 39 reefs, comprising 13 reefs where *A. laevis* had been recorded as abundant and 7 reefs where *A. laevis* had been absent, were re-surveyed in 2001. *Aipysurus laevis* were found on only 3 of these 20 reefs; all reefs where *A. laevis* had previously been recorded. I am investigating the population genetic structure of *Aipysurus laevis* throughout its range, including a detailed analysis of the small-scale population genetic structure of *A. laevis* in the Swain Reefs complex, using microsatellite genetic markers and mitochondrial DNA sequencing. Tissue samples from 35 to 40 individuals of *A. laevis* have been obtained from each of 4 reefs in the Swain Reefs complex and from Keppel Island, an inshore island 200km away. If populations are found to be genetically distinct at the level of individual reefs, then the loss of *A.laevis* populations from 10 of 13 reefs in the Swain Reefs complex, where *A. laevis* was found in large numbers 30 to 40 years ago, potentially represents a significant loss of genetic diversity, and thus a serious conservation concern. Information regarding the spatial scale at which population genetic structure occurs will be useful for determining the size and spatial scale of distribution of protected areas required to ensure viable populations of *A. laevis* in the Great Barrier Reef World Heritage Area, in the current development of the Representative Areas Program that is designed to protect biodiversity throughout the marine park.

The Effects of Habitat Fragmentation on The Log-dwelling Cockroach *Panesthia australis*

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The consequences of habitat fragmentation and restricted gene flow are common themes in conservation and evolutionary biology; however, the effects on log-dwelling invertebrates are virtually unknown. This study examined the effects of habitat fragmentation on a native wood-dwelling cockroach *Panesthia australis* with particular reference to fragment size and level of connectivity in a fragmented forest near Tumut, New South Wales. The only significant predictor of species abundance or allelic richness was the relationship between coarse woody debris and the abundance of animals, presumably owing to increased connectivity and resources provided by suitable habitat. Examination of four polymorphic allozymes from populations in remnant patches of native vegetation and an adjacent area of contiguous native forest indicated that high degrees of genetic variation existed among logs over small spatial scales (<30m), suggesting that there is locally patchy colonization, causing complex patterns of population substructure. On a broader scale, genetic differentiation was positively correlated with geographic distances above 10km in both fragmented and unfragmented habitat. However, habitat fragmentation was identified to significantly reduce gene flow between populations causing a strong pattern of isolation-by-distance. The geographic structuring present in the fragmented population indicates that habitat fragmentation has the potential to alter gene flow over as few as five generations in a species that is adapted to a naturally fragmented habitat. This result may have implications for other log-dependent invertebrates, which may be more susceptible to anthropogenic stress, indicating that human disturbance may have the potential to alter population dynamics and the microevolution of log-dwelling communities. Evidence from this study indicates that there is an urgent need for habitat conservation and research into the patterns of genetic variation into this functionally pivotal but relatively unstudied faunal community.

Inbreeding Contribution from Influential Ancestors: An Analysis of Milk Traits from Holstein Friesians in Australia

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Inbreeding in Holstein-Friesian (HF) dairy cattle [examples in Man *et al.* 14thAAABG, 325-328 (2001)] has been of increasing concern due to a diminishing current gene pool and subsequent increase in the rate of inbreeding. One of the consequences is inbreeding depression. Theoretically, the extent of inbreeding depression depends on the frequency of favourable alleles and the extent of their dominance. Since not all ancestors have the same set of alleles, the ancestral source of inbreeding may have an influence on the effect of inbreeding. The present analysis partitioned the contribution of influential ancestors to the inbreeding coefficient (*F*) of individuals using the path-searching method [Boyce, J. Hered. 74, 400-404 (1983)], which has been modified by the first author for this analysis. Selection of influential ancestors is described in Man *et al.* [7th WCGALP (2002)]. The software for computation of the overall *F* was written by Tier [Genet. Sel. Evol. 22, 419-430 (1990)] from the Animal Genetics and Breeding Unit (AGBU), Armidale.

Standardised production index [Jones and Goddard, 4th WCGALP, 8:382-385 (1990)] of 1st lactation milk yield and pedigree records from the Australian Dairy Herd Improvement Scheme (ADHIS) were kindly provided by Dr Les Jones and Dr Kevin Beard. The pedigree file has 65,919 HF cows born in 1992. Of these, 29,348 HF cows with at least two generations of complete pedigrees and 1st lactation records were used for this analysis. The effect of inbreeding contributed by the ten influential ancestors was estimated using ASReml [Gilmour *et al.*, ASReml Reference Manual (May 2002)], with the following model:

$$y_i = bF_i + b_jF_{ij} + hys_i + animal_i + \varepsilon_i \text{ , where;}$$

$_i$ = factor or variable for the ith individual in analysis,
y_i = standardized production index of milk volume (protein or fat),
F_i = overall inbreeding coefficient (*F*),
F_{ij} = part of *F* contributed by the jth common ancestor (j=1,2,...10),
hys_i = herd-year-season, and
$animal_i$ = random animal factor (heritability estimated in ASReml).

The effect of inbreeding contribution from the ten ancestors ranges from -56.9 ⊕92.1 to 32.0 ⊕100.8 L (volume), -1.82 ⊕2.86 to 1.40 ⊕1.13 kg (fat) and -5.75 ⊕3.22 to 1.64 ⊕1.40 kg (protein) per lactation for every 1% increase in *F*. The large standard errors (probably due to the small standard deviations in inbreeding contribution of these ancestors [Man *et al.*, 7th WCGALP (2002)]) indicate these effects are not significant in this analysis.

Phylogenetic reconstruction from ill-behaved sequences

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An intron in the leucine transfer RNA gene (trnL-UAA) in chloroplast DNA occurs in all land plant chloroplast genomes, as well as those of many eukaryotic algae, and the cyanobacteria believed to be related to those chloroplasts (Kushet *et al* 1990, Science 250:1570-1573; Xu *et al* 1990, Science 250:1566-1570).

This region of the genome is being widely used in the inference of phylogenetic relationships between plant species since 'universal' PCR primers for its amplification and sequencing were published by Taberlet *et al* (1991, Plant Mol Biol 17: 1105-1109). These universal primers are based on highly conserved sequences in the coding region of the trnL, and they amplify across the trnL-intron, which is observed to be relatively highly variable, by both point mutation, and by 'insertion/deletion events'.

Because of this variability, there is only a narrow 'window' of usefulness for this sequence in molecular phylogenetics, usually at taxonomic levels between genus and family. Above these levels, sequence alignment (that is, the identification of homology), by methods currently in use, is usually so difficult that believable phylogenetic inference from such comparison is impossible.

The principle of evolution by descent with modification implies that all representatives of this ubiquitous sequence are (in some way) 'homologous'. In this presentation, we explore ways in which regions of homology, and hence phylogenetic signal, can be recognised between more diverged trnL-intron sequences, thereby increasing the utility of this region for evolutionary reconstruction.

Phenotypic variation in mammals as an epigenetic effect of retrotransposon activity

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Phenotype in mammals is often considered to be directly derived from genotype, by means of the selective expression of genes tightly controlled by transcriptional regulatory elements. There is however abundant evidence that phenotype may vary, sometimes dramatically, between individuals with the same genotype, and this variation may sometimes manifest as disease. One possible source of such variation is stochastic defects in gene regulation created by active retrotransposons. Nearly half of the mammalian genome is made up of degenerate retroelements; virtually every gene has multiple retroelements in and around it. Transcriptional regulatory elements are adapted to function with enhancers and promoters interacting over long distances, and with intervening retroelements silent. There is however abundant evidence that the intrusion of an active promoter into a locus can disrupt its normal regulatory apparatus over substantial distances. Transcriptional activity of a retroelement in a locus may thus interfere with the regulatory apparatus and either suppress or increase expression of the gene. The human and mouse EST databases contain numerous transcripts originating in the promoters of retroelements of all classes, suggesting that degenerate retroelements have quite commonly retained transcriptional competence. It is likely that the activity reflected in dbEST is not programmed but instead is stochastic and mosaic (this view takes into account observations of transposon activity in a variety of species, including mice and maize). Imperfect silencing of retrotransposons may produce mosaic patterns of retrotransposon expression in somatic cells, and consequent mosaic alterations in gene expression. The stochastic nature of retrotransposon activity, and the very large number of genes that may be affected, will produce innate phenotypic differences between individuals (even genetically identical ones). These differences will be heritable if epigenetic alterations of retrotransposons are not cleared completely in each generation. The extent to which quantitative variation is produced by epigenetic rather than genetic effects remains to be established, and retrotransposon activity is potentially a significant factor.

Lysine 9 methylation of Histone H3 – either activation or silencing

Jennifer Cropley.

Molecular Evolution in the Paenungulates: Evidence from the Mitochondrial DNA

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Examination of the mitochondrial control region in the superordinal group Paenungulata has shown surprising structural similarity between taxa. Sequences of the complete mtDNA control region were sequenced from the tree hyrax, dugong and Caribbean manatee and aligned together with sequences from the Asian and African elephants available in Genbank. This alignment showed a high degree of similarity, including the presence of a large microsatellite in the CSB domain, of the control region in representatives from all the orders. This microsatellite repeat consists of a five base repeat (CGCATA) in Sirenia and Proboscidea and an eight base repeat (CACGTATA) in Hyracoidea. The Paenungulata has a higher degree of similarity than the Artiodactyla (represented by llama, giraffes, hippopotami, sheep, cows and pigs) - a group with a similar taxonomic position to the Paenungulata. The similarity ranges from 65% to 76% in the Artiodactyla and between 71% and 84% in the paenungulates over the entire control region. If data from the different species of elephants are included the percent similarity increases to 67% to 94%. These results indicate that the control region in the paenungulates is evolving more slowly than the Artiodactyla examined here. The control region may therefore be viewed as a type of DNA 'fossil' in the paenungulates and suggests a slow rate of evolution of the mtDNA in this group is plausible. These results indicate that studies using mitochondrial control region sequences should take into consideration this conservative rate of evolution to interpret patterns of diversification. This study supports manatee and dugong as sister taxa with substantial genetics differences between hyrax, elephants and the sirenians.

Use of EST sequence and comparative genomics to aid QTL gene discovery in farmed ruminants: a view from the trenches

John McEwan

AgResearch, Invermay, New Zealand

Many of the techniques we use are not new or novel, but they have been applied by us on a large scale, using computerised methods in sequence poor species, and as part of this we have spent a large effort trying to utilise information available from the human genome sequence.

Some potential areas to be covered
* Assembling and annotating ruminant ESTs - results and properties for cattle. (We have assembled up to 400K ESTs from one species in house)
* Expression clustering of assembled ESTs
A technique developed in plants but sadly overlooked in animals (some 6200 bovine EST contigs annotated by this method)
* Ortholog detection and annotation of ruminant EST contigs by comparison with the human genome. Results of a large scale comparison with the human genome (100,000 contigs and singletons)
* functional SNP identification from ruminant EST overlaps: prospects and problems. Results from a SNP discovery effort in bovine including properties of cSNPs (about 6000 SNPs)
* TIPs, a highly efficient method of generating SNPs to a targeted region of a ruminant genome based on human genome sequence and a ruminant EST contigs from a related species. About 50% success per primer pair designed for over 100 primers designed.
* Prospects of using LD in farmed ruminants to fine map QTL. The story is very different to humans.

The Mouse Genome Database: A Resource for Comparative Genomics

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The Mouse Genome Database (MGD) is a community resource dedicated towards providing an integrated representation of mouse genomic, genetic and biological information. MGD currently provides a scientifically curated homology dataset primarily extracted from literature for twenty mammalian species including a marsupial and a monotreme. MGD also provides a comparative mapping resource. Users have the ability to generate comparative map displays, Oxford Grid displays and composite listings of all mouse/human and mouse/rat homology data. Corresponding hypertext links are provided for mammalian species with an on-line genomic database. A feature of gene annotation in MGD is the use of controlled vocabularies for the description of the molecular function, biological process and cellular component of gene products as part to the Gene Ontology (GO) project. These terms can be used as attributes of gene products across species aiding in the development of comprehensive comparative maps and facilitation of queries across multiple databases. MGD continues to evolve, expanding its data coverage, providing new data manipulation and display tools as well as promoting data integration with other scientific resources to meet the growing needs of the scientific community.

Ring-a-Ring-a-Rosy: DNA Analysis of the Plague Bacillus from Late Medieval London

Anthony McKeough

Archaeological Sciences Laboratories, Institute for Molecular Biosciences, School of Social Sciences, University of Queensland

Plague bacillus (*Yersinia pestis*) is one of the most frequently documented diseases in modern history and has resulted in many suspected plague burials. However, the current lack of adequate methods in paleopathology prevents the cause of death being ascertained in plague victims, as *Y. pestis* leaves no visible manifestations on bone. Given the lack of methodology in identifying ancient plague (and ancient diseases as a whole), this project aimed to test the feasibility of DNA analysis in detecting plague in archaeological bone samples. The analysis utilised PCR in an attempt to detect *Y. pestis* (the causative agent of the plague) in six bone samples from a suspected plague burial in London, dating to 1348 during the Black Death epidemic. PCR allowed the identification of *Y. pestis* bacteria in one of the six bone samples, thus demonstrating this technique's viability in plague detection. Furthermore, this detection method can potentially be applied to virtually all blood-borne disease in archaeological and forensic samples.

Neuronal Ceroid Lipofuscinosis in Border Collie Dogs

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The neuronal ceroid lipofuscinoses (NCLs) represent a group of at least eight unique human genes that cause neurodegeneration by a largely unknown apoptotic pathway. The result of the breakdown is caused by an accumulation of ceroid lipofuscin within the lysosomes. A range of animal models, both naturally occurring and laboratory produced, are being studied to increase knowledge of the disease. Our goal is to characterise the gene mutation that causes NCL in Border Collies, and to develop a molecular test to screen carriers from the breeding population.

Microsatellites from work derived from the English Setter model show suggestions of linkage in the border collie, although the small pedigrees involved are a significant factor in our low LOD scores.

Haplotype analysis has so far justified the assumption that the same gene is responsible for the disease in both canine breeds. Degenerate primers to genes localised to the region from comparative mapping have been employed to isolate homologous canine genes that will be used to identify canine BACs containing the region. These can be used to find more microsatellites within our target region to refine mapping, and to identify sequence of canine candidate genes.

By identifying the gene responsible for NCL within Border Collies, it will be possible to identify carriers clearly and remove them from the breeding stock. This is more efficient than labelling all descendants of carriers as tainted stock.

Expansion and Duplication of the Serum Albumin Gene in Tuatara

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Previous studies have shown that the New Zealand tuatara (*S. punctatus*) has an enlarged albumin found in three polymorphic forms [Brown *et al.*, Biochem. Genetics 33, 189-204 (1995)]. In the present study, the A form and C charge variant were found to have masses of 155 kDa, equating to 7-domain proteins instead of the typical 3-domain structure. The B form has a mass of 205 kDa, implying a 9-domain structure. All three forms bound palmitate with high affinity, but not nickel and lacked carbohydrate sidechains. A fractionally more acidic plasma protein that bound palmitate and had the mass of a typical albumin, 68 kDa, was noted in all tuatara plasma samples. Anti-sera raised to tuatara A albumin reacted equally with A, B and C, but not at all with the 68 kDa protein. N-terminal protein sequence of this 68 kDa protein was highly similar to that of the A,B,C albumins, but differed in several residues, indicating it is the product of a different gene. Random screening of a liver cDNA library from an AB heterozygote resulted in the identification of two different albumin clones with PCR used to extend the known cDNA sequences. This resulted in the determination of 1788 nucleotides for the 68 kDa albumin encoding 527 residues of protein sequence, the stop codon and 3`UTR, and 1392 bases of A/B sequence encoding 401 residues of protein sequence, the stop codon and 3`UTR. Alignment of the deduced protein sequences with the albumin superfamily and subsequent phylogenetic analysis provided evidence that the 68 kDa albumin is encoded by a second albumin gene in the tuatara, which has arisen by means of a unique gene duplication event. A hypothesis of expansion and duplication of the albumin gene in tuatara is presented.

Physical mapping of the porcine milk proline-rich protein gene

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Proline-rich proteins (PRPs) account for 70% of the protein in human saliva and have been attributed with a variety of functions including the ability to bind fimbriae of various pathogenic organisms (e.g. *Porphyromonas gingivalis*, *Candida albicans*), to precipitate tannins, bind calcium and to act as a masticatory lubricant. Hall and colleagues from the Australian Equine Genetics Research Centre have previously reported the identification of a protein homologous with the human salivary PRPs (HsPRPs) in porcine milk. The protein was identified in sows' milk at all stages of lactation. It is characterised as being rich in proline, glutamine and glycine that together account for 76% of the amino acid residues. The porcine milk PRP (PmPRP) gene is at least 5kb and, like the human genes, consists of four exons with the third exon comprised almost entirely of tandemly repetitive sequence. The tandem repeat unit in the PmPRP gene sequence is 33 nucleotides in length and is repeated between 41 and 45 times, as determined by PCR and nucleotide sequencing. HsPRPs are the products of a six-gene cluster that spans approximately 700kb of HSA12p13.2, a chromosomal region that has been shown to have conserved synteny with the proximal part of SSC5q. Physical mapping of the PmPRP gene with a pig/rodent somatic cell hybrid panel has assigned this gene to SSC5q.

Development of an AFLP-based genetic map for cotton bollworm *Helicoverpa armigera*, with ribosomal protein genes as anchor loci comparative linkage mapping.

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We describe a linkage map consisting of 260 AFLP markers covering all 31 chromosomes of *H. armigera*. Because AFLPs cannot readily be transferred to other species, we are adding anchor loci that can. Ribosomal protein (RP) genes are useful for comparative purposes, as they are generally single-copy, highly conserved, and randomly dispersed throughout the genome. We describe our approaches to map these using RFLP analysis and denaturing HPLC, and present linkage results for approximately 20 RP genes.

Estimating Effective Population Size: A Comparison of Demographic and Pedigree-based methods in the Helmeted Honeyeater.

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The Helmeted Honeyeater *Lichenostomus melanops cassidix* is a sub-species in the Yellow-tufted Honeyeater complex. The last wild population was reduced to thirteen breeding pairs in 1989 at which time a recovery program was initiated. The program included wild habitat management, intensive population monitoring and captive breeding. This has led to an increase of the wild population to an apparent carrying capacity of 100 birds and a current emphasis on reintroduction to new sites.

Individual colour-banding of all wild birds was employed until 1997, and a genealogy incorporating both wild and captive birds has been maintained. We have estimated the genetically effective population size (N_e) of wild Helmeted Honeyeaters in two main ways: from the population data (1990-2000) using Lunney's method, and from the pedigree using Genedrop. The estimates are 33.0 and 23.9. In addition, a PVA simulation (Vortex) using the ecological data gave a heterozygosity loss equivalent to N_e of 31.7.

Reasons for differences between estimates will be discussed, as will year-to-year variations in N_e . The values imply a rate of loss of heterozygosity of 1.45% - 1.76% per generation. This prospect reinforces the priority of reintroduction and population expansion in the recovery program for Helmeted Honeyeaters.

Viability of fragmented *Macadamia integrifolia* populations

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Macadamia integrifolia is a vulnerable, sub-tropical tree species from the eastern coast of Queensland, however much of its distribution has been impacted by land clearing and urbanisation. The aim of this project is to determine the viability of remnant *M. integrifolia* populations. In particular, this paper will present results on the fine-scale genetic and demographic structure of three disturbed populations from the Brisbane region. Dominant and co-dominant RAF (randomly amplified DNA fingerprinting) markers were used to genotype all individuals in the study sites. Preliminary demographic results suggest that disturbed and isolated trees have greater fecundity than those within a protected canopy.

Effects of selection on variation in and around the insecticide resistance locus, *Rop-1*, in the sheep blowfly, *Lucilia cuprina*

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The hitchhiking model of molecular evolution predicts higher levels of linkage disequilibrium around genes under purifying selection than would be expected for a region of a chromosome with given levels of recombination. We have examined the levels of linkage disequilibrium around the insecticide resistance locus, *Rop-1* by sequencing regions of genes on chromosome IV of *L. cuprina* from isogenic (IV) lines that have already been characterised for their *Rop-1* haplotype status. Four genes were targeted initially, *AChE, Lcdsx, LcαE1, LcαE10*. *AChE* is genetically a large distance from *Rop-1* (*LcαE7*). *Lcdsx*, from correlations with its position in Drosophila, is closer to *Rop-1*. *LcαE1* and *LcαE10* lie at either end of the α-esterase cluster. From sequencing regions of these genes across 39 isogenic (IV) lines various levels of linkage disequilibrium with *Rop-1* were found, ranging from complete disequilibrium with *LcαE1* and *LcαE10* to intermediate levels with *Lcdsx* to equilibrium with *AChE*. Interesting a microsatellite within *LcαE10* shows higher levels of variation than surrounding point mutations suggesting either that this variation was carried through the selective sweep or that the variation has been generated very quickly since the sweep occurred some 50 years ago.

Charles Darwin in Australia

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Early in 1836 Charles Darwin spent two months in Australia as part of his round-the-world voyage on the *Beagle*. During this time he visited Sydney, travelled on horseback to Bathurst, visited Hobart, and called into King George Sound in Western Australia. Darwin met with several of the leading figures of the Australian colonies, including members of the King and Macarthur families in Sydney, and Alfred Stephen and George Frankland in Hobart. Darwin made extensive notes on the geology of the country through which he travelled, and recorded observations on natural history. The Australian flora and fauna, strikingly different from that with which he was a familiar elsewhere, made an impression on his mind which was to surface in later writings. This lecture provides a summary of Darwin's visit, illustrated with beautiful contemporary paintings by Augustus Earle and Conrad Martens, both of whom were shipmates of Darwin, having served on the *Beagle* during an earlier stage of her voyage.

Online Mendelian Inheritance in Animals (OMIA)

Frank W Nicholas

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Online Mendelian Inheritance in Animals (OMIA)[1] is a database of phenes[2] that have been documented in a wide range of animal species other than human and mouse. It is modelled on, is complementary to McKusick's Mendelian Online Inheritance in Man (OMIM)[3]. OMIA has been under construction since 1980. It contains references to publications on any trait or disorder for which familial inheritance has been claimed. The information on phenes includes their occurrence in different species, and a chronological list of papers describing that phene in each species. A number of other fields that are available for several traits include more detailed information on, e.g., synonyms, possible human homologues, clinical signs, pathology, inheritance, molecular genetics. The database is reciprocally 'hotlinked' to OMIM, which allows direct access to information on homologous human phenes, and, for OMIM users, direct access to animal models of human inherited disease. At the time of writing, OMIA contains 16,105 references on 1,211 phenes that have been reported in one or more of 206 species of animals. 106 of these phenes have been characterised at the molecular level, comprising 7 in cats, 23 in cattle, 8 in chickens, 29 in dogs, 1 in emu, 2 in fox, 5 in goat, 2 in hamster, 7 in horse, 1 in medaka, 1 in rhesus monkey, 8 in pig, 2 in quail, 3 in rabbit, 1 in rat, and 6 in sheep.

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[1] http://www.angis.su.oz.au/Databases/BIRX/omia

[2] A phene is a familial trait. For single-locus traits, the word(s) correspond to one of the phenotypes that arise from segregation at that locus. For example, CITRULLINAEMIA is the phene for the ARGININOSUCCINATE SYNTHETASE locus; and FECUNDITY, BOOROOLA is the phene for BONE MORPHOGENIC PROTEIN RECEPTOR 1B locus. OMIA also includes multifactorial traits and disorders. Thus, for example, HIP DYSPLASIA is a phene.

[3] http://www3.ncbi.nlm.nih.gov/Omim/

Approaches to studying copper homeostasis in *Drosophila melanogaster*

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Copper is an essential trace element to all organisms. However, intracellular copper concentrations must be finely balanced, as excessive amounts cause extensive cellular damage. The basic pathways for copper homeostasis have been defined and appear to be significantly conserved between species [Valentine and Gralla, Science 278, 817-8 (1997)]. However, much remains to be understood about copper homeostasis in multicellular organisms and *D. melanogaster* is likely to provide a useful model, avoiding the logistical and ethical implications of gene manipulation in mammals.

We are investigating three complementary approaches that should permit the identification and cloning of novel genes involved in copper homeostasis. The first of these uses gene chip technology to analyse the pattern of gene expression in the *D. melanogaster* genome following different conditions of exposure to copper. Preliminary data show a response from the expected pathways as well as indicating genes of interest for further study. A second approach employs a library of 196 strains each heterozygous for a different, but overlapping, large chromosomal deletion and provides the potential to screen ~80% of the genome for loss of function effects that lead to copper sensitivity or copper resistance. Screening to date of 110 deficiency strains has revealed 15 resistant and six sensitive strains. In the case of one of the strains, we have used overlapping deletions to narrow the region to ~83kb, containing 21 predicted genes. The final approach utilised chemical mutagenesis to generate 11 resistant strains, isolated by screening embryos on lethal levels of copper.

Ranking geographic areas for conservation with genetic criteria: a case study using the reptiles of the Northwestern slopes of New South Wales

Denis O'Meally, Don Colgan

Evolutionary Biology Unit, The Australian Museum. 6 College St. Sydney, NSW 2010

Decisions as to the best methods by which to conserve animal and plant species by habitat reservation draw upon many sources of information and many methods of ranking sites for reservation have been suggested (generally, comprehensiveness, adequacy and representativeness). More recently, interest in the inclusion of genetic information in such ranking has increased. The usual approach to date has been to consider, principally, one taxon, and to rank species or populations within this on the basis of their evolutionary distinctiveness or endogenous genetic variation. Here we examine metrics based on protein electrophoretic studies of eleven reptile species in the northwestern tablelands of New South Wales. Simpson and Shannon-Weiner indices of species diversity were also calculated. Values of the metrics within taxonomic groups suggested that those based on genetic distance are positively inter-correlated but all are negatively correlated with heterozygosity (highly) and the species diversity measures (intermediate values). Accumulation curves of the numbers of alleles represented as sites are added in order of their values for a particular metric do not discriminate between metrics. The average for a metric of the pairwise correlations between taxonomic groups has highest (but not large) values for the average genetic distance and average heterozygosity metrics, suggesting that these would be the better measures for if taxonomic groups are to be used as surrogates. The relatively low values of the correlations suggests however that taxonomic groups are not usually good surrogates for genetic prediction. Pye's Creek and The Flags/Riamukka areas were identified as sites of particular conservation genetic interest.

MJD White Presedential Address
Evolution of worker sterility in social insects

Ben Oldroyd

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Ever since Darwin conceded that the 'neuters' of social insects were potentially fatal to his theory of natural selection, biologists have puzzled over how 'altruistic' sterility genes can spread, and how social insect colonies evolved. A key breakthrough came in 1964 when Bill Hamilton elaborated his theory of inclusive fitness. He showed that if an allele causing sterility in one individual increases the reproductive success of a relative, then selection can favour the spread of the allele.

Hamilton's theory has shown how the haplodiploid genetics of Hymenopteran insects (bees, wasps and ants) favoured the evolution of social behaviour and worker sterility. In species where queens mate once, workers are more related to their sisters (r = 0.75) than their own daughters (r = 0.5). This asymmetry explains how workers can increase their inclusive fitness by refraining from mating and laying female-producing eggs. Instead they do better to rear their sisters from eggs laid by the queen. But what about males? Unmated workers can lay eggs that produce fully viable males, and if their colony loses its queen and brood, they do so with alacrity. Moreover, workers are more related to their own sons than those produced by the queen (their brothers). So why don't they lay male-producing eggs?

Francis Ratnieks [*Am. Nat.* 132:217] showed that in species like honey bees where queens mate many times, workers are finessed into an evolutionary stalemate wherein they are forced into rearing the queen's sons and not their own. Workers are related to their own sons by 0.5, and to those of the queen by 0.25. However, workers are related to the sons of their half-sisters by only 0.125. Thus workers grudgingly 'agree' to rear the queen's sons, refraining from personal reproduction. This saves them from being obliged to rear the sons of their half-sisters, as they would be required to do if other workers laid eggs.

Any system of self-restraint is open to abuse by cheats, and must be policed by the society. Recalcitrant workers are prevented from reproducing by 'worker policing', in which workers eat any worker-laid eggs. This mechanism is extremely efficient, and in honeybees, worker reproduction is very rare. However, in about 1 in 10,000 colonies, worker sterility breaks down due to a behavioural mutation. From one of these colonies we have bred a line of bees that allows us to investigate the genetics by which worker sterility is normally controlled. We are using this line to uncover the mechanisms of and the genes that control worker sterility.

Experimental and genetic investigations into graft compatibility within and between the gymnosperm families Podocarpaceae and Pinaceae

George Orel

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Interspecific, intraspecific and interfamilial graft compatibility within Podocarpaceae and between Podocarpaceae and Pinaceae was tested during the period of 29 weeks. *P. dispermus* White., *P.elatus* R. Br. ex Mirb. and *Afrocarpus falcata* (Thunb.) R. Br. ex Mirb. were chosen for rootstocks and *P. dispermus*, *P.elatus* and *Afrocarpus falcata* and three not identified *Podocarpus* species, U1, U2 and U3, together with *Keteleeria evelyniana* Mast., were chosen for scions. Only actively growing grafts were considered, although a number of scions were still alive, but not exhibiting active growth. An overall success rate, after 29 weeks, of 86.66% was recorded for the combination of intraspecific grafts between *P.elatus* and *P. elatus* and a 53.33% success rate for *P. dispermus* and *P.dispermus*.The success rate for interspecific grafts was as follows; scion U1 on *P. dispermus* - 75%, scion U2 on *A. falcata* - 72% and scion U3 on *P.elatus* 65%. Some 70% of the interfamilial grafts between *K. evelyniana* and *P. dispermus* were successful. The results confirmed the existence of intraspecific and interspecific compatibility within and between the Podocarpaceae, suggesting a close genetic relationships. The high success rate of the Podocarpaceae and Pinaceae grafts suggests relatively close taxonomic relationship. However, these results should be viewed in the light of the short duration of this experiment, which did not take into account the effects of delayed graft compatibility.

Population Structure in *Macrobrachium australiense*

Sonja Parsonage, Amanda Dimmock, Peter Mather

Queensland University of Technology, Brisbane.

Macrobrachium is one of the most challenging genera within the decapod crustacean group from a systematic point of view. We examined population structure at the CO1 mitochondrial gene and compared patterns of genetic variation to patterns of variation in traditional taxonomic characters for the most common Australian species, *Macrobrachium australiense*. Genetic structure within and between river catchments was investigated to discriminate between competing hypotheses for the origins of this ubiquitous species. Results show considerable morphological variation at the smallest spatial scale examined (tributaries within a river catchment) compared with a low level of genetic differentiation among populations within a river catchment and a pattern of isolation by distance between river catchments in eastern and northern Australia.

Cryptic speciation in Australian *Drosophila serrata*

Yvonne Parsons, K. Viduka, M. Schiffer, M. Carew & A. Hoffmann

Centre for Environmental and Stress Adaptation Research (CESAR), La Trobe University

Drosophila serrata is being increasingly used as a model system for evolutionary studies of speciation and climatic adaptation. Populations of *D. serrata* are found over wide geographic distances but limited information is currently available on the genetic structure of these populations. Here we assess microsatellite variation in *D. serrata* from eastern Australia to understand levels of population structuring and to test for genetic discontinuities across the Burdekin gap at the nuclear level. We are interested in levels of gene flow because good evidence for clinal variation in *D. serrata* has been obtained for a number of traits including wing shape and stress resistance. We obtained high F_{ST} values between populations in Far North Queensland and all more southern populations, suggesting the presence of a cryptic species, but a relatively low level of sub structuring elsewhere. We pursued this further by setting up crosses between lines of *D. serrata* collected above and below the Burdekin Gap. These indicated unequivocally that there are two cryptic species of *D. serrata* sympatric in far north Queensland but only one species south of the Burdekin gap.

Harnessing the versatility of *Saccharomyces cerevisiae* to identify novel mechanisms that influence glutathione homeostasis

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In many organisms glutathione (L-γ-glutamyl-L-cysteinylglycine) is the major non-protein thiol, participating in numerous cellular functions including protection from reactive oxygen species and xenobiotics, maintenance of cellular redox homeostasis, mitochondrial related apoptotic signalling and ageing. In humans altered GSH homeostasis has been associated with a number of diseases including: Parkinson's disease, cystic fibrosis, myocardial infarction, ischemic reperfusion injury, hemolytic anemia and HIV to name only a few. In yeast glutathione deficiency leads to a decreased tolerance to various stress conditions. Although glutathione biosynthesis and degradation have been well studied the genetic mechanisms influencing intra/intercellular glutathione homeostasis have not been fully elucidated.

The availability of a comprehensive set of yeast deletion mutants provided an opportunity to perform a genome-wide analysis of cellular mechanisms influencing glutathione homeostasis. Briefly, the screen identified a large number of genes (~250) whose deletion resulted in altered glutathione metabolism. Of these many mutants could be grouped according to the respective function of their encoded gene product. This approach identified several distinct classes of genes that allowed us to propose a model of the genome-wide factors influencing glutathione homeostasis in yeast. The implications of this model in terms of certain human diseases are discussed.

Polygene Discovery for Body Weight Regulation in Animal Models and Relationship to Human Gene Discovery

Daniel Pomp

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Body weight and fatness are complex traits controlled by relatively equal contributions of polygenic and environmental influences. Despite the mapping of many QTL for these traits across several mammalian species including mouse, rat, pig, sheep, cattle and human, little can be learned from comparative analysis due to the inherent inaccuracy of QTL localization and the inability to differentiate between colocalization and coincidence. Elucidation of the identity and nature of the underlying polygenes is required, yet such success has remained frustratingly elusive.

Long-term selective breeding is a powerful approach to concentrate allelic variants explaining significant variation for quantitative traits. We study body weight, fatness and energy balance using two sets of such selection lines in mice. The M16 line was selected 27 generations for rapid 3-6 wk weight gain (Gene Eisen, NC State), while the MH/ML lines were selected 16 generations for high/low heat loss using direct calorimetry (Merlyn Nielsen, UNL). Marker genotyping has identified regions harboring putative polygenes regulating relevant phenotypes in these lines. Fine mapping of QTL is progressing using a combination of congenic lines, advanced intercrosses and recombinant progeny testing. Gene expression analysis using microarrays is defining correlated responses to selection in the transcriptome, while a parallel approach evaluates selection response in the proteome. These efforts are combined with detailed physiological and metabolomic phenotyping in an integrated approach to polygene discovery. For example, key transcriptional, proteomic, metabolomic and endocrine pathways are being phenotyped in large, segregating F2 (n=1,200) and AIL (n=2,000) populations for which STR genotypes are available. Such analysis will facilitate correlation of predisposition (QTL) genes with those regulating key physiological events controlling body weight and fatness, will enable estimation of heritabilities and genetic correlations among multiple sub-phenotypes, and will lead to a better understanding of the overall genetic architecture of complex traits. Bridging the gap between predisposition and physiology will be required to overcome the substantial obstacles that have thus far rendered polygene discovery an elusive goal. We propose to combine the power of a large number of recombination events with detailed functional analysis in order to help achieve this goal. Full characterization of polygene identity and function in animal models will provide significant benefits towards comparative understanding of complex traits in agricultural animals and in humans.

Comparative QTL mapping in sheep and applications in cattle

Herman Raasdma

CRC for Innovative Dairy Products and REPROGEN, Faculty Veterinary Science, Sydney Univ, NSW 2006

Telomere maintenance in human cancer cells

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The organisation of human and other genomes into linear chromosomes has two major consequences. First, cells must be able to distinguish the chromosome ends, ie. telomeres, from DNA breaks. This is achieved by proteins that specifically bind to telomeric DNA and form a cap structure. The second consequence is that normal DNA replication in somatic cells causes progressive shortening of telomeres due to an intrinsic property of DNA replication. In cells of the germ-line, there is a complex multi-subunit enzyme, telomerase, that synthesises new telomere sequence to counteract telomere shortening. Some rapidly dividing normal somatic cells have low levels of telomerase activity; this seems to slow down, but does not completely prevent, telomere shortening. When a normal cell's telomeres become short, it permanently ceases dividing and becomes "senescent"; this may contribute to ageing of tissues. In addition, the normal replication-linked telomere shortening process acts as a major barrier to the development of cancer. Even if a cell has undergone pre-malignant genetic or epigenetic changes, the senescence barrier will prevent its progeny from dividing a sufficient number of times to form a clinically significant tumour. The great majority of tumours have activated a mechanism to avoid telomere shortening. In most cases, this is achieved through activation of telomerase, but tumours can also use at least one other mechanism referred to as Alternative Lengthening of Telomeres (ALT). The ability to prevent telomere shortening is one of the most striking and consistent known differences between cancer cells and normal somatic cells, and presents a target for the development of new anticancer drugs. This presentation will focus on recent findings regarding telomerase and ALT.

Phylogeography of southeast Queensland populations of the Wallum froglet, *Crinia tinnula.*

Juanita Renwick, Peter Mather.

Queensland University of Technology, Brisbane,.

The wallum froglet, *Crinia tinnula*, is a small ground dwelling frog that inhabits coastal wallum heathlands and associated Melaleuca swamps in southeast Queensland and northeastern New South Wales. Commonly referred to as an 'acid' frog because it is restricted to acidic waters of the wallum, this species is currently listed as Vulnerable due to recent fragmentation and loss of wallum habitat in mainland coastal areas.

This study addressed the phylogeographic history and population structure of wallum froglet populations in southeast Queensland using mitochondrial 12S rRNA and COI sequence data. Analysis of sequence data suggests that at least two distinct evolutionary lineages exist. Southeast Queensland populations divide into two monophyletic clades and a significant level of genetic divergence exists between these clades. A model for evolution of *C.tinnula* is presented, specifically in relation to recent historical changes in sea level and implications for current taxonomy are discussed.

The evolution of the mitochondrial DNA control region in the Adélie penguins of Antarctica

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The Adélie penguins (*Pygoscelis adeliae*) of Antarctica possess a mitochondrial DNA control region that is unusually long (1768 bp) and contains a repeat complex at the 3'-end. We sequenced the hypervariable region I of the mtDNA control region for 560 modern Adélie penguins, and revealed the presence of two distinct mitochondrial lineages that differed by an average of 8.3% (±1.1). One lineage was present in all populations around Antarctica (*A*) and the other was only recorded in the Ross Sea (*RS*), suggesting Adélie penguins were restricted to two ice-age refugia during the Pleistocene glacial cycles. Ancient DNA was extracted and sequenced from 96 sub-fossil Adélie penguin bones (^{14}C dates ranged from 310-6082 years before present) from 16 locations on the coast of the Ross Sea. The ancient DNA from these frozen bones was extraordinarily well preserved, due mainly to the dry and cold conditions experienced in Antarctica. Using both modern and dated ancient DNA sequences we estimated a rate of nucleotide evolution using a full-likelihood approach in a Bayesian framework. We estimated that the *A* and *RS* lineages shared a common ancestor approximately 75,000 years before present. The event that led to the lineage sorting and the present distribution of the Adélie penguin mitochondrial types occurred during the last glacial cycle.

The consequences of habitat fragmentation on rainforest trees (*Elaeocarpus*)

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In its original form, the 'Big Scrub' in Northern NSW was the largest extent of lowland subtropical rainforest in Australia. Unfortunately, extensive clearing for forestry and agriculture has reduced this highly significant ecological community to less than 1 % of its original distribution. Less than 40 small isolated fragments remain. Within these, flora diversity is still surprisingly high but little is known about the population-level consequences of such extreme environmental degradation.

The 'Gene Flow and Habitat Fragmentation' project aims at contrasting genetic diversity across a number of representative populations within fragmented and undisturbed sites in North Eastern NSW. Microsatellites were used to investigate and compare the consequences of fragmentation on one common and two rare *Elaeocarpus* species. Overall diversity and population dynamics were assessed using direct and indirect measures of gene flow across a large number of individuals and their progenies.

This study will provide insights on the conservation, management and regeneration of rainforest remnants and on the factors affecting gene flow across different ecological constraints.

Attractive traits and adaptive sex allocation in Zebra Finches revisited: what was all the hurley-burley about?

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Two decades ago, controversial laboratory studies on Zebra Finches by Burley [Burley, Science, 211,721-722 (1981); Burley, Evolution, 40, 1191-1206, (1986)] suggested that natural selection favours the production of offspring of the same sex as that of the most attractive parent. Despite numerous criticisms of Burley's studies, replication of her experiments have not been published, although there is some support for her differential parental attractiveness hypothesis from other species.

More recently, the development of a 'universal' avian molecular sex marker has enabled the investigation of adaptive sex allocation in a range of species under various selection pressures, many at the primary sex ratio stage (the sex ratio at conception). Some of these studies have provided empirical support for the hypothesis that natural selection will favour parental control of offspring sex ratios when fitness returns from investing in each sex are unequal.

We semi-replicated Burley's studies to test whether the differential parental attractiveness hypothesis was supported at the primary sex ratio stage in Zebra Finches. Parental attractiveness was manipulated by the application of coloured leg bands and sex ratios were determined by using a molecular sex marker on DNA extracted from early stage embryos. Statistical analysis using a generalized linear model did not indicate any significant effect of attractiveness on offspring sex ratios (17 pairs, 72 clutches, 287 embryos, $P = 0.71$); sex ratio bias was 48 % male when males were attractive and 50 % male when females were attractive. We conclude that Zebra Finches do not adjust offspring primary sex ratio in response to parental attractiveness.

Mapping the genes controlling resistance to turnip mosaic virus in *Brassica*

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Turnip mosaic virus (TuMV) is the second most economically important virus infecting field vegetable crops worldwide and is responsible for causing significant economic losses in *Brassica* crops each year. The *Brassica* A genome possesses a number of genes that confer resistance to different spectra of the twelve distinct pathotypes of TuMV. Genetic mapping has identified the genomic positions of three of these resistance genes; *TuRB01b*, a single dominant gene controlling strain specific resistance and *retr01* and *ConTR01* which together confer broad-spectrum resistance to TuMV on *Brassica rapa*. Genetic markers linked to these genes will allow gene pyramiding and the marker-assisted selection of durable resistance to TuMV in *Brassica*. The genetic dissection of resistance determinants in near-isogenic lines carrying defined resistance genes will facilitate the biological evaluation of different resistance mechanisms. In combination with the ability to construct hybrid virus genomes carrying defined segments of two or more TuMV isolates, this represents an excellent system for resolving the molecular biology of pathogenesis and host resistance for an economically important crop pathogen. High resolution mapping of these *Brassica* genes for resistance to TuMV is also bringing cloning of these genes within reach.

Candidate resistance genes mined from an EST database prove a rich source of markers for genes conferring resistance to major apple pests and diseases

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The HortResearch apple EST database contains approximately 22,200 non-redundant cDNA sequences derived from 50 cDNA libraries representing several cultivars and a range of tissues. ESTs mined from this database for homology to the major resistance gene classes have proved an excellent source of novel markers linked to previously identified resistance genes to apple scab, powdery mildew and wooly apple aphid (including *Vf*, Pl_2, Pl_{MIS} and Er_3). This direct, targeted approach has enabled us to rapidly identify novel closely linked markers and possible *R* gene candidates for a number of resistance genes, with some clusters of mapped ESTs from different *R* gene classes often extending over several centimorgans on one linkage group. After initially screening candidate ESTs across selected individuals representing subsets of our populations and testing these markers on enlarged populations to confirm linkage, closely linked markers are converted to PCR based markers (SCAR or SNP) to enable high-throughput population screens and immediate implementation in our apple resistance breeding programme. This method of identifying new novel closely linked markers is also being employed in the identification of candidate markers linked to other economically important traits in apple such as fruit quality and tree architecture.

Molecular phylogenetics of Southern Ocean cephalopods

Chester Sands[1] and Simon Jarnan[2].

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Example abstract. The phylogenetic relationships of seven species within the family Onychoteuthidae (Cephalopoda: Oegopsida) were examined using sequence data from two mitochondrial DNA regions. The cytochrome c oxidase subunit 1 region (CO I) appeared to be more informative than the 16S rDNA region used previously in other studies. Combining the two data sets provided less ambiguous phylogenetic relationships than either region used separately. Members of the genus *Moroteuthis* represented in this study appear to be closely related apart from *M. knipovitchi* which seems to be a more distant relation. *Kondakovia longimana*, the sole member of its genus, clusters within *Moroteuthis* and appears to be a closer relative than *M. knipovitchi*. A morphological variant *M. robsoni* was found to be a potentially distinct genetic species. Interestingly, the degree of relatedness between members of the genus *Moroteuthis* appears to correlate with the frontal zones of the Southern Ocean, giving some clues as to the evolutionary history of the group.

Immunoglobulin chain interactions

Kwame Sarfo

University of Ghana

Mcg and Weir are monomeric subunits of the human immunoglobulin chains. They combine to form a hybrid product that crystallizes as trygonal bipyramids in ammonium sulphate. The Mcg phenotype, lack of binding of bis(nitrophenyl)lysine, is dominant over the Weir phenotype, which is binding. Both proteins belong to the same genetic subclass. The difference in binding properties lies in the amino acid sequences of the proteins. In their immunoglobulin formation, the Mcg protein (light-chain analogue) and the Weir protein (heavy-chain analogue) combine. Since in the Mcg light chain dimer bis(dinotrophenyl)-lysine spans two relatively well separated subsites (A and B), one subsite needs to be blocked to allow binding. This is done by replacement of valine 48 and serine 91 in Mcg by the amino acids from equivalent Weir sites, leucine and methioninine. This replacement in effect blocks access to subsite B in Mcg's and thus the binding activity for bis(dinitrophenyl)lysine in the Mcg/Weir hybrid is restored.

Comparative Microbial Genomics

Neil Saunders

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The first complete genome sequence of a free-living micro-organism, that of *Haemophilus influenza*, was obtained in 1995. Since then the genomes of almost 100 more micro-organisms have been completely sequenced and upwards of 200 are in the process of being sequenced. The complete sequences obtained include representatives of all three domains of life, the Eukarya, Archaea and Eubacteria. Many of the bacterial genomes are those of medically significant pathogens, whilst those of the Archaea include organisms from extreme environments such as hot submarine vents.

Developments in DNA sequencing technology and computational methods for genome assembly mean that a complete microbial genome sequence can be obtained in a short time. However, the development of methods for analysis of these data lags some way behind the sequencing effort. Although there are many web-based services for genomic analysis, a genuine bioinformatics effort requires novel, project-specific tools based around free, open-source programming languages and software. This need is fulfilled in part by the Bio* projects (such as BioPerl, BioPython and BioJava). Another barrier to progress is the many, varied and often incompatible data formats used by different sequencing projects and sequence databases.

This talk will cover 3 main areas: (1) a general survey of what we have learned from the microbial genomes sequenced to date, (2) the use of open source bioinformatics software in microbial genome projects and (3) the comparative analysis of microbial genomes with special reference to the methanogenic Archaea.

Conservation genetics of rare Graptophyllum species (Acanthaceae) from Queensland

Alison Shapcott

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There are four species of *Graptophyllum* in Queensland; *G. spinigerum* (widespread), *G. excellsum* (rare), *G. ilicifolium* (vulnerable) and *G. reticulatum* (endangered) covering a range of distributions from widespread to restricted and endangered. All of these species have been grown in cultivation, *G. excellsum* being one of the earliest native plants cultivated in Queensland being a shrub with spectacular scarlet flowers. All species grow in localised scattered populations but differing is geographic extent with *G. reticulatum* being known from only three locations only one within a National park , and *G. ilicifolium* only known from 3 locations in the Mackay region. They are all rainforest species but span an ecological gradient from *G. reticulatum* and *G. ilicifolium* occurring in CNVF to *G. excellsum* in vine thickets on limestone outcrops such as near Mt Etna. This project was focussed around understanding the populations genetics of the endangered species *G. reticulatum* found on the Sunshine Coast and comparing the levels of genetic diversity and inbreeding with its congeners which varied in abundance and distribution. Results indicate all species have quite high levels of genetic diversity (He, A, P) the endangered species *G. reticulatum* having the lowest diversity but the highest diversity was found in the vulnerable *G. ilicifolium*. All species were significantly effectively inbred but the two more restricted species *G. ilicifolium* and *G. reticulatum* had the highest allelic fixation. The genetic differentiation among populations F_{ST} was highest in the most geographically restricted *G. reticulatum* but lowest in vulnerable and locally restricted *G. ilicifolium* thus population differentiation was not related to geographic distribution of species populations. The results may have implications for conservation management especially the urban population of *G.reticulatum* on Buderim Mountain. The results will also have implications for the retail nursery industry and local species recovery programs.

Use of molecular data to investigate species diversity in the centipede genus *Henicops*

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The lithobiomorph genus *Henicops* represents one of the most common centipede groups found in wet forest habitats in eastern Australia, southwest Western Australia, and New Zealand. A recent combined analysis of data from morphology and multiple molecular markers supported monophyly of *Henicops* and identified appropriate outgroups for investigating the internal phylogeny of *Henicops*. However, within the genus, separate morphological analysis inferred different relationships among species compared to results from molecular and combined analyses. In particular, it is unclear if a morphologically defined group of samples from northern NSW and southern Queensland represents a single new species or multiple taxa. In this study, 24 samples from seven populations throughout the range of this morphological group, together with samples representing the other species of *Henicops* from Australia and New Zealand, were analysed using sequence data from 16S LSU mtDNA, protein-coding COI mtDNA, and 28S rDNA (D1 expansion region). Parsimony and likelihood analyses were used to investigate species boundaries and interrelationships.

Sequenced- based genotyping at major histocompatibility complex (SLA) loci in Westran pigs

Denbigh Simond[1], Jun-Heon Lee[1], Stacy Walters[2], Tina Patel[2], Richard Allen[2], Wayne Hawthorne[2], Phil O'Connell[2], Jeremy Chapman[2], Douglas Smith[3] and Chris Moran[1]

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For practical, ethical, financial and safety reasons, pigs are being evaluated and modified as a source of organs and tissues for xenotransplantation. Since SLA molecules play an important role in xenoreactive responses, eight loci within the MHC were examined in inbred Westran (Westmead Hospital Transplantation) pigs, produced for transplantation research and as possible xenotransplantation donors. Westran pigs show very low microsatellite heterozygosity, a reflection not only of their recent deliberate full sib inbreeding, but also their derivation from a feral stock from Kangaroo Island, South Australia, established by the release of a single male and female in 1803. Four class 1 loci (SLA 1, SLA 6, SLA 7, SLA 14) and four class 2 loci (DQA, DQB, DRA and DRB) were assessed for variation, with only the SLA 1 locus appearing to be segregating in the most inbred generation. A sequence based genotype test was designed to detect a dinucleotide polymorphism identifying the two alleles. Animals from a number of generations have been genotyped to identify the segregating gene. The SLA genotypes are being compared with sequence based genotypes from other breeds and populations of pigs. They may be beneficial in designing modulation of immune responses in xenotransplantation.

Transposable Element in the Lungfish Genome

Nick Sirijovski, Jean Joss

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Lungfish genomes are the largest of any vertebrate genomes. The Australian lungfish (*Neoceratodus forsteri*) has a genome size of 105 pg DNA/somatic cell nucleus, which compares with the human genome of 4.5 pg DNA/nucleus. The current surge of genome research is concentrating on very small to medium-sized genomes. Comparison of these with similar studies on very large genomes, might be expected to produce considerable insights into genome evolution. The lungfish genome appears to have increased over the last 200 my. There is good evidence from fossil cell size that Devonian lungfish had much smaller genomes than the living genera. Of these, the more highly derived lungfish from Africa and South America have genomes approximately twice that of *Neoceratodus*, which strongly suggests that large genome size has been positively selected for in this lineage.

As part of a study, which seeks to understand the evolution of large genomes, this presentation will describe the identification of a highly repeated transposable element in the genome of *Neoceratodus.* Following restriction enzyme digests of genomic DNA, a single band was distinguished from *Eco*R1, which when cloned and sequenced was found to be a CR1-like non-LTR retrotransposon (LINE), similar to that found in chicken, reptiles and most recently in *Fugu*. We are calling this element NfCR1. Characterisation of NfCR1 will be presented in terms of sequence analysis, evolutionary analysis and estimation of its contribution to the genome size, as estimated by real-time PCR assay. NfCR1 appears to contribute a significant percentage to the *Neoceratodus* genome, as CR1-like elements do in *Fugu* and a closely related LINE, L1 type does in human (17%).

A new transcription factor gene of *Arabidopsis thaliana* involved in trichome and seed coat development

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In *Arabidopsis*, mutants of the *TRANSPARENT TESTA GLABRA2* (*TTG2*) gene show reduced numbers of leaf hairs that are unbranched. Also seed coat mucilage is absent, and tannins do not accumulate in the endothelium.

The *TTG2* gene was tagged by the endogenous transposon *Tag1* and shown to encode a member of the plant-specific transcription factor family WRKY. In trichome development, the TTG2 protein apparently acts downstream of TTG1 (an accessory protein) and GLABROUS1 (a MYB transcription factor), but it shares functions with GLABRA2 (an HD-Zip transcription factor). In seed coat development, TTG2 requires TTG1 function for tannin production. TTG2 is also expressed strongly in the specialised epidermal cells of roots in which root hair development is suppressed, but these cells are unaffected in *ttg2* mutants, and this expression is not dependent upon TTG1 or GL2 function. Another gene may share the same function as *TTG2* in root epidermal cell development.

The expression of all WRKY genes examined to date is activated by pathogen infection, and by wounding and during senescence. However *TTG2* expression is not stimulated by salicylic acid, wounding or aging. This suggests that its function has diverged markedly from other members of this large family, and that it now regulates the expression of genes involved in cell differentiation. Interestingly, the three cell types involved are all originally derived from the epidermis.

The *cryptochrome* gene in two species of Tephritid fruit fly, *Bactrocera tryoni* and *Bactrocera neohumeralis*

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School of Biological Sciences, University of Sydney, NSW 2006

Bactrocera tryoni and *Bactrocera neohumeralis*, two closely related species of Australian native fruit fly lay eggs in a wide range of nature and cultivated fruits. The destruction of commercial fruit crops caused by the two species of fruit fly in the eastern part of Australia is a serious problem. Although the two species are almost identical in their morphology and show very little genetic variation, *B. tryoni* is a much more serious pest than *B. neohumeralis*, as *B. tryoni* has a much wider geographical distribution than *B. neohumeralis.* This indicates the adaptability of *B. tryoni* to a wider geographical range than *B. neohumeralis*. The two species are differentiated by the colour of the humeral callus and the distinctive difference in their mating time- *B. tyoni* mates at dusk while *B. neohumeralis* mates at noon. In order to identify the genetic basis for the mating time difference, we are investigating the *cryptochrome (cry) gene*, one of the strong candidate genes from the circadian clock. *cry* encodes a blue light receptor and is thought to entrain the circadian clock. Sequencing of the *cry* gene in both species is close to completion and the sequence comparison has been made between the two species for any possible difference. The expression and regulation of the *cry* gene has also been studied using semi-quantitative RT-PCR from flies sampled over a 24 hour period, and found that the *cry* mRNA is transcribed at constant levels. This finding is somewhat different from findings from *Drosophila*, which shows an oscillating pattern of *cry* mRNA in a circadian manner. Further investigations are being carried out on the expression level of the *cry* mRNA using more sensitive detection methods such as REAL-TIME PCR.

The floating genome: The role of mobile gene cassettes in bacterial evolution

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It has long been known that horizontal gene transfer (HGT) plays an important role in bacterial evolution. It is only with the advent of the genomics era however that the true extent of HGT has been fully appreciated in that it has been revealed that a typical bacterium acquires 15 - 20% of its genome from elsewhere. For cells to incorporate foreign DNA into their genome they must, firstly, acquire it by conjugation, transduction or transformation. Secondly, they must incorporate it into a resident replicon. This can occur by either transposition or site-specific recombination. To what extent do these processes interact to produce the observed level of foreign DNA in bacteria? Gene cassettes are the smallest mobile elements known and consist only of a single gene and a recombination site. These cassettes can be mobilised by a site-specific recombinase encoded by another genetic element known as an integron. Integrons and gene cassettes are well known in the context of hospital acquired infections as many different antibiotic resistance genes are contained within gene cassettes. By the assembly of several cassettes into an integron, pathogenic bacteria can readily acquire a multi-drug resistance phenotype. Using, a novel PCR strategy, we have recently shown that gene cassettes are both ubiquitous and abundant in the many diverse bacterial environments tested. Analysis of these cassettes revealed that the predicted products of the associated genes are all novel and the vast majority cannot even be assigned to known protein families. These findings, together with other analysis showing that some cells possess integrons with hundreds of gene cassettes, demonstrates that a vast untapped genetic resource is contained within mobile cassettes. This extensive floating population of genes has important ramifications for understanding bacterial evolution. It also suggests that the genomic sequencing of defined cell lines is unlikely to recover anything but a very small fraction of this bacterial novelty.

Family group structure in wild populations of Cunningham's Skink (*Egernia cunninghami*): revealed by genetic determination of parentage and site fidelity.

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There are very few studies on lizards showing long-term aggregations of parents and offspring, or the persistence of breeding pairs beyond a single breeding season. This study utilises genetic and capture-mark-recapture techniques to investigate aspects of the mating system and individual movement of the rock dwelling Australian lizard *Egernia cunninghami.* Sampling was carried out at two study sites on the Central Tablelands of New South Wales. To investigate anthropogenic effects on breeding pair fidelity, and philopatry of parents and offspring, sampling at each site was conducted in a deforested and naturally vegetated habitat. Analysis of the mating system using 10 microsatellite loci, and capture-mark-recapture, show high levels of site fidelity by parents and their offspring, in both deforested and naturally vegetated habitats. Parentage assignment reveals low levels of multiple breeding partners within breeding seasons and pair fidelity across two or more breeding seasons. No habitat differences were evident in the level of multiple breeding partners both within and across seasons. High levels of site fidelity, and breeding pairs associated beyond one breeding season, result in aggregations of parents and offspring over several age cohorts and thus a family-like group structure.

Comparative phylogeography of saproxylic invertebrates

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To conserve evolutionary processes within taxa as well as local co-evolutionary associations among taxa, habitat reservation needs to take account of natural genetic-geographic patterns. These patterns are likely to vary greatly among taxa. Most research to date has been focussed on vertebrate species and rarely multi-species assemblages. While vertebrates tend to have at least moderate dispersal and geneflow on a landscape scale, hence rather limited potential for local adaptation and chance differentiation, there are good reasons to expect many small invertebrates to be strongly subdivided. Strong local genetic structure will tend to promote both local adaptation and (currently) non-adaptive differentiation. It is a major challenge in conservation biology to develop efficient tools and model systems to understand genetic and evolutionary spatial patterns in less mobile, habitat-specific organisms. Further, it is necessary to investigate the relative importance of local adaptation (which may be replaceable if evolutionary processes are maintained) and historical differentiation (which will be more irreplaceable). I present an overview of collaborative research into an exciting model system – the saproxylic (rotting log) habitat in a large block or forest centred on Tallaganda State Forest NSW. In this system, velvet worms (onychophora) showed extreme and unexpected levels of local genetic subdivision. These animals offer a fine-scale phylogeographic benchmark against which codistributed saproxylic animals can be compared. I outline our current and future investigations.

IS wide shut: copy number control of IS*6110* in *Mycobacterium tuberculosis*

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The insertion sequence IS*6110* is a source of genetic variability among *Mycobacterium tuberculosis* isolates, making it a suitable marker for population studies. In order to use a genetic marker, it is important to understand its underlying biology. For example, we must know the rate at which the marker changes to draw conclusions about clusters of DNA fingerprints. From the genetic and evolutionary points of view, it is evident that the copy number of transposable elements is usually controlled (regulated). While some mechanisms of regulation of IS elements have been characterised under laboratory conditions it would be useful to know whether regulation can be detected in natural settings. In this study, we statistically detect regulation, and quantify its strength. The quantification is direct and in vivo in that we use longitudinal data of naturally occurring strains of a pathogen. We propose several alternative quantitative models of IS transposition. These include: the independent action of separate copies, perfect homeostasis, copy number-dependent repression, and strain heterogeneity. Using the maximum likelihood framework we estimate not only the rate of change of IS*6110*, but also the parameters involved in control. The models that include some degree of control fit the data significantly better than models without control. The next stage of the project involves the use of the Akaike Information Criterion (AIC) as the basis for comparing many alternative models of regulation. Preliminary results show that heterogeneity among strains in their ability to regulate IS*6110* may be the best explanation of the observed pattern of changes.

A Genomic Screen for Oxidative Stress Tolerance in *Saccharomyces cerevisiae*

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All cells growing in an aerobic environment encounter potentially pernicious Reactive Oxygen Species (ROS). The main source of ROS is leakage from the electron transport chain, and these ROS are able to damage many cellular constituents, including DNA, proteins, and lipids. Cells exposed to this environmental insult have evolved defence mechanisms to protect against harmful oxidation reactions. These defence mechanisms may be quite specific, such as detoxification of an oxygen radical, or can be general, such as adjustment of cellular metabolism. *Saccharomyces cerevisiae* is a good eukaryotic model organism for the study of oxidative stress defence, since there is a comprehensive set of isogenic mutants available for study. Identification of sensitive mutants will facilitate in developing an understanding of the cellular responses to oxidative stress, indicating the most important cellular functions required for normal tolerance to oxidative injury. A high throughput method was designed in which growth of control and mutant strains were compared on agar plates containing various oxidants. Preliminary results indicate that different cellular functions are required for tolerance to distinct types of oxidative damage. This could be due to various oxidants causing different kinds of cellular damage, which in turn may activate defences of discrete specificities. These results will contribute to the elucidation of a global network of stress and oxidative stress signalling and defence pathways.

Sharing of nuclear and cpDNA variation across eucalypt species

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The biogeographic pattern of cpDNA variation in the Tasmanian *Eucalyptus* species is consistent with reticulate evolution, involving at least 12 Tasmanian species from subgenus *Symphyomyrtus*. Intraspecific cpDNA polymorphism in 14 out of 17 species is coupled with extensive sharing of identical haplotypes, some of which are unique to Tasmania, across populations of different species in the same geographic area. The cpDNA results suggest that eucalypt evolution should be reassessed to allow for the effects of interspecific hybridisation and introgression. This hypothesis is now supported by a study of the pattern of variability in cinnamoyl CoA reductase (CCR), a nuclear gene encoding the first dedicated enzyme in the lignin biosynthesis pathway. Again, extensive sharing of phylogenetically closely related alleles supports the hypothesis that extensive hybridisation has occured between *E. globulus*, a widespread species, and *E. cordata*, a Tasmanian endemic. This is a key result, since it is the first molecular evidence for introgression having a major influence on nuclear gene diversity in a eucalypt species. Indeed, *E. globulus* may have assimilated genes from several co-occurring species, which may have increased its adaptive range, consistent with the early 'compilospecies' concept.

Homozygous and heterozygous fitness effects of clonally transmitted genomes in waterfrogs

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The hemiclonal waterfrog *Rana esculenta* is a natural hybrid between *R. ridibunda* and *R. lessonae*. It eliminates the *lessonae* genome from the germline and clonally transmits the *ridibunda* genome (hybridogenesis). Lacking recombination, *ridibunda* genomes in hybrids are prone to accumulate and fix spontaneous deleterious mutations through Muller's ratchet. Fixed recessive deleterious mutations may explain why *R. ridibunda* offspring from matings between hybrids are typically inviable and die at an early larval stage. From this hypothesis results a straightforward prediction: Matings between different hemiclones, i.e. between *R. esculenta* possessing different *ridibunda* genomes of independent origin, should produce viable *R. ridibunda* offspring, because it is unlikely that different clonal lineages fix the same mutations. I tested this prediction by comparing survival and larval performance of tadpoles from within- and between-population crossings using *R. esculenta* from three widely separated populations in Switzerland. Results were in general agreement with the hypothesis I tested: within-population crosses were mostly inviable, between-population crosses were mostly viable. Some exceptions to this general pattern revealed that one hemiclone occurred in two of the populations and that several coexisting hemiclones in one population may not be evolutionarily independent. When backcrossed with the parental species *R. ridibunda*, hybrids from all three populations produced viable offspring with a larval performance comparable to that of normal, sexually produced *R. ridibunda* tadpoles, suggesting that in the heterozygous state, the deleterious mutations in clonal genomes do not reduce tadpole fitness. However, environmental stress can enhance the negative effects of mutation accumulation. A second experiment therefore compared the fitness of tadpoles possessing either one clonal and one sexual or two sexual *ridibunda* genomes under benign and stressful conditions. Again, there was no indication that tadpoles with a clonal genome were inferior, indicating that although there is strong evidence for the fixation of deleterious mutations, their heterozygous effects are weak and may not impair the evolutionary perspective of the hybrid taxon *R. esculenta*.

dsRNA-mediated genetic interference studies of sister-chromatid cohesion in *Drosophila*

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'Cohesin' is an evolutionarily conserved multi-protein complex thought to be a primary effector of sister cohesion in all eukaryotes. In yeast, cohesin is loaded onto chromosomes in S-phase where it acts to maintain cohesion until the metaphase-anaphase transition. Sister-chromatid separation is then triggered by the site-specific cleavage of the Scc1p/Rad21p cohesin subunit. In higher species including *Drosophila*, the bulk of nuclear cohesin dissociates from chromosomes in prophase, leaving only a minor pool of centromere-associated cohesin to maintain sister-chromatid cohesion until anaphase. How the various cohesin subunits and their regulators orchestrate these events has yet to be fully elucidated.

To further investigate the role of the *rad21* cohesin subunit in mediating sister-chromatid cohesion in *Drosophila*, we have examined the consequences of reduction of DRAD21 function by RNA interference in both S2 cells in culture and preblastoderm embryos. In S2 cells, *Drad21* RNAi was unexpectedly shown to cause mitotic arrest with sister-chromatids still co-joined. Examination INCENP staining indicates that these cells arrest prior to the onset of anaphase. In comparison, time lapse confocal studies of *Drad21i* embryos show a range of mitotic phenotypes, including delayed chromosome condensation, unequal chromosome segregation, metaphase arrest, nuclear collisions, micronuclei formation as well as mitotic failure. Expression of DRAD21 truncations in embryos leads to a similar range of phenotypes, and expression in developing eye tissues causes dominant developmental abnormalities. We are currently investigating these phenotypes in more detail. Our data suggests that impairment of *Drad21* function prevents the timely resolution of sister-chromatids in prophase, as chromosomes condense, which prevents or impairs their correct segregation in anaphase. When cohesin dysregulation occurs in a developmental context, genomic instability, aberrant gene expression and increased levels of cell death ensue.

Genome-wide mutagenesis screen for non-random X-inactivation phenotypes in the mouse

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Genome-wide mutagenesis of pre-meiotic spermatogenic stem cells of male mice induced by the chemical mutagen N-ethyl-N-nitrosourea (ENU) is an effective method of generating novel mutant phenotypes.

We have designed a sensitised screen to detect mutations that affect the pattern of X chromosome inactivation using the C57BL/6J strain of mice. ENU treated founder male mice were mated to females to produce F_1 progeny. F_1 female mice, which have inherited the mutation-bearing X-chromosome and autosomes from the founder male, were crossed to untreated males to produce F_2 progeny. F_2 males were then screened for potential mutations that alter the random pattern of X-inactivation in the female F_3 embryos. F_2 males were crossed with female mice that are homozygous for an X-linked *LacZ* transgene. E8.5-9.0 embryos were examined macroscopically and histologically for β-galactosidase expression to reveal the pattern of X-inactivation. The sex of the embryo was determined by PCR analysis for the presence of *Zfy* sequence in yolk sac samples.

Altogether, 97 male and 102 female F_3 embryos derived from 15 F_2 males have been analysed. From 7 of the 15 F_2 males (descended from 4 F_1 females and 2 founder males), there were 16 female embryos displaying an extremely skewed pattern of X-inactivation. In addition, at least 8 female littermates of these 16 highly skewed embryos also showed a non-random but less skewed X-inactivation pattern. In both types of female embryos, the paternally derived allele was preferentially inactivated. Distinctive patterns of skewed X-chromosome activity were derived from two different founders. This raises the possibility that two different mutations may have arisen in the founders. We are currently investigating the heritability and mode of inheritance of the putative mutations, and the degree of skewness by scoring X-linked transgenic activity at the cellular level. A likely outcome of this mutagenesis screening is the identification of genetic loci that regulate the process of X-chromosome inactivation.

Adaptive radiation and dispersal in the seagrass genus *Halophila*

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The marine angiosperms ('seagrasses') represent a series of three or four adaptive radiations to a submerged marine habit from freshwater and saline origins. The majority of seagrass genera show a bi-ocean spilt in their species distributions: tropical genera such as *Halodule*, *Syringodium*, and *Thalassia* occur in the Caribbean and the Indo-Pacific; *Posidonia* occurs in the Mediterranean and the Australian Indo-Pacific. One genus, *Halophila* has a global distribution. Based on historical biogeographic analysis these radiations may have occurred either prior to the breakup of the tethys sea during the late Cretaceous or following the closure of the Panamanian isthmus in the Eocene.

In this study the relationships among members of the seagrass genus *Halophila* (Hydrocharitaceae) were investigated using phylogenetic analysis of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA and chloroplast *rpL16* sequences. In addition, population genetic data using AFLP analysis and microsatellite data was generated to better understand the microevolutionary processes in operation.

Evolutionary trends in *Halophila* appear to be toward a more reduced simple phyllotaxy. In addition, long distance 'jump' dispersal between major ocean systems may have occurred at least in the globally distributed *H. decipiens*. Results of ITS analyses also indicate that the widespread pacific species *H. ovalis* is paraphyletic and may contain cryptic species. Likewise, the geographically restricted species *H. hawaiiana* and *H. johnsonii* could not be distinguished from *H. ovalis* with these data and warrant further investigation.

Analysis of the population genetic variation of *Halophila johnsonii*, a species restricted to the Florida coast, indicates a high level of genetic similarity and perhaps is an introduction to the region. It's status as a 'threatened species' may need revising once it is established if this species is a relict of more widespread occurrence or a 'recent' introduction.

Investigating the evolution of the mistletoe family Loranthaceae using a molecular phylogenetic approach

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The Loranthaceae is a large family of mostly woody perennials which occur as aerial parasites known as mistletoes, and three putatively primitive species which are terrestrial root parasites. Believed to be of ancient origin, well established before the rifting of the Gondwana continents, this family is widely distributed throughout the tropics but with many representatives in southern temperate regions.

Mistletoes are a prominent feature of the Australian flora which is recognised as being comprised of both Gondwanan and intrusive elements. There are Gondwanan groups of Australian Loranthaceae, some relictual others derived, believed to have been evolving in Australia for a long period and is rich in endemics. In addition there exists an intrusive element that is thought to have arrived during the mid-Miocene to early Pliocene when there were suitable continuous land surfaces to the north with Malesia. Consequently, the derived Australian lineages, which are predominantly tropical or arid radiations, may be derived from these two distinct origins. Other Australian genera may be easily recognised as ancestral, possibly remnants of a former paleoaustral flora, for example *Atkinsonia* and *Nuytsia* which exhibit plesiomorphic character states including, importantly, the terrestrial habit.

Here evolutionary and biogeographic trends among the Australian members of the Loranthaceae are examined using inferred phylogenetic relationships generated with 18S rDNA and *trn*L cpDNA sequence data. There is support for more than one lineage among the Australian mistletoes, one an apparently derived group including *Dendrophthoe* and a paraphyletic *Amyema*. The putatively primitive terrestrial *Atkinsonia* and *Nuytsia* appear to be allied with the only other terrestrial mistletoe *Gaiadendron* from South and Central America. The Australasian/Malesian 'Amylothecae' occur in a poorly resolved clade with these terrestrial taxa and other American and New Zealand mistletoes. The molecular phylogenetic approach is resolving the complex evolutionary relationships in this important family which has been used to model biogeographic trends in the evolution of the Australian flora.

The use of molecular techniques for conservation of endangered species

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Molecular genetic techniques are increasingly being used to address significant conservation questions. I discuss how specific techniques are being used to help with endangered species management in the field and captivity. The questions addressed by molecular techniques range from forensic identification of samples to issues of phylogenetic distinction and population differentiation. I discuss how specific molecular approaches are appropriate at different levels of evolutionary divergence and demonstrate with examples how molecular techniques can address specific problems in species management. I conclude with a perspective on future problems in conservation and the need for development of new genetic approaches to address these problems.

Metastable Epialleles in Mammals

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It is well recognised that there is a surprising degree of phenotypic variation among genetically identical individuals even when the environmental influences, in the strict sense of the word, are identical. Genetic textbooks acknowledge this fact and use different terms such as "intangible variation" or "developmental noise" to describe it. We believe that this intangible variation results from the stochastic establishment of epigenetic modifications to the DNA nucleotide sequence. These modifications, which may involve cytosine methylation and chromatin remodelling, result in alterations in gene expression which, in turn, affects the phenotype of the organism. Recent evidence, from our work and that of others in mice, suggests that these epigenetic modifications, which in the past were thought to be cleared and reset on passage through the germline, may sometimes be inherited to the next generation. This is termed epigenetic inheritance, and while this process has been well recognised in plants, the recent findings in mice force us to consider the implications of this type of inheritance in mammals. At this stage we do not know how extensive this phenomenon is in humans but it may well turn out to be the explanation for some diseases which appear to be sporadic or show only weak genetic linkage.

A Comparative Analysis of Insect Genomes: Contrasting the Sheep Blowfly with the Vinegar Fly

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Chromosome number is variable within the genus *Drosophila*, however six linkage elements remain conserved. In *Drosophila* species, each linkage element is present as either an entire chromosome or as a chromosome arm. A number of markers, consisting principally of morphological markers, mapped in *Lucilia cuprina*, *Musca domestica* and *Ceratitis capitata* suggest the six linkage elements conserved in *Drosophila* remain conserved among other members of the higher Dipteran genera (Weller and Foster 1993). A more detailed map of *L.cuprina* was generated using molecular markers. The molecular map allows comparisons between *L.cuprina* and *D.melanogaster*, hence investigations into the extent of synteny conservation between *L.cuprina* and *D.melanogaster*. Molecular markers were mapped within *L.cuprina* chromosome 3 (corresponding to *D.melanogaster* chromosome X) and *L.cuprina* chromosome 4 (corresponding to *D.melanogaster* chromosome 3R). Our results were consistent with synteny between *L.cuprina* and *D.melanogaster* remaining conserved. Indeed, we have not found a single exception to the synteny rule. However, within a linkage element gene order has not been conserved at all in the100 million years of evolution from the common ancestor that gave rise to *L.cuprina* and *D.melanogaster*.

Reference: Weller, G.L. and Foster, G.G., 1993, Genetic maps of the sheep blowfly *Lucilia cuprina*: linkage group correlations with other dipteran genera, Genome 36:495-506

The Origin of the Australian Dingo

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The precise ancestry, place of origin and time of arrival in Australia of the dingo have not been determined, nor whether, on its arrival, it was a domesticated or a truly wild dog. We obtained a detailed picture from the study of mitochondrial (mt) DNA.

In a sparse archaeological record, the earliest substantiated finds of dingoes are from 3500 yr ago. Finds are absent in Tasmania which was separated from Australia by the rise of the sea level ~12,000 yr ago. Archaeological data therefore indicate the arrival of dingoes between 3,500 and 12,000 yr ago. In skeletal morphology dingoes resemble pariah dogs of south Asia.

We studied mtDNA D-loop in 38 dingoes from all states of Australia and in 389 dogs from all continents and 27 wolves. The sequence variation among dingoes was very restricted; a minimum-spanning network shows one ancestral sequence type (d18) from which 12 other types differ by just one substitution or one or two indels. Domestic dogs originate from several maternal wolf lines, and the dingo sequences fall into the main cluster of dog sequences containing ~70% of domestic dog mtDNA types

From these results we conclude that the dingo originated from a population of East Asian dogs. Type d18 was one of several mtDNA types brought into Island Southeast Asia, but only d18 reached Australia. The founding of the dingo population was probably the last trickle of domestic dogs through a series of bottlenecks in the Southeast Asian archipelago and may even have been a single chance event. The dingo population has then remained isolated from other dog populations.

Assuming that d18 was the only founder type, the time for the introduction of dingoes to Australia is estimated to 5,800 yr BP, using the mean distance (substitutions) of dingo sequences to d18 (0.263, SE=0.072) and the mutation rate of the analyzed region (7.4% per Myr). This date agrees reasonably with the archaeological record of the region and suggests that the dingoes arrived in connection with the expansion into Island Southeast Asia of the Austronesian culture. After more than 3,000 years of isolation the dingoes represent a unique isolate of early undifferentiated dogs.

Bioinformatics approaches to candidate gene selection in familial dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is a heart muscle disease that is a major cause of morbidity and mortality in our society. DCM may result from a diverse variety of factors that impair cardiomyocyte function, including coronary artery disease, viral infections and systemic diseases. DCM may also occur as a primary muscle disorder. Recent molecular genetic studies suggest that at least one third of cases of primary DCM may result from an inherited gene defect. Familial DCM may occur as an autosomal dominant, autosomal recessive or X-linked disorder, with autosomal dominant inheritance most common. Linkage studies performed to date in families with autosomal dominant DCM have shown that this disorder is genetically heterogeneous. Although multiple chromosomal loci have been identified, relatively few disease-causing genes have been found. In our laboratory, we have an ongoing program to identify new disease genes in familial DCM using linkage analysis and candidate gene approaches. The traditional method of identifying candidate genes in chromosomal loci defined by linkage studies has been positional cloning. This approach is time-consuming, labour-intensive and expensive. It has been widely anticipated that the recent release of sequence data from the Human Genome Project would greatly facilitate the process of candidate gene screening in chromosomal disease intervals. We have used computational analyses to evaluate genes in two chromosomal intervals, 6q23 and 10q23, that have been associated with the autosomal dominant DCM phenotype. To determine the reliability and accuracy of published sequence data, we have compared the Celera database with the public databases (Ensembl and Entrez) in these two chromosomal loci. Surprisingly, we found several significant problems in all of the databases, including incorrect annotations, discordance of marker positions, lack of sequence coverage resulting in missing exons in genes, and assembly errors (eg a known gene with the 5′ and 3′ ends separated by 70MB). These problems highlight the incompleteness of the available human genome databases and indicate that current annotations should be interpreted with caution.

Evolutionary Relationships and Intergeneric Variation of Branching Pattern within the Brown Algal Order Sporochnales

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Members of the brown algal order Sporochnales are an aesthetically interesting group of plants and display a spectacular array of habits and iridescent thalli, the latter due to the distinctive ordinal feature of trichothallic tufts. The temperate coastal waters surrounding the Australian continent host nearly the entire compliment of 10 genera and 24 species. Initial surveys based on a comparison of growth habit within and between the genera highlight significant variation in overall branching pattern. A preliminary morphometric analysis of species within the genus *Sporochnus* suggests that there is room for much synonymy and that some of the other genera require further critical comparison. We are combining a molecular approach with studies of morphological variation to help resolve the evolutionary relationships of the Sporochnales to better understand the intra and inter ordinal phylogeny. Specifically, we plan to reconstruct phylogenies based on the rbcL, rubisco spacer regions, and 5'end of the rbcS region of cdDNA to determine whether traditional generic concepts based on morphology are congruent or challenged by molecular results.

Maintenance of fitness in small mate-limited populations of the grassland herb *Rutidosis leptorrhynchoides* suggests negligible effects of biparental inbreeding

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Genetic influences on the viability of plant populations may occur in a variety of ways including: effects of genetic erosion on mate availability in self-incompatible species; loss of adaptive potential; increased hybridisation with disturbance and; elevated inbreeding. *Rutidosis leptorrhynchoides* (Asteraceae) is a self-incompatible perennial herb that occurs in remnant grasslands in southeastern Australia. Previous research has shown that small populations (<200 plants) exhibit significant reductions in seed set owing to mate limitation due to low *S* allele richness. However, crossing studies indicate that mating among full and half-sibs is still possible (though at reduced frequency) because of dominance relationships among *S* alleles, and studies of seed dispersal and spatial genetic structure show that substantial family structure builds up within populations. The combination of these two factors suggest that, in addition to the observed effects of *S* allele erosion on seed set, biparental inbreeding may reduce seedling fitness in small populations. To examine this, seeds from up to ten open-pollinated families from populations ranging in size from 5 to >100 000 reproductive plants were germinated and grown in pots for six months in a common environment. No significant differences were found in germination, survivorship or seedling growth as measured by biomass. These results indicate that primary genetic effects on population viability in *R. leptorrhynchoides* are likely to be through effects of *S* allele limitation rather than through subsequent effects of biparental inbreeding on progeny fitness. This result contrasts with data from the self-compatible grassland herb *Swainsona recta*. In that species, seed set is relatively unaffected by population size, however elevated inbreeding in small populations is correlated with reductions in several components of seedling fitness. This comparison highlights the importance of breeding system in determining the way in which genetic processes influence demography and, ultimately, population viability.

Modelling Effects of Self-incompatibility on Plant Population Viability

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Theoretically, genetically controlled self-incompatibility systems may impose significant demographic constraints on small plant populations through loss of *S* allele richness and reduced mate availability. Such constraints represent one of the few direct links between genetic diversity and population viability that are of immediate importance for plant conservation. We use a spatially explicit individual-based simulation approach to investigate the demographic and genetic consequences of different self-incompatibility systems for plants that also vary in their reproductive capacity and lifespans. The results support the idea that, in the absence of inbreeding effects, populations of self-incompatible species will often be smaller and less viable than self-compatible species, particularly for shorter-lived organisms (high death rates) or where potential fecundity (ovule production) is low. While possessing some form of self-incompatibility reduces population size and persistence for a broad range of conditions, our results show that the actual number of *S* alleles is important for a more limited set of life-histories. In these situations increasing population viability through the addition of new *S* alleles may be the most effective approach to conservation. Comparison of model results to empirical data on disassortative mating provides a method for estimating *S* allele numbers in wild populations and assessing long-term effects of current mate limitation.

The First Comprehensive Genetic Linkage Map of a Marsupial – the Tammar Wallaby (*Macropus eugenii*)

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The construction of the tammar wallaby (*Macropus eugenii*) genetic linkage map carried out in this study is the first near complete genome map for any Australian marsupial which incorporates both functional and anonymous genetic markers. The impetus for the development of this map has primarily been to develop a system for comparative and quantitative trait mapping. This study used a total of 353 informative meioses and 64 genetic markers to construct a framework genetic linkage map for the tammar wallaby. Nearly all markers (93.8%) formed a significant linkage (LOD > 3.0) with at least one other marker indicating that the majority of the genome had been mapped. In fact, when compared with chiasmata data more than 70% (828 cM) of the genome had been covered. Nine linkage groups were identified, with all but one (LG7; X-linked) allocated to the autosomes. These groups ranged in size from 15.7 cM to 176.5 cM, and have an average distance of 16.2 cM between adjacent markers. Of the autosomal linkage groups, LG2 and LG3 were assigned to chromosome 1 and LG4 localized to chromosome 3 based on physical localization of genes. Significant sex-specific distortions towards reduced female recombination rates were revealed in 22% of comparisons. When comparing the X-chromosome data to closely related species it is apparent that it is conserved both in synteny and gene order.

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Designing siRNAs

AA followed by 19 nucleotides followed by TT overhang

TT ——— antisense

Target site positioning is crucial

siRNA are potent suppressors even at concentrations as low as 0.125 nM

siRNA inhibition of 3 different targets has been established

Homemade siRNA - using T7 construct
? get details

Long dsRNA ⇒ RNAse L / Active PKR ⇒ Apoptosis (- in cytoplasm)
>30bp

Transferable suppression - something expressed in culture media → suppressive effect on other cells

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Short hairpin RNA - work better with larger loops

Expression of sh RNA

Nature AOP J

Jaque

Applied Biosystems

an Applera Corporation Business