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51st Annual Meeting of Genetics Society of Australia

Melbourne

July 11-14, 2004

Conference Abstracts

GSA
Genetics Society of Australia Inc.

DRSmyth

51st Annual Meeting of Genetics Society of Australia Inc. July 11-14, 2004

University of Melbourne

GSA
Genetics Society of Australia Inc.

Organized by

Philip Batterham and Wayn Wong

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&

GSA Committee

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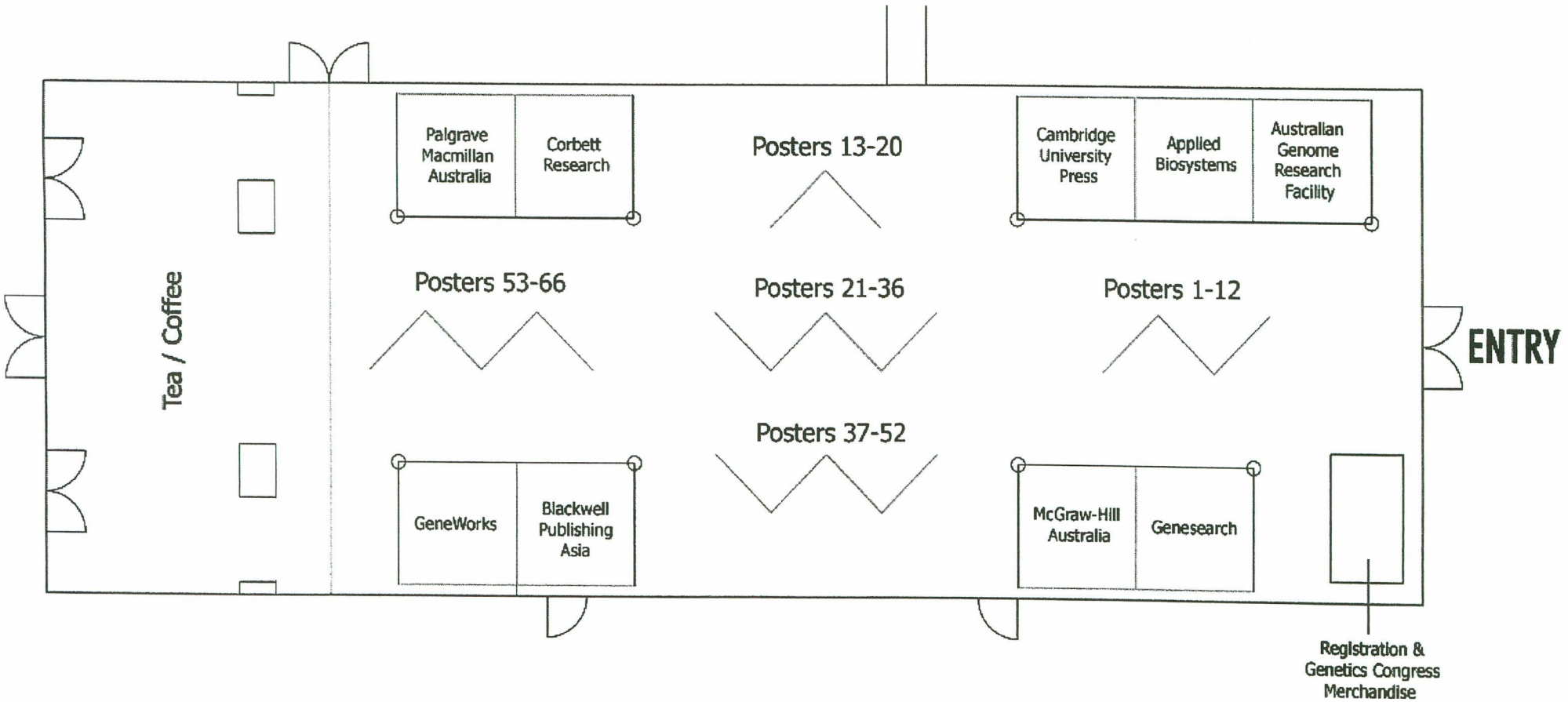
GSA values the support of the Sponsors and Exhibitors who have made a substantial contribution to this Conference. Please visit each of the Exhibition booths and remember the support given to GSA as you purchase products and services over the next twelve months

Posters & Exhibition Area
Grand Buffet Hall, Union House - Second Level



No.	Grid	Building / Department
198	K20	**1888 Building (UMPA)**
394	O14	**139 Barry Street**
220	N11	**780 Elizabeth Street**
148	G13	Accounting & Business Information Systems
152	G16	Administration, Employees & Students
167	I18	Advanced Minerals Products Centre
162	I19	**Alice Hoy Building**
181	L12	Anatomy and Cell Biology
132	F17	Anthropology
133	F18	**Architecture, Building & Planning**
395		Archives (120 - 122 Dawson St, Brunswick)
199	K21	**Arts Centre Building**
158	I21	Asialink
106	R15	Asian Law Centre
337	O14	**Australian Centre (131 / 137 Barry St)**
193	I16	Australian Photonics CRC Processing
139	F13	**Babel Building**
177	I13	**Baillieu Library**
113	D17	**Baldwin Spencer Building**
101	C15	**Beaurepaire Centre**
404	H4	**BIO21 Institute**
185	I11	**Biochemistry and Molecular Biology Building**
115	E18	Biology Laboratory
177	H13	**Bookroom**
122	E13	**Botany Building**
123	D12	**Botany North Extension**
182	J13	**Brownless Bio Medical Library**
249	Q19	**Building G (183 Bouverie St)**
385	O25	Centre for Communications Skills & English as a Second Language (138 Cardigan St)
368	M18	Centre for Study Health & Society (205 / 209 Grattan Street)
106	R15	Centre for Comparative Constitutional Studies
106	R15	Centre for Corporate Law & Securities Regulation
106	R15	Centre for Energy & Resources Law
176	L18	Centre for Environmental Applied Hydrology
106	R15	Centre for Employment & Labour Relations Law
175	L16	Centre for Geographic Information Systems & Modelling
128	F14	Centre for Indigenous Education
106	R15	Centre for Media, Communications & Information Technology Law.
356	E9	Centre for Studies in Australian Music (21 Royal Parade)
165	I18	**Chemical Engineering Building 1**
167	J18	**Chemical Engineering Building 2**
153	G17	**Chemistry Building**
154	G18	**Chemistry East Wing Building**
174	L17	**Civil Engineering Building**
601	K9	Clinical School (Royal Melbourne Hospital)
105	P14	Computer and Software Engineering
141	H11	**Conservatorium of Music**
263	U17	Contemporary Europe Research Centre
176	L18	CRC for Catchment Hydrology
123	D12	CRC for Bioproducts
193	I16	CRC for Senior Signal & Information Processing
263	U17	Criminology (234 Queensberry St)
161	H18	**C.S.H.E. Building**
700	M9	Dental Science (Royal Dental Hospital)
379	O18	Development Office
168	J19	**Doug McDonell Building**
200	E23	**Earth Sciences Buildings**
333	F24	**Eastern Precinct Carpark**
148	G13	**Economics & Commerce Building**
162	I19	Education Policy & Management (Alice Hoy)
263	U17	Education Policy & Management (234 Queensberry St)
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175	L16	**Engineering Block B**
174	L17	**Engineering Block C**
176	L18	**Engineering Block D**
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133	F18	Environmental Planning
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152	F16	Financial Operations
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199	K21	French & Italian Studies
260	O13	General Practice & Physiotherapy (200 Berkeley St)
194	H12	**Genetics Building**
132	F17	Geography & Environmental Studies
200	E23	Geology
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199	K21	German & Swedish Studies
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196	K20	Graduate Centre
465	M17	Graduate House (210 / 214 Leicester St)
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343	G23	Grounds Office
191	J15	History (John Medley)
149	G14	History & Philosophy of Science
191	J15	Horwood Language Centre
183	K11	**Howard Florey Institute**
152	F16	Human Resources
136	F21	**Ian Potter Museum of Art**
105	P14	**Information & Communication Technology Building**
142	E11	**Institute of Land & Food Resources**
191	J15	**John Medley Building**
197	J21	**John Smyth Building**
168	J19	Language, Literacy & Arts Education
106	R15	**Law Building**
217	T21	**Lincoln Square Building A (623 / 629 Swanston Street)**
218	T21	**Lincoln Square Building B (631 / 645 Swanston Street)**
162	I19	Learning and Education Development (Alice Hoy)
263	U17	Learning and Education Development (234 Queensberry Street)
348	N21	Learning Skills Unit (723 Swanston St)
199	K21	Linguistics and Applied Linguistics
343	G23	**Maintenance Section** Deliveries (270 Faraday St)
388	N14	Major Projects (151 Barry Street)
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160	G20	Mathematics & Statistics
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163	I17	Melbourne Research & Innovation Office
197	J21	Melbourne Scholarships
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203	M17	Melbourne University Private (213 / 217 Grattan Street)
184	J11	**Microbiology and Immunology Building**
184	J11	Microbiological Diagnostic Unit
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357	E9	Music (23 Royal Parade)
358	E9	Music (25 Royal Parade)
359	E9	Music (27 Royal Parade)
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206	N13	Music (214 Berkeley Street)
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149	G14	**Old Arts Building**
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156	H19	**Old Geology South Building**
166	J17	**Old Metallurgy Building**
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150	G15	**Old Quadrangle Building**
164	J18	**Old Radiation Laboratory Building**
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181	K12	Physiology
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191	J16	School of Languages
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207	O13	202 / 206 Berkeley Street
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112	E13	**University House**
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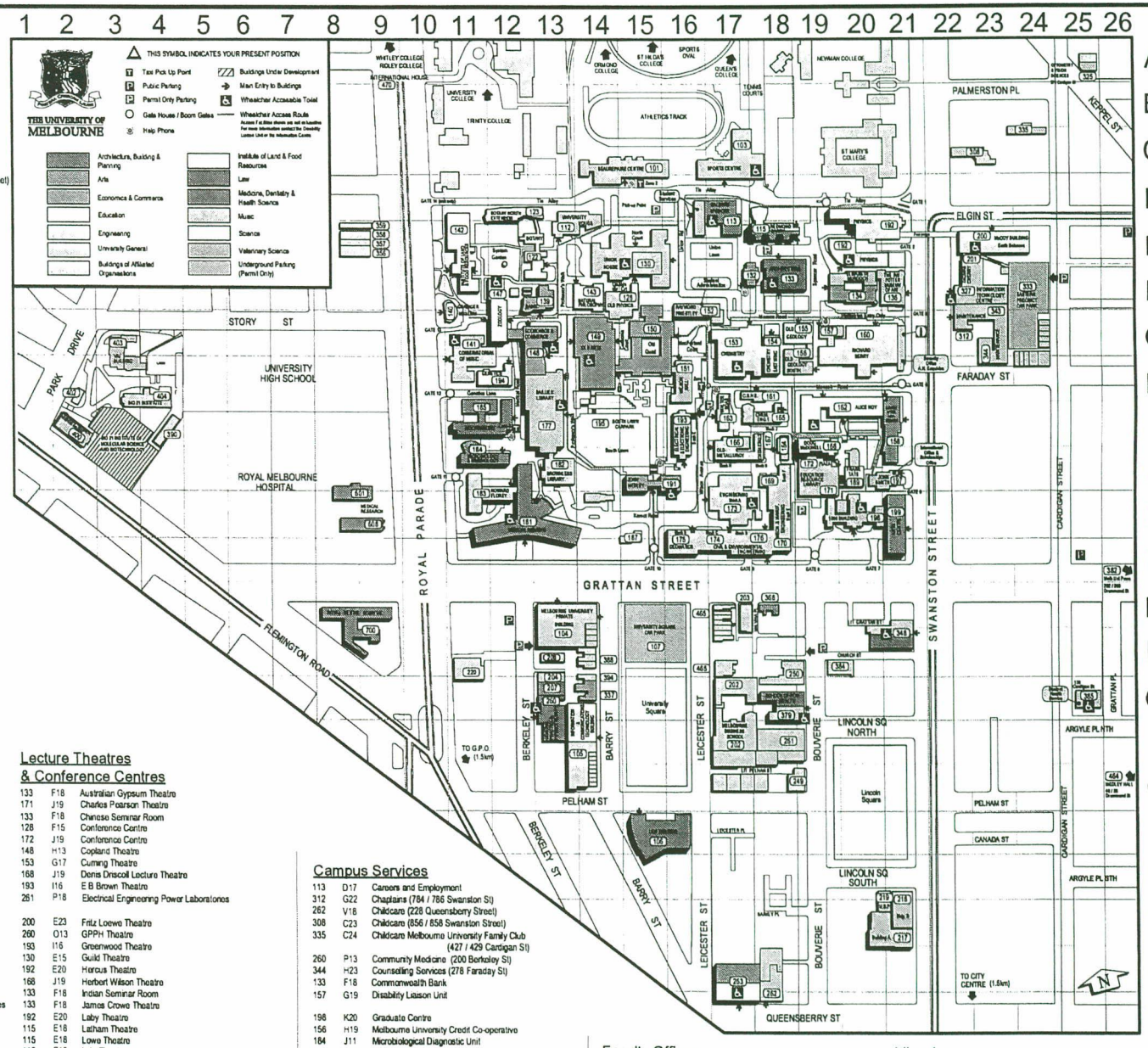
163	I17	**Walter Boas Building**
151	H16	**Wilson Hall**
390	I4	**W.E.H.I. Building**
147	G12	**Zoology Building**



Lecture Theatres & Conference Centres

133	F18	Australian Gypsum Theatre
171	J19	Charles Pearson Theatre
133	F18	Chinese Seminar Room
128	F15	Conference Centre
172	J19	Conference Centre
148	H13	Copland Theatre
153	G17	Cuming Theatre
168	J19	Denis Driscoll Lecture Theatre
193	I16	E B Brown Theatre
261	P18	Electrical Engineering Power Laboratories
200	E23	Fritz Loewe Theatre
260	O13	GPPH Theatre
193	I16	Greenwood Theatre
130	E15	Guild Theatre
192	E20	Hercus Theatre
168	J19	Herbert Wilson Theatre
133	F18	Indian Seminar Room
133	F18	James Crowe Theatre
192	E20	Laby Theatre
115	E18	Latham Theatre
115	E18	Lowe Theatre
115	E18	Lyle Theatre
153	G17	Masson Theatre
115	E18	Medley Theatre
141	H11	Melba Hall
128	F15	Old Physics Conference Centre
199	K21	Open Stage
172	J19	Plaza Conference Centre
133	F18	Prince Philip Theatre
149	G14	Public Lecture Theatre
133	F18	Sasakawa Theatre
199	K21	White Theatre
151	H16	Wilson Hall

Campus Services

113	D17	Careers and Employment
312	G22	Chaplains (784 / 786 Swanston St)
262	V18	Childcare (228 Queensberry Street)
308	C23	Childcare (856 / 858 Swanston Street)
335	C24	Childcare Melbourne University Family Club (427 / 429 Cardigan St)
260	P13	Community Medicine (200 Berkeley St)
344	H23	Counselling Services (278 Faraday St)
133	F18	Commonwealth Bank
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184	J11	Microbiological Diagnostic Unit
143	F14	National Australia Bank
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196	K20	University of Melbourne Postgraduate Assoc (UMPA)
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385	O25	Student Dental Health (138 Cardigan St)
385	O25	Student Health Service (138 Cardigan St)
385	O25	Student Legal Aid Service (138 Cardigan St)
113	D16	Student Support Services (Employment, Housing & Financial Aid)
130	E15	Student Union
	H16	Taxi Pick Up Point Zone 1

Faculty Offices

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149	G14	Arts Faculty Office
148	G13	Economics & Commerce Faculty Office
162	I19	Education Faculty Office
173	K17	Engineering Faculty Office
142	E11	Institute of Land & Food Resources
106	R15	Law Faculty Office
155	G19	Science Faculty Office
181	K12	Medicine, Dentistry & Health Sciences Faculty Office
141	H11	Music Faculty Office
400	I2	Veterinary Science Faculty Office

Libraries

133	F18	Architecture Building & Planning Library
177	I13	Baillieu Library
182	J13	Brownless Biomedical Library
154	G18	Chemistry Library
200	E23	Earth Sciences Library
173	K17	Engineering Library
171	J19	E.R.C. Library
148	G13	Giblin Economics & Commerce Library
106	R15	Legal Resource Centre
192	E20	Physics Library
403	H3	Veterinary Science Library

Map created by Property & Buildings : Asset Data Unit Date : Nov 2003

Parking @ uniMelb

Eastern Precinct
The entrance is in Cardigan Street just south of Elgin Street.

University Square
Enter from Berkeley Street approximately 50 metres from the corner of Grattan Street on the east side.

Public Transport

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The City Saver Metcard is a new product designed for travel around the city centre as well as to key Melbourne landmarks such as the MCG and Melbourne Museum.

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Source: www.victrip.com.au

Mixer – INU bar, Union House

The Conference Mixer will be held at INU in the Union House [**map ref**. E15, building 130] at the University of Melbourne on Sunday July 11, commencing at 7.30pm. Drinks and canapés will be served.

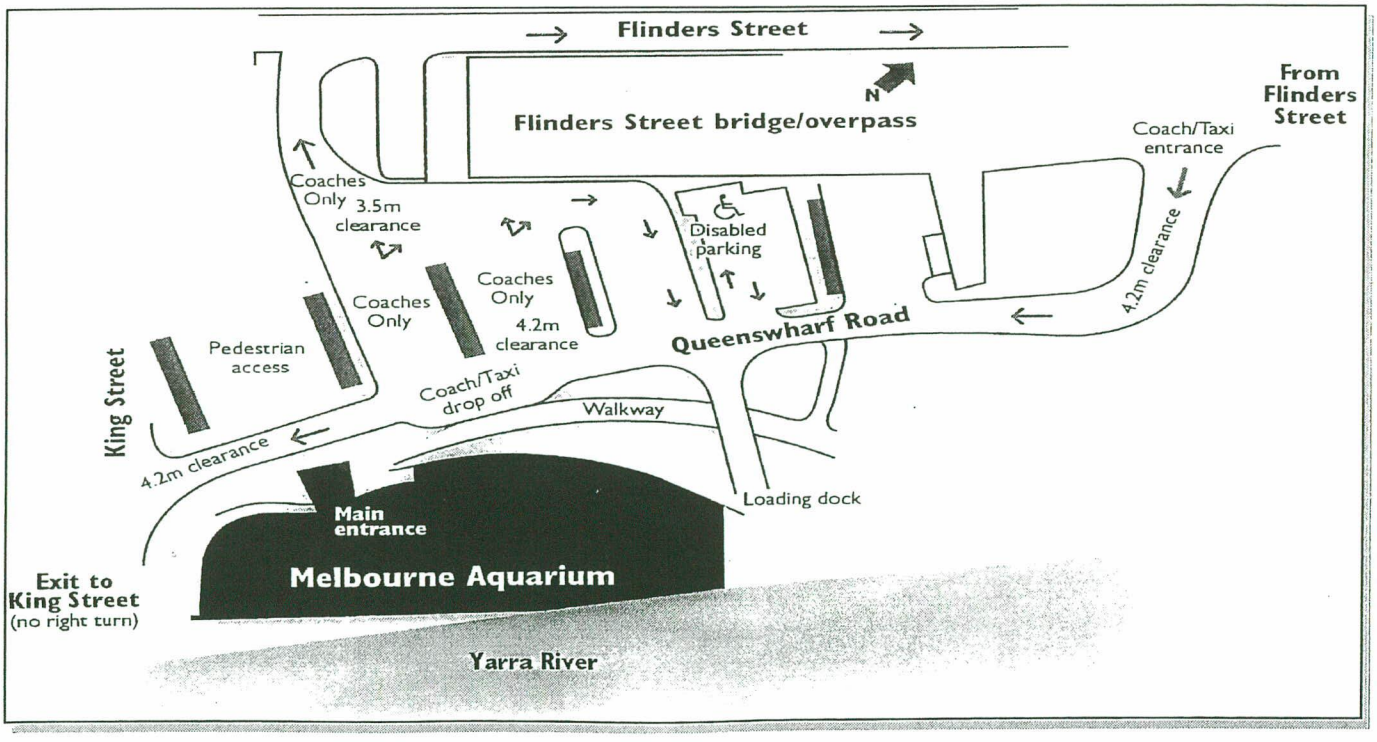
Conference Dinner –

Melbourne Aquarium, Tuesday July 13 at 7.00pm

Event	Area	Time
Pre-Dinner Drinks	Jellies	7:00 pm
Dinner	Coral Atoll	7:30 pm

A three-course meal and drinks (Hardy's Sparkling Wing, Chardonnay, Shiraz Cabernet, Victoria Bitter, Fosters Light Ice, Orange Juice & Soft Drinks)

Address: Corner Queenswharf Rd & King St, Melbourne.



Student Prizes

Smith-White Travel Prize

Spencer 'Spinny' Smith-White was a founding member of the Genetics Society of Australia. The Smith-White Travel Prize provides for an annual award of up to $1,750 to assist a postgraduate student member of the GSA to attend overseas conference. Applications for this Prize were received before the conference and will be evaluated by the committee

Mayo Prize

Valued at $500 this Prize will be awarded for the best talk given by a Ph.D. student at the GSA conference. The Prize honours the contribution to GSA and, more generally, to Australian Genetics by Drs Jean and George Mayo.

Sidney James Prize

Valued at $500 this Prize will be awarded for the best poster presentation by a Ph.D. student. The Prize honours the contribution to GSA and, more generally, to Australian Genetics by the late Dr. Sid James.

AGRF Prizes

Thanks to the generous support of the Australian Genome Research Facility two other prizes each worth $300 will be awarded for student presentations at the Conference.

Elsevier Australia P/L Prizes

Thanks to the generous support of the Elsevier Australia P/L two book vouchers each worth $275 will be awarded for student presentations at the Conference.

McGraw-Hill Australia Prize

Thanks to the generous support of the McGraw-Hill Australia one book voucher worth $250 will be awarded for a student presentation at the Conference.

Sunday July 11th

12:00 – 2:00 pm **Registration**
Copland Theatre, Economics and Commerce Building

2:00 – 2:15 pm Official Opening
Professor John McKenzie, Dean of Science, University of Melbourne

Session 1 - *Plenary Address*

Chair: Joan Kelly
Venue: Copland Theatre, Economics and Commerce Building

2:15 – 3:15 pm **Martin Kreitman**, Department of Ecology and Evolution, University of Chicago
Functional analysis of evolved differences in the Drosophila even-skipped stripe two enhancer

3:15 – 4:05 pm **Sue Forrest**, Australian Genome Research Facility
Jumping into the evolutionary "sweet spot": sequencing of the kangaroo genome

4:05 – 4:30 pm TEA BREAK
Foyer Copland Theatre

Session 2A - *Genetic Variation in Natural Populations & Livestock*

Chair: Steve Donnellan
Venue: Copland Theatre, Economics and Commerce Building

4:30 – 4:50 pm **Sean MacEachern, Paul Sunnucks, John McEwan & Mike Goddard**
Genetic hoofprints: is there molecular evidence of selection in domestic cattle?

4:50 – 5:10 pm **Jaime Gongora, Dendigh Simond, Daniel White, Stewart Lowden & Chris Moran**
Phylogenetic relationships of African, Asian and European Suids

5:10 – 5:30 pm **Sam Ingram, Pauline Garnier-Géré and Peter Ades**
Interpreting Provenance by Environment interaction in *Eucalyptus regnans* F.Muell.

5:30 – 5:50 pm **Luke G. Barrett, Tianhua He, Byron B. Lamont & Siegfried L. Krauss**
Genetic variation within the aerial seed bank of the shrub *Banksia hookeriana* (Proteaceae)

5:50 – 6:10 pm **J. Tupac Otero, Nicola S. Flanagan, Mark Clements, Allen Herre & Paul Bayman**
Phylogeography of an orchid mycorrhizal mutualism in the Caribbean

Session 2B – *Cell death and Development*

Chair: Stephen Gregory
Venue: Old Arts Theatre D, Old Arts Building

4:30 – 4:50 pm **Jörg Heierhorst, Carolyn J. McNees, Brietta L. Pike, Lindus A. Conlan & Nora Tenis**
ASCIZ regulates homologous recombination and apoptosis in response to DNA damage

4:50 – 5:10 pm **Vanessa S. Marsden, Paul G. Ekert, Mark Van Delft, Lorraine A. O'Reilly, David L. Vaux, Jerry M. Adams, Andreas Strasser**
Initiation of the caspase cascade in mammalian cells.

5:10 – 5:30 pm **Nicole Siddall, Kristine Behan, Jennifer Crew, Tara Cheung, John A. Pollock and Philip Batterham**
The role of the lozenge gene in apoptosis and cell recruitment in the developing Drosophila eye

5:30 – 5:50 pm **Nirmal Lorensuhewa and Robert Saint**
Characterisation of mapmodulin: A novel tumour suppressor homolog in Drosophila melanogaster

5:10 – 5:30 pm **Stephanie Bunt, Nicole Siddall and Gary Hime**
Identifying signals that regulate male reproduction in Drosophila

Session 2C - *Gene Expression I*

Chair: Richard Todd
Venue: Old Arts Theatre E, Old Arts Building

4:30 – 4:50 pm **Karen Bunting, Sudha Rao, Thomas Parks and M. Frances Shannon**
A molecular analysis of the role of c-Rel in chromatin remodelling and cytokine gene transcription in T cells

4:50 – 5:10 pm **Janine Deakin, Margaret Delbridge, Patrick Kirby, Matthew Wakefield, Paul Waters and Jennifer A. Marshall Graves**
Unravelling the evolution of mammalian dosage compensation

5:10 – 5:30 pm **Ruth N. Kaplan-Levy and David R. Smyth**
Mode of action of PETAL LOSS, a transcription factor involved in regulating sepal and petal development in *Arabidopsis thaliana*

5:30 – 5:50 pm **Calida Neal, Sue Shien Tan, Alan Neale**
The regulation of a gene family involved in glucosinolate biosynthesis in *Arabidopsis thaliana*

5:50 – 6:10 pm **Zhenzhong Chen, Katherine Smith, John Damiano, Jayne Lydall, Phil Batterham & Charles Robin**
No nonsense with insecticide resistance: cloning a resistance gene with orthology to nonsense mediated decay genes of *C. elegans* and humans.

6:10 – 7:30 pm GSA COMMITTEE MEETING

7:30 – 10:30pm **MIXER**
INU Bar, Union House, University of Melbourne

Monday July 12th

Session 3 - *Plenary Address*

Chair: Bill Warren
Venue: Public Lecture Theatre, Old Arts Building

9:00 – 10:00 am **Nancy Bonini**, Department of Biology, University of Pennsylvania
Drosophila as a model for human neurodegenerative disease

10:00 – 10:40 am MORNING TEA AND POSTERS
Grand Buffet Hall, Union House

Session 4A - *Phylogeography*

Chair: Belinda Appleton
Venue: Public Lecture Theatre, Old Arts Building

10:40 – 11:00 am **Jeremy Pulvers and Don Colgan**
Molecular phylogeography of *Melonycteris* in the Solomon Islands

11:00 – 11:20 am **Sherryn Ciavaglia, Mark Blacket and Paul Sunnucks**
Comparative phylogeography of two terrestrial flatworms at Tallaganda, NSW

11:20 – 11:40 am ✓ **Amber Beavis**
A comparative phylogeographic study of two genera of funnel web spider (*Hadronyche & Atrax*) in Tallaganda State Forest (NSW)

11:40 – 12:00 pm ✓ **Mark de Bruyn, John C. Wilson and Peter B. Mather**
Testing biogeographic hypotheses using intraspecific molecular data: phylogeography of giant freshwater prawns

12:00 – 12:20 pm ✓ **Shannon Corrigan and Luciano B. Beheregaray**
Phylogeography of the pencilfish *Nannostomus unifasciatus*, a flooded-forest fish from central Amazonia

12:20 – 12:40 pm ✓ **Kate Hodges, Scott Keogh, David Rowell**
Comparative phylogeography of the water skinks *Eulamprus heatwolei* and *Eulamprus tympanum* in southeastern Australia, with comparisons of co-distributed invertebrates at Tallaganda, NSW.

Session 4B - *Multigene Families*

Chair: Charles Robin
Venue: Old Arts Theatre D, Old Arts Building

10:40 – 11:00 am **B.G. Fry and W. Wüster**
Assembling an arsenal: Origin and evolution of venom in snakes

11:00 – 11:20 am **Katherine Belov, Rachael Woodward, Hannah Siddle, Don Colgan, Teena Browning, Mark Eldridge and Janine Deakin**
Marsupial and monotreme MHC class II genes

11:20 – 11:40 am **Donald M. Gardiner, Anton J. Cozijnsen, Leanne M. Wilson, M. Soledade. C. Pedras, Barbara J. Howlett**
Comparative analysis of gene clusters encoding secondary metabolites of filamentous fungi

11:40 – 12:00 pm **Daniel Hovan, Charles Robin, Philip Batterham and David G. Heckel**
Insecticide resistance and esterase patterns in two field populations of Australian *Helicoverpa armigera.*

12:00 – 12:20 pm **Lloyd Low, Charles Robin, Phil Batterham**
Molecular evolution of GSTs in the *Drosophila* genus

12:20 – 12:40 pm **Peter Smibert and Robert Saint**
Reverse Genetic analysis of the *RGK* gene family of *Drosophila melanogaster*

Session 4C - *Symbionts & Parasites*

Chair: Andrew Weeks
Venue: Old Arts Theatre E, Old Arts Building

10:40 – 11:00 am **Natalie P Leo, Jane M Hughes, Xiaoye Yang, Shree KS Poudel, William G Brogdon and Stephen C Barker**
The head and body lice of humans are separate species: evidence from double infestations.

11:00 – 11:20 am **Scott L. O'Neill**
Wolbachia Genomes: A Key To Understanding a Ubiquitous Symbiosis of Insects

11:20 – 11:40 am **Elizabeth McGraw and Scott O'Neill**
Drosophila melanogaster gene expression in response to a virulent endosymbiont

11:40 – 12:00 pm **Warwick Grant, Steve Skinner, Chuck Shoemaker, Jan Newton-Howes, Kirsten Grant & Susan Stasiuk**
The first successful genetic manipulation of an animal parasitic nematode.

12:00 – 12:20 pm **H.S.L.Roberts, A. Reineke, O. True, J. Belatti and O. Schmidt**
Two genetically distinct lines co-exist in populations of the endoparasitoid *Venturia canescens* .

12:20 – 12:40 pm **M. S. Bulmer and R. H. Crozier**
Duplication and diversifying selection among termite antifungal peptides

12:40 – 3:40 pm FREE LUNCH, POSTERS, TRADE DISPLAYS & AFTERNOON TEA
Grand Buffet Hall, Union House

Session 5 - *Plenary Address*

Chair: David Smyth
Venue: Public Lecture Theatre, Old Arts Building

3:40 – 4:30 pm **Chris Cobbett**, Department of Genetics, University of Melbourne
Mechanisms of heavy metal detoxification and homeostasis in plants

4:30 – 5:20 pm **Brandon Wainwright**, Institute of Molecular Bioscience, The University of Queensland
Patching up common human cancer

5:20 – 7:00 pm GSA Annual General Meeting

Tuesday July 13th

Session 6 - *Plenary Address*

Chair: Jennifer Marshall Graves
Venue: Public Lecture Theatre, Old Arts Building

9:00 – 10:00 am **Alan Templeton**, Department of Genetics/Department of Biology, Washington University, St. Louis
Recent Human Evolution: What Genes Really Tell Us.

10:00 – 10:40 am MORNING TEA AND POSTERS
Grand Buffet Hall, Union House

Session 7A - *Conservation and Biodiversity*

Chair: Michael Kearney
Venue: Public Lecture Theatre, Old Arts Building

10:40 – 11:00 am **David Runciman, Christina Schmuki, Sean MacEachern & Paul Sunnucks**
Landscapes and log-dwellers: A cockroach's eye view of the genetic effects of habitat fragmentation.

11:00 – 11:20 am **Tristan Armstrong and Peter J. De Lange**
Population genetic diversity and structure in the endangered New Zealand shrub *Hebe speciosa*: implications for conservation and ethnobotany

11:20 – 11:40 am **Iman Lissone, Alison Shapcott & Jenny Ovenden**
The use of RAFs enables determination of genetic structure within and among catchments in the Australian lungfish *Neoceratodus forsteri*

11:40 – 12:00 pm **R. C. Garrick, C. J. Sands, D. M. Rowell, N. N. Tait, P. Greenslade & P. Sunnucks.**
High local endemism of a saproxylic 'giant' springtail (Collembola) from SE Australia

12:00 – 12:20 pm **Steven J.B. Cooper, Remko Leys, John Bradbury, Kathy Saint, Chris H. S. Watts & William F. Humphreys**
Subterranean islands in the desert: evolutionary history of stygofauna from calcrete aquifers of central Western Australia

Session 7B - *From Cell Division to Development*

Chair: Alex Andrianopoulos
Venue: Old Arts Theatre D, Old Arts Building

10:40 – 11:00 am **Rebecca Keall & William D. Warren**
A genetic screen for novel regulators of chromosome segregation

11:00 – 11:20 am **Stephen Gregory, Tatiana Shandala, Hazel Dalton, and Robert Saint**
Genetic analysis of cytokinesis

11:20 – 11:40 am **Ryan Herbert and Robert Saint**
Identification and characterisation of novel interactors of cytokinesis

11:40 – 12:00 pm **Rachael J. Rutkowski and William D. Warren**
deflated encodes a novel, highly conserved *Drosophila* protein, implicated in regulation of cell proliferation.

12:00 – 12:20 pm **Masha Smallhorn, Michael Murray and Robert Saint**
The Rho GTP exchange factor Pebble is required for the Drosophila mesoderm epithelial to mesenchymal transition

Session 7C - *Solving Problems with Genetic Markers*

Chair: Kathie Raphael
Venue: Old Arts Theatre E, Old Arts Building

10:40 – 11:00 am **Leigh.A. Nelson**
The molecular identification of closely related calliphorids (Diptera: Calliphoridae) of forensic importance.

11:00 – 11:20 am **T.M. Crowley, M.S. Muralitharan and T.W. Stevenson**
Cyclaneusma minus – A molecular characterisation.

11:20 – 11:40 am **Simon Jarman, Bruce Deagle, Abraham Passmore and Kevin Redd**
Reconstruction of predator diet from prey DNA diversity

11:40 – 12:00 pm **Angela Corrie and Ary Hoffmann**
Using genetic markers to understand the biology and population structure of grapevine phylloxera

12:00 – 12:20 pm **Stuart Gilchrist, Alison Ling, XiuMei Liang, Alan Meats and John Sved**
Tracking fruit flies in inland Australia

12:40 – 1:40 pm LUNCH

Session 8A - *Speciation & Development*

Chair: Ross Crozier
Venue: Public Lecture Theatre, Old Arts Building

1:40 – 2:00 pm **C. J. Sands, D. M. Rowell, N. N. Tait, D. Briscoe, M. Blacket, R. C. Garrick and P. Sunnucks.**
Deep divergence in Australian Onychophora: A tale of two species

2:00 – 2:20 pm **Lynne van Herwerden, Philip L. Munday and Christine L. Dudgeon**
Sympatric speciation by host shift in the sea

2:20 – 2:40 pm **Michael Kearney and Mark Blacket**
The origin and spread of the parthenogenetic grasshopper *Warramaba virgo*: evidence from mitochondrial DNA sequences

2:40 – 3:00 pm **K.A. Raphael, X. An, and M. Frommer.**
Circadian behaviour and speciation in tephritid fruit flies.

3:00 – 3:20 pm **Annette Becker and David Smyth**
Evolutionary genetics of carpels: Using California poppy (*Eschscholzia californica* Cham.) as a basal eudicot model system

3:20 – 3:40 pm **Yvonne Parsons and Hatch Stokes**
Leaky species and the devolution of sex

Session 8B - *Genes & Disease*

Chair: Jim Camakaris
Venue: Old Arts Theatre D, Old Arts Building

1:40 – 2:00 pm **Steve Callaghan and Richard Cotton**
Verifying Existing Mutation Data

2:00 – 2:20 pm **Teena L. Browning, Kyall R. Zenger and Mark D.B. Eldridge**
Is susceptibility to infectious disease written in the genes?

2:20 – 2:40 pm **Kavita Praveen, Terence O'Brien, Cassandra Szoeke, Wendyl D'Souza, Mark Cook, Simon Foote**
Linkage Analysis of a Family with Familial Partial Epilepsy with Variable Foci

2:40 – 3:00 pm **Alan Wilton, Marina D'Sa, Erica McAuley, Carol Ting and Stephen Lillioja**
Gestational Diabetes as a model for Type 2 Diabetes

3:00 – 3:20 pm **Melanie Norgate, Adam Southon, Ashley Farlow, Esther Wei, J. Camakaris, P. Batterham, Richard Burke**
Characterisation of the dmATP7 copper transporter in *Drosophila melanogaster*

3:20 – 3:40 pm **Jack da Silva**
Predicting Adaptive Molecular Evolution: Simulation of HIV-1 Adaptation to Antibody Surveillance

Session 8C - *Insect Pests & Insecticide Resistance*

Chair: Joanne Daly
Venue: Old Arts Theatre E, Old Arts Building

1:40 – 2:00 pm **Emilie Cameron and Stuart Gilchrist**
Waiter there's a fly in my Ti Tree: Characteristics of a fruit fly outbreak

2:00 – 2:20 pm **Simon W. Baxter, Jian-Zhou Zhao, Linda J. Gahan, Anthony M. Shelton, Bruce E. Tabashnik, & David G. Heckel**
Novel genetic basis of field-evolved insect resistance to Bt toxins

2:20 – 2:40 pm **G. Ma, H.L.S. Roberts, N. Featherstone and O. Schmidt**
Tolerance to the *Bacillus thuringiensis* endotoxin Cry1Ac in a laboratory *Helicoverpa armigera* strain is based on immune induction, which is transmitted by a maternal effect.

2:40 – 3:00 pm **Trent Perry, Michael Bogwitz, Philip Batterham and Phillip Daborn**
Insecticide Resistance in *Drosophila melanogaster*; the role of *Cyp6g1* and friends

3:00 – 3:20 pm **Erica J. Crone, Tara D. Sutherland, Robyn J. Russell and John G. Oakeshott**
Juvenile hormone esterase of *Drosophila melanogaster.*

3:20 – 3:40 pm **C. W. Wee and D. G. Heckel**
Altered Gene Expression Levels Linked to Fenvalerate Resistance in Cotton Bollworm, *Helicoverpa armigera*

3:40 – 4:20 pm AFTERNOON TEA AND POSTERS
Grand Buffet Hall, Union House

Session 9 - *Plenary Address*

Chair: Richard Newcomb
Venue: Public Lecture Theatre, Old Arts Building

4:20 – 5:10 pm **John Oakeshott**, CSIRO Entomology, Canberra
Paradigm Shifts in Adaptive Enzyme Evolution

5:10 – 5:50 pm **Neil Murray**: Polluting the Scientific Atmosphere? Peppered moths, spiced stories and the problem of scientific fraud.

7:00 – 12:00 am CONFERENCE DINNER
Melbourne Aquarium

Wednesday July 14th

Session 10 - *Plenary Address*

Chair: Michael Hynes
Venue: Public Lecture Theatre, Old Arts Building

9:00 – 10:00 am **Kevin Struhl**, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School
Transcriptional regulatory mechanisms in yeast and human cells

10:00 – 10:40 am MORNING TEA AND POSTERS
Grand Buffet Hall, Union House

Session 11A - *Conservation*

Chair: Luciano Beheregaray
Venue: Public Lecture Theatre, Old Arts Building

10:40 – 11:00 pm **Paul Mitrovski, Dean Heinze, Kathryn Guthridge & Ary Hoffmann**
Conservation genetics and biogeography of the endangered mountain pygmy-possum, *Burramys parvus.*

11:00 – 11:20 pm **Alan Wilton, Britt-Louise Carlsson & David Jenkins**
Are the dingoes on Fraser Island the last remaining pure dingoes in the wild?

11:20 – 11:40 pm **Paul Sunnucks, Ryan Garrick, Chester Sands, Sherryn Ciavaglia, Mark Blacket, Noel Tait & David Rowell**
Phylogeographic congruence and long-term environmental refuges: did a Regional Forest Agreement cream off a biodiversity-generating hotspot?

11:40 – 12:00 pm **David Field, Andrew Young, Rob Whelan & David Ayre**
Swamping the Swamp gum: Habitat fragmentation and disturbance promotes hybridisation in *Eucalyptus aggregata*

12:00 – 12:20 pm **C. Schmuki, S. MacEachern, D. Runciman, C. Vorburger & P. Sunnucks**
Beetles on islands of bush in a sea of pine: impacts of habitat fragmentation on two species of Adeliini at Tumut, SE Australia.

Session 11B - *Gene Expression II* DRS

Chair: TBA
Venue: Old Arts Theatre D, Old Arts Building

10:40 – 11:00 pm **Kathleen De Boer, Robin Brimblecombe, Angela Siu, Melanie Hand, Suzy Ryan, and John D. Hamill.**
Regulation of genes important in pyridine alkaloid metabolism in plants

11:00 – 11:20 pm **G.S. Khew, S.L. Murray, M.A. Davis, M.J. Hynes.**
Analysis of genes involved in fatty acid b-oxidation in *Aspergillus nidulans*

11:20 – 11:40 pm **Richard B. Todd, James A. Fraser, Meryl A. Davis and Michael J. Hynes**
Nuclear Export of the Transcriptional Activator AreA occurs via the CrmA Exportin in *Aspergillus nidulans.*

11:40 – 12:00 pm **Michael Groszmann, Teodora Paicu and David Smyth.**
Functionally significant promoter and protein regions of SPATULA, an *Arabidopsis* gene involved in gynoecium development.

12:00 – 12:20 pm **Lily Pereg-Gerk**
FlcA of *Azospirillum* controlling cell differentiation and attachment to plant roots

Session 11C - *Responding to the Environment*

Chair: John Syed
Venue: Old Arts Theatre E, Old Arts Building

10:40 – 11:00 pm **Alisha R Anderson, Ary A Hoffmann and Stephen W McKechnie**
Response to selection for chill coma recovery in *Drosophila melanogaster*

11:00 – 11:20 pm **Julia Jones, Mary Myerscough, Sonia Graham, Ben Oldroyd**
Thermoregulation in honey bee colonies: diversity promotes stability

11:20 – 11:40 pm **Travis K. Johnson, Steve W. McKechnie & David J. Clancy**
Water balance in *Drosophila*: can early physiological decline predict aging and longevity?

11:40 – 12:00 pm **Kathryn Guthridge, Rebecca Hallas, Marina Telonis and Ary Hoffmann**
SSR- and candidate gene- based linkage analysis of resistance and susceptibility to desiccation stress in *Drosophila melanogaster*

12:00 – 12:20 pm **Angela. P. Van De Wouw, P. J. Daborn, D.G. Heckel and P. Batterham**
The effects of cyromazine treatment on Drosophila melanogaster ñ a genetic approach

12:20 – 1:20 pm LUNCH

Session 12A - *Gene Flow, Clines and Adaptation*

Chair: Stuart Gilchrist
Venue: Public Lecture Theatre, Old Arts Building

1:20 – 1:40 pm **Carla M. Sgrò, Aston Arthur and Andrew R. Weeks**
Latitudinal clines without chromosomal inversions?

1:40 – 2:00 pm **David Hurwood, Mike Heasman and Peter Mather**
Gene flow in wild populations of the native flat oyster (*O. angasi*): a species being considered for culture as an alternative to the Sydney rock oyster.

2:00 – 2:20 pm **Melinda Pickup and Andrew Young**
Local adaptation and success of transplanted populations of *Rutidosis leptorrhynchoides* (Asteraceae)

2:20 – 2:40 pm **Joanna Wiszniewski, Luciana Möller, Simon Allen and Luciano Beheregaray**
Fine-scale genetic structure of inshore bottlenose dolphins (*Tursiops aduncus*) in the Hunter Region, NSW

2:40 – 3:00 pm **Andrew R. Weeks and Ary A. Hoffmann**
Frequency-dependent selection maintains clonal diversity in asexual organisms: evidence from natural populations of the earth mite species *Penthaleus major*

Session 12B - *Evolution of Genomes* DRS

Chair: David E. Catcheside
Venue: Old Arts Theatre D, Old Arts Building

1:20 – 1:40 pm **Rami Stiglec, Shargal Tsend-Ayush, Frank Grützner, Tariq Ezaz, Anne Gaeth, Steve Sarre, Arthur George, Jennifer A. Marshall Graves**
Sex chromosomes and *DMRT1* in the tiger snake

1:40 – 2:00 pm **Jennifer A. Marshall Graves and Horst Hameister**
Wide genome comparisons reveal the origins of the human X chromosome

2:00 – 2:20 pm **Hayley Sharp and Dave Rowell**
Double sex-linked translocation chains: segregation in a new race of *Delena cancerides.*

2:20 – 2:40 pm **Tony Brown, Jason Rauscher, Simon Joly, Jane Doyle and Jeff Doyle**
Nuclear ribosomal DNA evolution in polyploid *Glycine tomentella* (Leguminosae)

2:40 – 3:00 pm **Jeremy N. Timmis, Michael A. Ayliffe, Chun Y. Huang and William Martin**
Cytoplasmic organellar DNA has contributed massively to the genetic complexity of the nucleus during endosymbiotic evolution

Session 12C - *From Genetic Maps to Phenotypes*

Chair: Alan Wilton
Venue: Old Arts Theatre E, Old Arts Building

1:20 – 1:40 pm **Andrew Dubowsky and Warwick Grant,**
SNP loci association mapping in *Onchocerca volvulus*

1:40 – 2:00 pm **Amanda Chamberlain and Mike Goddard.**
Estimating the number of real QTL.

2:00 – 2:20 pm **Sara McClintock, Kevin Beard, Michael Goddard**
Calving difficulty in dairy cows: the genetic background

2:20 – 2:40 pm **Maxy Mariasegaram, Nick Robinson, Mike Goddard**
Empirical evaluation of the sensitivity of selective DNA pooling to detect QTL in a half-sib design

3:00 – 3:30 pm AFTERNOON TEA

Session 13 - *Plenary Address*

Chair: Ary Hoffmann
Venue: Public Lecture Theatre, Old Arts Building

3:30 – 4:30 pm MJD White Address
Joan Kelly, School of Molecular and Biomedical Science, Adelaide University
Genetic Dissection of a Regulatory Network

4:30 – 4:45 pm Presentation of Student Prizes
Ary Hoffmann

Poster Program

1 **Papoulis M., Achter R.C., Herbert S.C., Ziino M.N., Stevenson P.L., Ewen-White K.R.**
DNA Quality and Your Results

2 **Marlien van der Merwe, Peter H. Thrall and Jeremy Burdon**
Evolutionary relationships inferred from the TEF1 gene among the rust fungi.

3 **S. Singh[1], M.Muralitharan, G. Kadkol and D. Cahill**
Field assessment of black leg disease among genotypes of *Brassica napus*

4 **Renée Jarvis, Donald Gardiner and Barbara Howlett**
Gene silencing using RNAi in *Leptosphaeria maculans*, the blackleg fungus of canola

5 **Samiya Al-Jaaidi, Margaret Katz, David Beckhouse and Lily Pereg-Gerk**
Transformation of *Thielaviopsis basicola*: A tool to study the host-pathogen interaction at a molecular level.

6 **Ellen Fox, Donald Gardiner and Barbara Howlett**
Determining the biosynthetic pathway for sirodesmin production

7 **Donald M. Gardiner, Renee S. Jarvis, Barbara J. Howlett**
Transport proteins for the fungal secondary metabolites, sirodesmin and gliotoxin

8 **Donald M. Gardiner, Anton J. Cozijnsen, Leanne M. Wilson, M. Soledade. C. Pedras, Barbara J. Howlett**
Gene clusters involved in biosynthesis of fungal epipolythiodioxopiperazine toxins

9 **Dawar Hussain, Sarah M. Sherson, Edwin Wong, Scott A. Sinclair, James Camakaris and Christopher S. Cobbett.**
P-type ATPase heavy metal transporter genes involved in essential zinc homeostasis in *Arabidopsis thaliana*

10 **Michael J. Haydon and Christopher S. Cobbett**
Genetic and molecular characterisation of a novel Major Facilitator Superfamily protein implicated in zinc homeostasis in *Arabidopsis*

11 **Narelle Cairns and Chris Cobbett**
Characterisation of T-DNA insertion mutants of γ-glutamylcysteine synthetase in *Arabidopsis*

12 **Ashley Farlow, Esther Wei, Melanie Norgate, Adam Southon, Jim Camakaris, Phil Batterham, Richard Burke**
Markers of copper deficiency in *Drosophila melanogaster*.

13 **M. Muralitharan, B. Konsak, S.F. Chandler, B.A. Kunz, B.G. Tatham, E. Ostrowska and F.R. Dunshea.**
Altering flavonoid gene pathways in linseed and mustard to enhance their properties as functional foods for human health

14 **Paul A. Pickering, Mrinal Bhave.**
Analysis of Genetic Factors Determining Grain Hardness in Australian Wheat Cultivars.

15 **Graham Eariss, Maria Hrmova, Geoffrey Fincher & David E. A. Catcheside**
Directed evolution of barley β-D-glucan endohydrolases

16 **Steven Henderson, Briony Forbes, Leah Cosgrove & David E. A. Catcheside**
Directed evolution of human growth factors in *Neurospora crassa*

17 **F.J. Bowring, P.J. Yeadon, R.G. Stainer and D.E.A. Catcheside.**
Mutation of *spo-11* and its effect on meiotic recombination in *Neurospora crassa*.

18 **Edward J. Vonarx, Lisa J. McCarthy, Nyree Mathe, Peter Mohr and Bernard A. Kunz.**
AtRev7, an *Arabidopsis* homologue of the human and yeast polymerase zeta Rev7 subunit, restores UV resistance in a yeast *rev7* mutant

19 **Heather J. Anderson, Antonio Di Rubbo, Edward J. Vonarx, Desma M. Grice and Bernard A. Kunz.**
Interaction of two *Arabidopsis* PCNA homologues with *Arabidopsis* AtRad30, a putative homologue of human and yeast polymerase eta

20 **Edward J. Vonarx, Heather J. Anderson, Megan J. Osmond, Emma K. Tabone, Bernard A. Kunz**
Arabidopsis AtXPD and Atp44 participate in transcription and/or nucleotide excision repair in yeast

21 **Davis MA, Askin MC, Hynes MJ.**
The *Aspergillus nidulans sarA* gene encodes an L-amino acid oxidase

22 **T. Betteridge, J. Yang, A. J. Pittard, and J. Praszkier**
Interactions of the replication initiator protein RepA with the origin of replication in IncB plasmid.

23 **Sally Isberg, Peter Thomson, Frank Nicholas, Emily Gray, Fredoun Ahmadi-Esfahani, Stuart Barker and Chris Moran**
Genetic Improvement of Saltwater Crocodiles

24 **Fahri Fahri, Liz Nugent Mark Jois and Brendan Tatham**
An *in vitro* model for intramuscular fat in beef cattle is effected by sire genetic estimated breeding value, steer age and cell treatment with thiazolidinedione

25 **M. Cornish, M. Muralitharan, E. Ostrowska, F. Dunshea and B. Tatham**
Comparative mechanisms of hypertrophy between ovine and murine muscle models

26 **D. Hulett, M. Muralitharan, E. Ostrowska, F. Dunshea and B. Tatham**
Effects of genetic polymorphisms on gene expression and muscle hypertrophy

27 **C. Ketses, M. Muralitharan, E. Ostrowska, B. Tatham and F.R. Dunshea**
Effects of bovine whey and soy protein supplemented diets on biochemical signalling pathways involved in muscle development in pigs

28 **J. Ferrari, M. Muralitharan, E. Ostrowska, B. Tatham and F.R. Dunshea**
Expression Peroxisome Proliferator Activated Receptor (PPAR) and Lipogenic enzyme activity on bovine whey and soy protein isolates fed minipig

29 **B.Konsak, M. Muralitharan, T. Crowley, E.Ostrowska, F.R. Dunshea and B. Tatham**
Comparative genome mapping of pig and sheep for economic traits

30 **Hollingsworth KS, Duff RM, Ly T, Wilton SD.**
Identification of susceptibility loci in complex disorders

31 **Robert Flegg, M. Luisa Ashdown and Martin L. Ashdown**
HIV and the Human Genome

32 **Jaclyn Aldenhoven, Yizhou Chen and Chris Moran**
Comparative mapping of HSA1q with respect to porcine chromosomes further refines evolutionary breaks

33 **Kerryn E. Slack, Frederic Delsuc, Gillian C. Gibb, Mary Morgan-Richards, Steve Trewick, Gabrielle L. Harrison, Matthew J. Phillips, Patricia A. McLenachan, Alan Cooper, Ulfur Arnason, David Penny**
Avian Evolution Using Complete Mitochondrial Genome Sequences

34 **Joseph GR. Cross, Gavan A. Harrison, Jennifer A. Marshall Graves.**
Cloning and Mapping of the Genomic Region containing the Tammar Wallaby (Macropus *Eugenii*) Orthologues of MHC genes.

35 **JT Fong, M. Delbridge, JAM Graves**
Detailed genomic comparison of the human Xp and wallaby 5p regions

36 **Edda Koina, Matthew Wakefield, Cristina Walcher, Christine M. Disteche, Jennifer Marshall Graves**
X inactivation of the Marsupial SLC16A2 Gene

37 **Tariq Ezaz, Simon Harvey, Brendan McAndrew and David Penman**
Sex determination in Nile tilapia (*Oreochromis niloticus* L.): isolation and physical mapping of sex-linked DNA markers

38 **Lynn Jones, Hamilton Fraval, Masha Smallhorn and Robert Saint.**
Identification of genetic interactors of the *Drosophila* Rho-GEF, *pebble*.

39 **Tetyana Shandala, Stephen Gregory, Hazel Dalton, Masha Smallhorn and Robert Saint**
The cytokinetic role of *Drosophila* Citron kinase in Rho GTPase signalling

40 **Istvan Belecz and Robert Saint**
The role of RacGAP50C during cytokinesis and in the organisation of the interphase cytoskeleton

41 **Jane Sibbons, Len Kelly and Robert Saint**
The *Drosophila dead ringer* gene is required for adult fly vision

42 **Adam Wall, Marie Phillips, Len Kelly**
Translation of a second open reading frame in *drosophila* dicistronic mRNAs is dependent on the absence of internal *AUG* codons in the first open reading frame.

43 **David A. F. Loebel, Bonny Tsoi, Nicole Wong and Patrick P. L. Tam**
A conserved intronic antisense transcript at the mouse *Dnm3* locus

44 **Derek Collinge, Steve Whyard, Carolyn Behm**
Weapons of Mass Transformation: Biolistic DNA Delivery in *Helicoverpa armigera*

45 **Ariadne Tan-Kristanto, David Heckel, Phil Batterham**
Lipase Expression in *Helicoverpa armigera*

46 **John Damino, Lorin Magoc, Gerald Full, Philip Daborn, Philip Batterham**
Towards positional cloning of RST(1B) CYR, a mutant conferring cyromazine and lufernuron resistance in drosophila melanogaster

47 **Zhenzhong Chen, Ayscha Hill-Williams, John A. McKenzie, Narelle Sales, Garry Levot, Philip Batterham**
Overexpression of cytochrome P450 genes and insecticide resistance in the sheep blowfly, *Lucilia cuprina*

48 **Charles Robin, Jayne Lydall, Michael Bogwitz, Trent Perry, Jason Fair, Phil Batterham, Belinda Appleton.**
Gene duplication, selective sweeps and fitness costs of P450s implicated in insecticide resistance.

49 **Payam Arasta, Yizhou Chen, Yuandan Zhang , Richard Kerr , Chris Moran**
Elimination of CYP21 as a candidate gene for androstenone boar taint

50 **R.J.H. Hallas, M. Telonis-Scott, A.A. Hoffmann**
Correlated responses following selection for desiccation and starvation resistance: towards genetic dissection and gene identification

Speaker Abstracts

(in alphabetical order)

Response to selection for chill coma recovery in *Drosophila melanogaster*

Alisha R Anderson[1], Ary A Hoffmann[2] and Stephen W McKechnie[1].
1. School of Biological Sciences, Monash University, Victoria 3800 Australia 2. CESAR, La Trobe University, Bundoora 3083 Australia

Invertebrate distributions are often related to their tolerance of low temperatures and this can vary between and within species. Cold tolerance has been difficult to study genetically mainly because exposure to sub-zero temperatures results in low and variable fitness of offspring from cold-stressed mothers. One measure of cold tolerance that varies geographically in *Drosophila melanogaster* and that is amenable to genetic analysis is chill-coma recovery. Three replicate lines of *D. melanogaster* were selected every second generation, for over 30 generations, for fast recovery following exposure to 0°C. Lines responded rapidly to the intermittent selection regime with realized heritabilities varying from 33% to 46%. Selected lines showed faster recovery after exposure to a range of low temperatures and also had a lower mortality following a more severe cold shock, indicating that a general mechanism of tolerance had been selected. The selection response was independent of plastic changes in cold tolerance because the selected lines maintained their ability to harden (ie, a short-term exposure to cool temperature resulted in faster recovery in subsequent assays). Heat tolerance of the lines was not changed by the selection. These results indicate that chill-coma recovery can be rapidly altered by selection, as long as selection is undertaken every second generation to avoid carry over effects, and suggest that lower thermal limits can be shifted towards increased cold resistance independently of upper thermal limits.

Population genetic diversity and structure in the endangered New Zealand shrub *Hebe speciosa:* implications for conservation and ethnobotany

Tristan Armstrong[1] and Peter J. De Lange[2]
1. Landcare Research, Private Bag 92170, Auckland, New Zealand. 2. Science & Research Unit, Department of Conservation, Private Bag 68908, Newton, Auckland, New Zealand

AFLP (Amplified Fragment Length Polymorphism) markers were used to investigate the population genetic structure and phylogeography of the highly endangered endemic New Zealand shrub *Hebe speciosa* (named titirangi by Maori). The six remaining wild populations are threatened by significant habitat modification and the loss of native pollinators and dispersers. Genetic diversity was positively correlated with population size, but also varied considerably between geographic regions. Among-population genetic differentiation was high (mean pairwise Fst = 0.47) indicating complex historical relationships and negligible contemporary gene flow between populations. Extremely low genetic diversity in southern populations relative to northern sites suggests that southern populations may be more recent in origin. Patterns of phylogenetic structure, together with archaeological evidence, indicate that some populations are likely to have originated from pre-European Maori dispersal and cultivation of the species. Low seed set and recruitment in small populations of this self-incompatible *Hebe* may be associated with the loss of s-alleles through drift. Active management of the species' remaining genetic diversity may be necessary.

Genetic variation within the aerial seed bank of the shrub Banksia hookeriana (Proteaceae)

Luke G. Barrett[1], Tianhua He[1], Byron B. Lamont[1] and Siegfried L. Krauss[2]
1. Department of Environmental Biology, Curtin University of Technology, Perth, WA 6845, Australia. 2. Botanic Gardens and Parks Authority, Kings Park and Botanic Garden, Fraser Avenue, West Perth, WA, 6005, Australia; School of Plant Biology, Faculty of Natural and Agricultural Systems, The University of Western Australia, Crawley, WA, 6009, Australia.

We examined the genetic structure of the seed bank of *Banksia hookeriana* within a single 15-year-old population in southwestern Australia, and compared genetic variation between adult and seed bank populations using AFLPs. *B. hookeriana* is ideal for the study of seed bank dynamics due to the canopy storage of its seeds, and because each annual crop can be identified. A total of 304 seeds, representing 9 crop years, were genotyped from the aerial seed bank of five maternal plants, along with the entire adult population of 113 plants. The number of polymorphic loci increased logarithmically within the seed bank over time, while overall genetic diversity as indicated by gene diversity (H_j), and Shannon's index (I) did not change. The mean genetic dissimilarity of seedlings from the seed bank was significantly less than mean genetic dissimilarity of all plants in the adult population. However, there were no significant differences for gene diversity or Shannon's index between adults and the seed bank. Linear regression analysis showed that the mean number of non-maternal fragments decreased significantly with seed age, indicating that offspring became relatively more outbred on average as the population aged. These results are discussed with regard to the potential for variable fire frequencies to influence the amount of genetic variation stored within the seed bank.

Novel genetic basis of field-evolved insect resistance to Bt toxins

Simon W. Baxter[1], Jian-Zhou Zhao[2], Linda J. Gahan[3], Anthony M. Shelton[2], Bruce E. Tabashnik[4], & David G. Heckel[1-5]
1. CESAR, Department of Genetics, University of Melbourne, Parkville, Victoria 3010, Australia; 2. Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA; 3. Department of Biological Sciences, Clemson University, Clemson, SC 29665, USA; 4. Department of Entomology, University of Arizona, Tucson, AZ 85721, USA; 5. Max Planck Institute of Chemical Ecology, Hans-Knöll-Strasse 8, Beutenberg Campus, Jena D-07745, Germany.

Insecticidal toxins from *Bacillus thuringiensis* (Bt) are widely used in sprays and transgenic plants to control insects that attack crops and transmit diseases, but evolution of pest resistance threatens their continued efficacy. Diamondback moth (*Plutella xylostella)* is the only pest that has evolved Bt resistance in open field populations, although several others have been selected for resistance in the laboratory or glasshouses. The most common type of resistance ("Mode 1") is characterized by recessive inheritance, >500-fold resistance to at least one Cry1A toxin, and negligible cross-resistance to Cry1C. The primary mechanism of Mode 1 resistance is reduced binding of Bt toxins to midgut membrane target sites. Mutations affecting a Cry1A-binding midgut cadherin protein are tightly linked to laboratory-selected Mode 1 resistance in two major pests, *Heliothis virescens* and *Pectinophora gossypiella*. The cadherin gene has thus been considered the prime target for DNA-based screening for resistance. Here we show that field-evolved Mode 1 resistance in diamondback moth has a different genetic basis, indicating that screening for resistance in the field should not be restricted to searching for cadherin mutations.

A comparative phylogeographic study of two genera of funnel web spider (*Hadronyche & Atrax*) in Tallaganda State Forest (NSW)

Amber Beavis
Division of Botany & Zoology, Australian National University, ACT, 0200, Australia.

Decomposing logs are habitat to invertebrate species occurring across a spectrum of ecological niches. A collaborative research project is examining patterns of genetic endemism among saproxylic (log-dwelling) invertebrates across Tallaganda State Forest (NSW). Previous study of the onychophoran *Euperipatoides rowelli* revealed strong population structuring, identifying five areas of local endemism. Subsequently, *E. rowelli* has been used as a base-line study species for a comparative phylogeographic study of saproxylic invertebrates. To date, analysis of the mitochondrial gene Cytochrome Oxidase I (mtCOI) has revealed that a number of taxa display phylogeographic congruence with *E. rowelli*, suggesting that the saproxylic habitat has been a major factor in the evolution of these invertebrate species.

Hadronyche and *Atrax* are two ecologically similar genera of funnel web spider found across Tallaganda State Forest, however, *Hadronyche* species are log-dwelling whereas *Atrax* species are ground-burrowing. The niche partitioning of unnamed species within these genera allows the separation of species and habitat as contributing factors to the patterns of local endemism found in this system. The resolution of this question is central to defining the impact of habitat on the evolution of log-dwelling invertebrates and critical to the establishment of conservation priorities for invertebrate species in Tallaganda State Forest.

Evolutionary genetics of carpels: Using California poppy (*Eschscholzia californica* Cham.) as a basal eudicot model system

Annette Becker and David Smyth
Monash University, School of Biological Sciences, Wellington Road, Clayton, VIC 3800

All flowering plants have carpels, female reproductive structures that enclose the eggs and develop into seed pods and fruits. Carpel development genes are being defined in *Arabidopsis*, a higher eudicot. But the evolutionary origin and the molecular basis of the enormous variety of carpel morphologies among flowering plants is not clear. Here, we describe the characterisation of genes that control carpel development in a more primitive plant, the California poppy (*Eschscholzia californica*). This new model species is a basal eudicot that can be manipulated transgenically allowing for functional analysis of gene functions.
We have been able to identify homologs of the *Arabidopsis* carpel development genes *AGAMOUS* and *CRABS CLAW* in the Californian poppy. Comparison of carpel genes in California poppy and *Arabidopsis* will help reveal core genes that underlie carpel development in all dicots.

Marsupial and monotreme MHC class II genes

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The Major Histocompatibility Complex (MHC) plays a central role in controlling immune responsiveness and susceptibility to disease in vertebrates. The MHC is divided into three classes (I, II and III). In order to study the evolution of the mammalian MHC, we are characterizing MHC genes from marsupials and monotremes. Among eutherian orders orthologous relationships can be discerned between MHC class II gene families. However, orthologs have not been identified in marsupials or monotremes and it appears that there has been rapid gene turnover in this region during mammalian evolution.

Five tammar wallaby (*Macropus eugeii*) and one platypus (*Ornithorhynchus anatinus*) MHC class II loci have been identified. I will describe their characterization through isolation of cDNAs and BACs. I will address nomenclature of new genes and discuss our plans for discovering further genes. I will present fluorescence *in situ* hybridization results which localize the tammar wallaby MHC to chromosome 2q. We have found high levels of MHC diversity at the DZB locus in mainland and Tasmanian platypuses. However, the King Island population is monomorphic at this locus raising conservation concerns.

Drosophila as a model for human neurodegenerative disease

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Human neurodegenerative diseases, such as Alzheimer's, Huntington's and Parkinson's diseases, are late-onset progressive degenerative disorders for which few treatments are available. In order to pioneer new approaches to these diseases, my laboratory developed *Drosophila* as a model for human neurodegenerative disease. We initially did this for the polyglutamine disease spinocerebellar ataxia type 3, where an expanded run of the amino acid glutamine within the protein causes neurodegeneration. We subsequently applied the same approach to Parkinson's disease, where dopaminergic neural degeneration is observed upon _expression of α-synuclein. We have used these models in order to learn about the mechanisms by which the proteins are toxic to neurons, and to develop new approaches to treatment. Our findings indicate that polyglutamine and α-synuclein toxicity share some common mechanisms, and that upregulation of stress pathways may be of benefit in the treatment of human neurodegenerative disease.

Nuclear ribosomal DNA evolution in polyploid *Glycine tomentella* (Leguminosae)

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Polyploidy is a widespread feature of plant genomes that has major effects on molecular evolution and genomic interactions. In particular the composition of nrDNA tandem arrays is subject to repeat loss, interlocus concerted evolution, and differential expression (nucleolar dominance). We used direct sequencing of PCR products from universal and repeat-specific primers to characterize nrDNA internal transcribed spacer (ITS) homoeologues from multiple accessions of the *Glycine tomentella* complex. This species complex includes at least six tetraploid and five diploid taxa, and ITS phylogenies pointed to multiple origins for four of the polyploid races. In most tetraploids, both homoeologous nrDNA repeats are present, but often in highly biased amounts, which F2 segregation data indicate can be due to repeat loss or interlocus concerted evolution with both processes occurring within a taxon. Allotetraploid populations of the same genome combination can differ as to which repeat predominates. Using an RT-PCR technique, ITS expression was compared among tissues, individuals, polyploid lineages, and in synthetic amphiploids. Nucleolar dominance was the rule in natural polyploids, usually incomplete and for the predominant repeat. The results suggest that the establishment of nuclear dominance is a complex process involving both repeat-specific and stochastic events.

Is susceptibility to infectious disease written in the genes?

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Conservation genetics theory predicts that genetic diversity and/or the possession of particular alleles will play a major role in selection and fitness, for example, susceptibility to infectious disease. Hypotheses such as these, are difficult to evaluate quantitatively with direct measures of disease-related mortality.
Tammar wallabies (*Macropus eugenii*) are medium sized macropods endemic to southwestern Australia. Tammars are susceptible to an orbivirus, which causes Tammar sudden death syndrome (TSD) and results in a morbidity of ~50%. Disease onset is undetectable until the animal collapses and death rapidly follows. In the spring of 1998 an outbreak of TSD caused an epidemic in a captive colony of tammars. Mortality was ~50% of 80 animals. MHC class II genes (*DAB* and *DBB*) from the tammar wallaby have been characterised. Characterisation of class I genes is in progress. I will discuss the correlation between microsatellite diversity, MHC diversity and TSD epidemic victims and survivors. Microsatellite data suggest an association between genetic diversity, relatedness and susceptibility to this infectious disease.

Duplication and diversifying selection among termite antifungal peptides

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The innate immune system provides the last line of defence against pathogens in invertebrates and therefore components of this system are likely to be focal points for host-pathogen conflicts. Selection on molecular components of the innate immune system may be particularly strong in social insects because group living increases their vulnerability to disease. We have recently identified the mRNA sequence of defensin-like peptides from several termite species in Australia. Comparison of the relative fixation rates of synonymous (silent) and nonsynonymous (amino acid altering) mutations indicates that these defensins are positively selected. Moreover, duplication and parallel divergence among these defensins appears to be a repeated strategy for coping with the evolution of fungal resistance.

Identifying signals that regulate male reproduction in Drosophila

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Gametes are produced throughout the adult life of *Drosophila* and as a consequence the adult *Drosophila* testis contains germ cells at different stages of development. Different signals are shared between germ cells and somatic support cells at different stages of spermatogenesis in order to facilitate the production of functional spermatozoa from germ line stem cells. The ordered differentiation of cells observed in the *Drosophila* testis makes it an excellent system for identifying signals that are utilized during germ cell differentiation. In contrast to many renewing tissues, the identity of the germ line stem cells and their morphology, position in the gonad, and physical relationship to surrounding somatic cells are known. We have used the GAL4-UAS system to ectopically express genes in testis stem cells and surrounding somatic cells. Expression of the BMP2/4 homologue, *decapentaplegic*, in germ line stem cells resulted in an overproliferation of stem cell-like cells. Expression of activated BMP-family or activin-family receptors confirmed that these signaling pathways directly affected germ cells. Activation of either signaling pathway in germ line stem cells also resulted in the formation of clusters of proliferating cells in the basal testis, removed from the normal site of stem cell proliferation. These clusters displayed early germ cell characteristics but were heterogeneous for the expression of a stem cell marker. We suggest that disruption of signalling in the stem cell niche can allow stem cells to escape the niche. Once displaced, these cells may proliferate in a manner allowing some cells to retain stem cell identity and some to display an intermediate phenotype. An endogenous role for TGF-beta signaling in regulation of stem cell proliferation was shown by the ability of an inhibitory Smad (*Dad*) to cause cells to lose stem cell identity. We have also identified other pathways that may play roles in regulating germ cell differentiation.

A molecular analysis of the role of c-Rel in chromatin remodelling and cytokine gene transcription in T cells

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Regulation of inducible cytokine genes in T lymphocytes occurs largely at the level of altered chromatin and activated gene transcription. We have previously shown that c-Rel, an NF-κB/Rel family member, is critical for chromatin remodelling across the IL-2 gene promoter in T cells and more recently, that c-Rel is required for the regulation of a whole array of genes. To better understand the role of c-Rel in inducible gene regulation, we are undertaking a structure/function analysis of the human c-Rel protein. We have identified a series of mutations in c-Rel which exhibit decreased transcriptional activity and/or dominant negative activity in reporter assays. We have shown that these mutants have normal expression in T cells and have characterised the effects of the mutations on c-Rel binding to DNA. Having demonstrated a novel interaction between c-Rel and the histone acetylase CBP/p300, we speculate that chromatin remodelling on the IL-2 gene may require the recruitment of remodelling complexes by c-Rel and/or the binding of c-Rel to DNA to stabilise a remodelled state. Interestingly, a mutation in serine267, a conserved phosphorylation site in c-Rel, reduces c-Rel transcriptional activity but may be important for transcriptional synergy with CBP/p300. To dissect the role of c-Rel in IL-2 promoter remodelling, we are using CHART-PCR analysis of the IL-2 gene in activated T cells over-expressing c-Rel mutants. These approaches will define more clearly the function of c-Rel in the regulation of cytokine gene transcription in T cells.

Strike
Good
logically
organised
some study
busy

Chromatin remodelling focus, but
expts not too convincing that
CBP has a role - only 5x only?
technically challenging

2 questions Richard N Merzel answered OK.

Testing biogeographic hypotheses using intraspecific molecular data: phylogeography of giant freshwater prawns

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Biogeographic patterns are characterised by distinct faunal and floral assemblages, but relationships among groups of taxa often vary and may not be discrete. Historical biogeography as a consequence, while providing crucial insights into the relationship between biological diversity and earth history, has some limitations. Patterns of intraspecific molecular variation, however, may show unambiguous evidence for geographically mediated isolation or interchange events, and can be used to test competing biogeographic hypotheses (often based on the dispersal-vicariance debate). Here we utilise this method to test the hypotheses that: 1.) a major biogeographic transition zone between the Sundaic and Indochinese biotas, located just north of the Isthmus of Kra in SE Asia, is the result of Neogene marine transgressions that breached the Isthmus in two locations for prolonged periods of time; 2.) Lake Carpentaria (*circa* 80 000-8 500 BP) facilitated genetic interchange among aquatic organisms during the Late Pleistocene; 3.) New Guinea's Fly River changed course from its current easterly outflow to flow westwards into Lake Carpentaria during the Late Pleistocene. Phylogeographic analyses of giant freshwater prawn (*Macrobrachium rosenbergii*) populations supports: 1.) the historical existence of the more northerly postulated Isthmus of Kra Seaway; 2.) that Lake Carpentaria facilitated interchange; but contests 3.) the westward diversion of the Fly River.

Verifying Existing Mutation Data

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This work describes some preliminary results of an ongoing survey of data and query quality on general mutation and locus specific databases (LSDBs) and outlines a method for automatically verifying consistency between mutation description correctness, i.e. consistency with the affected wild type nucleotides. The survey is part of a project to provide a single point of entry for and enquiry on all known mutation data by integrating the existing mutation databases. For a mutation description to be meaningful it must include the mutation's nucleotide position within a specified reference and the effect of the change - preferably as a *standardised name* such as **PAH c.117C>T** or **BRCA1 r.1224_1226delGAT**: correctness requires further that (for the latter example) nucleotides 1224 to 1226 of the BRCA1 mRNA reference sequence are in fact GAT. Survey results to date indicate that most but not all descriptions are correct, but manual verification is time-consuming especially for intronic mutation descriptions such as **c.IVS2+5G>T** or **c.2238-3delT**, for which knowledge of exon boundaries is required to determine actual genomic nucleotide positions. So we are prototyping a method that (where possible) automatically verifies mutation descriptions: for intronic mutations this uses exon boundaries typically included in GenBank genomic reference sequences.

Waiter there's a fly in my Ti Tree: Characteristics of a fruit fly outbreak

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Until recently the northwest of Australia was thought to be relatively free of fruit fly pests. However from 2000 there have been a number of outbreaks in developing horticultural regions in both the Northern Territory and Western Australia. One such outbreak occurred in Ti Tree, a grape and mango growing town north of Alice Springs, where the outbreak was detected and contained relatively quickly. Although suspected of coming from Alice Springs, finding the exact cause of the outbreak was complicated by the fact that very little is known about the fruit flies in the area. We have used microsatellite analysis to firstly characterise the populations of fruit fly in the northwest and then used these profiles to identify the source of outbreak populations. Assignment testing and cluster analysis have confirmed that the most likely source of the Ti Tree flies is Alice Springs. Since the source of the Ti-Tree flies could be identified the outbreak provides an excellent opportunity to model the characteristics of an outbreak and to predict the size of the outbreak population. The mitotypes of outbreak flies and background populations were used in simulations to provide a model of the outbreak in terms of changes in heterozygosity, allele numbers and linkage disequilibrium.

Estimating the number of real QTL.

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Classical quantitative genetics assumes an infinitesimal model, in which the overall genetic variance is explained by many genes each with small effect. QTL mapping experiments have demonstrated that this model is incorrect, that some genes do have a finite effect on the trait. In the absence of experimental error, QTL mapping experiments would identify how many genes control the genetic variance for a trait, and what the gene substitution effect is for each QTL. However, sampling errors and significance tests lead to several biases in the number of QTL reported and their effects. Generally only significant QTL are reported, underestimating the number of QTL and overestimating their effects (Beavis, 1994; Georges et al., 1995). Unbiased estimates of the number of QTL controlling typical traits would improve our understanding of the genetics of quantitative traits and allow accurate correction of the biased QTL effects, resulting in larger gains from marker assisted selection (MAS). Inaccurate estimates of QTL effect erode the benefits from marker assisted selection (MAS) (Ruane and Colleau, 1996; Spelman and van Arendonk, 1997).
We have conducted a genome scan for QTL affecting milk yield and composition in 6 large half sib families of dairy cattle, and developed methods that yield unbiased estimates of the number of QTL. We found that the average animal is segregating for 9 QTL affecting one of these traits and that on average 20% of the 6 sires are heterozygous for each QTL.
We have also developed methods to estimate bivariate distributions. The three independent traits used measure three biological scenarios, altered milk yield of fixed composition, lactose synthesis and fat synthesis. Results revealed that the majority of detected QTL appear to have a substantial effect on only one of the three traits.

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No nonsense with insecticide resistance: cloning a resistance gene with orthology to nonsense mediated decay genes of *C. elegans* and humans.

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Nonsense Mediated Decay (NMD) is a molecular proof reading system that degrades transcripts with premature termination codons (PTC). In *Caenorhabditis elegans* the key NMD genes (*Smg1-7*) were originally uncovered as suppressors in screens for three unrelated characters. All seven genes have orthologs in mammals and appear to have conserved functions in NMD. By knocking out any one of these genes it is possible to have PTC transcripts produce functional albeit truncated proteins and thus suppress the PTC mutation. Recessive mutations can be rendered dominant by allowing dominant negative proteins to be produced. Cyromazine is an insecticide with an unknown molecular target. In a screen for cyromazine resistance we have isolated mutants in the ortholog of *Smg1* in *Drosophila melanogaster*. Our analyses of these mutations support other studies showing that NMD pathway is significantly different in *D.melanogaster* compared to *C. elegans* and humans.

Comparative phylogeography of two terrestrial flatworms at Tallaganda, NSW

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As part of a study of diverse invertebrates, phylogenetic and comparative phylogeographic analysis was performed on terrestrial flatworms *Caenoplana coerulea* and *Artioposthia lucasi* at Tallaganda in the Great Dividing Range in SE NSW. *Caenoplana coerulea* is larger and faster-moving, and thus is expected to show a shallow phylogeographic structure, relative to *Artioposthia lucasi*. A region of the mtCO1 gene was screened with SSCP and sequencing. AMOVA and spatial autocorrelation analyses revealed four mitochondrial genetic subregions within Tallaganda, closely following the predictions of a model of past and present environmental variation at the site. Very few haplotypes were shared across the subregional boundaries, but individuals within each region exhibited identical or similar haplotypes. Whilst recognizing the same genetic subregions and the same apparent long-term environmental refuge, the two flatworms showed quantitatively different patterns consistent with their size and mobility. The less mobile *Artioposthia lucasi* showed deeper phylogeographic structure compared to *Caenoplana coerulea*. Assuming a molecular clock, *Caenoplana coerulea* mtDNA at Tallaganda dates back to Early / Mid Pleistocene with subregional radiations in the Late Pleistocene. *Artioposthia lucasi* shows similar episodes, but also an older split around the Late Miocene.

Mechanisms of heavy metal detoxification and homeostasis in plants

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Heavy metals, such as zinc and copper, are essential but toxic in excess or, such as cadmium and mercury, are simply toxic. Plants, like all organisms, have evolved mechanisms for responding to heavy metal toxicity and for the provision of essential metals to their tissues and cells. Such mechanisms include the expression of metal-binding compounds and numerous families of heavy metal transporters.

We have used the model genetic organism, *Arabidopsis thaliana*, to explore the genetic, biochemical and physiological basis for some of these mechanisms in plants. This has involved both forward genetic approaches and, with the completion of the Arabidopsis genome sequence, reverse genetic approaches.

The isolation and characterisation of cadmium-sensitive mutants led to the identification of genes involved in the biosynthesis of a family of heavy-metal binding compounds, the phytochelatins (PCs) (1,2). These genes include those involved in the biosynthesis of the substrate for PC biosynthesis, the tri-peptide reducing agent, glutathione (3), and the gene encoding PC synthase itself (4). This work established the central role of PCs in heavy metal detoxification mechanisms in plants and led to the identification of similar mechanisms in some animal species. Work continuing in our laboratory is aimed at exploring the roles of glutathione in various cellular functions (5).

Among the families of heavy metal transporters, the P_{1B} ATPases are well recognised. In most eukaryotes there are one or two such transporters involved in copper transport. In contrast, in Arabidopsis there are eight P_{1B} ATPases. Recent work has focussed on a group of three of these, HMA2, HMA3 and HMA4. Genetic analysis has shown HMA2 and HMA4 are essential for zinc homeostasis: an *hma2,hma4* double mutant is severely zinc deficient (6). Symptoms include chlorosis, stunting and failure to develop pollen, and the phenotypes are reversible by the application of exogenous zinc. In contrast, the role of HMA3 *in vivo* is less clear. Promoter-GUS reporter constructs show *HMA2* and *HMA4* have parallel expression patterns in vascular tissues and in developing anthers. These HMA transporters appear to have important roles in the vascular transport of zinc from root to shoot and in the delivery of zinc to developing pollen. Continuing work in our laboratory is exploring the functions of the HMA transporters and to identify other genes involved in zinc homeostasis. For example, a genetic screen for zinc-sensitive mutants affected in zinc homeostasis has identified a member of the Major Facilitator Superfamily (MFS) group of transporters.

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PhD Uni Melb E.coli tyrosine ~~tryptophan~~ operon Tim Pittard

84 Lecturer Uni Melb Heavy metals

~~88~~ 89 Chris Somerville Uni Michigan Arabidopsis

Uni Melb Dept Genetics
Associate Professor
Head of ~~Biology~~ Dept from 1 Jan 05

Using genetic markers to understand the biology and population structure of grapevine phylloxera

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Phylloxera are important pests of grapevines overseas and in Australia, capable of devastating vineyards that are not planted on tolerant rootstocks. The Australian viticulture industry relies on quarantine to restrict phylloxera to a few isolated regions. Despite its importance, there is a poor understanding of phylloxera biology and the genetic structure of phylloxera populations. Here we use microsatellite markers to demonstrate that phylloxera in Australia reproduce asexually, and we use mtDNA data to show that several phylloxera lineages have been introduced from overseas. We demonstrate that most recent infestations are associated with only two widespread phylloxera genotypes, while other genotypes with more restricted distributions can persist on tolerant rootstocks. Some genotypes appear restricted to leaves, while others are always found on vine roots. These findings help in devising effective quarantine protocols, methods for assessing rootstock responses, and tools for predicting phylloxera spread within vineyards.

Phylogeography of the pencilfish *Nannostomus unifasciatus*, a flooded-forest fish from central Amazonia

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The richest biota in the world is found in the lowland forests of Amazonia. Despite its enormous importance as a source of biodiversity, very little is known about the evolutionary processes that have shaped diversification in Amazonia. This is particularly true for the extremely diverse and understudied Amazonian fish fauna. We are using genetic and biogeographical information to investigate spatial and temporal patterns of evolutionary diversification in six small fish species inhabiting the forests of the Rio Negro floodplain (RNF), in central Amazonia. One of our target taxa is the pencilfish *Nannostomus unifasciatus*, a popular aquarium fish used as fishery resource by riverine communities from the RNF, one of the world's major fishing grounds for ornamental fish. Here we present results based on analyses of microsatellite and mitochondrial DNA sequence data obtained from 360 individual pencilfish sampled from 19 tributaries along ~2,000 km of the RNF. Our study, which probably represents the first molecular phylogeographic survey of an Amazonian flooded-forest fish, unravels extremely high levels of genetic diversity and fine-scale population divergences. The implications of these results for conservation management of the RNF's ornamental fish resources and for understanding patterns of population diversification in flooded-forest fishes are discussed.

Subterranean islands in the desert: evolutionary history of stygofauna from calcrete aquifers of central Western Australia

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Thirty-four isolated groundwater calcrete (limestone) aquifers of central Western Australia (WA) have been recently shown to contain a diverse subterranean invertebrate fauna (stygofauna), including the world's most diverse collection of subterranean dytiscid diving beetles. Over 60 dytiscid species have now been described and each appears to be unique to an individual calcrete aquifer, with between one and four species per calcrete. We have investigated the evolutionary relationships of these species with surface diving beetle species using molecular phylogenetic and molecular clock analyses of a 1644 bp region of mitochondrial DNA. These analyses indicate that the subterranean lineages diverged from surface ancestral species between 5-10 mya, coinciding with a major period of aridification of the Australian continent during the Miocene. The analyses further revealed the possibility that each calcrete represents a "subterranean island", each with a highly endemic stygofauna that evolved independently through processes such as convergent evolution and both allopatric and sympatric modes of speciation. We test this hypothesis further using mtDNA sequence and morphological data from a second group of stygofauna, the crangonyctoid amphipods. Preliminary analyses indicate that populations of amphipods within calcretes have been isolated over a long time period consistent with the subterranean island hypothesis.

Juvenile hormone esterase of *Drosophila melanogaster.*

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The aim of this postgraduate project was to identify and characterise esterase genes potentially involved in juvenile hormone degradation from *Drosophila melanogaster*. Four genes were identified in the *Drosophila* genome as potential *Jhe* candidate genes. One was shown to be the *D. melanogaster Jhe* by peptide mass fingerprint analysis (Campbell *et al.*, 2001), while another (CG8424) appeared to be a *Jhe* gene duplication. Phylogenetic analysis of the four genes showed the putative *D. melanogaster Jhe* and the *Jhe* duplication gene products were most closely related to the JHE of Coleoptera. As Lepidopteran and Coleopteran JHEs do not cluster in phylogenetic analyses I expressed the two other potential JH esterases, CG7529 and CG9858, that clustered closer to the Lepidopteran JHEs.

The acyl chain length preference of the four expressed proteins was examined using artificial substrates, and the ability of the expressed proteins to hydrolyse different forms of JH was examined. Only one of the expressed proteins (JHE) kinetically resembled a JH esterase. Chiral selectivity in the hydrolysis of JHIII enantomers was observed by two of the expressed proteins (JHE and CG7529).

Cyclaneusma minus – A molecular characterisation.

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Pinus radiata is the most widely planted pine in the world. Rapid growth and desirable lumber and pulp qualities enable it to be the leading introduced species in Australia, New Zealand, and Spain, and a major species in plantations of Argentina, Chile, Uruguay, Kenya, and the Republic of South Africa. In these countries, *P. radiata* is a mainstay of the forest economy, serving internal markets, generating valuable foreign exchange reserves as an export, and reducing cutting pressure on native forests. However, profit and productivity of *P. radiata* plantations can be reduced due to the needle cast pathogen *Cyclaneusma minus*. *Cyclaneusma minus* is a relatively difficult trait to assess, and to date, minimal molecular research has been undertaken, highlighting the need to study and understand this pathogen. We have found there to be considerable cultural and genetic variation between isolates within Australia and New Zealand. This variation has been documented and investigated using techniques such as incompatibility testing and several molecular techniques have been explored including RAPDs, microsattelites, ITS and IGS regions. In addition, we have utilized PCR techniques to allow rapid detection of *C. minus* on *P. radiata*.

Regulation of genes important in pyridine alkaloid metabolism in plants

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There is increasing interest in understanding natural chemical defence strategies used by plants to protect them against disease and predators[1] and in the genetic engineering of secondary metabolic pathways [2,3] . The pyridine alkaloid anabasine, derived from amino acids aspartate and lysine, is toxic to many insect and mammalian predators and is present at high levels in foliage of a limited number of species. These include *Anabasis aphylla* from S. Eurasia, *Nicotiana glauca* from S. America and *N. debneyi* from S.E. Australia. Unlike the related alkaloid nicotine which must be transported from its site of synthesis in roots of *Nicotiana* species, recent studies suggest that increased synthesis of anabasine occurs directly in foliage of *N. glauca* following wounding and involves the transcriptional upregulation of key biosynthetic genes, such as QPRTase and A622[4]. Using regulatory and coding sequences from these genes, we are studying the molecular processes controlling their expression in foliage vs roots of different *Nicotiana* species with the aim of using gene up-regulation and/or down-regulation strategies to genetically alter alkaloid levels and/or profiles in host plants. Knowledge thus gained may facilitate the transfer of these chemical defence capacities into the foliage of non-alkaloid producing crops

N. glauca genomic library made — great northerns — 2 copies of QPT12 work ongoing — expression (will do RNAi) — 1 copy ODC2 " "

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Qs alkaloid function ?
common elements in promoters ?
how damaged ?
diploid ?

Predicting Adaptive Molecular Evolution: Simulation of HIV-1 Adaptation to Antibody Surveillance

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The vast amount of experimental data on the molecular interactions between HIV-1 and the immune system and on the population genetics of HIV-1 provides a unique opportunity to examine in detail the factors affecting the rate of adaptation and to predict evolutionary change. Although HIV-1 is known to adapt to antibody surveillance, the nature of selection by antibodies and the effects of protein functional constraints, cell superinfection, and viral recombination on adaptation are poorly understood. I use an individual-based, Monte Carlo computer simulation approach to investigate these issues. Preliminary results of simulations will be presented. Of special interest are (1) trade-offs between adaptation to antibody surveillance and adaptation to chemokine coreceptors, (2) the weakened link between virion genotype and phenotype due to cell superinfection and the production of hybrid virions, and (3) the effect of viral recombination on the rate of adaptation. The aims of these simulations are to predict the evolution of HIV-1 in response to antibody selection and to help design experiments to test the predictions.

Unravelling the evolution of mammalian dosage compensation

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Dosage compensation equalises the expression of genes on the X chromosome between male and female mammals. The mode of dosage compensation has long been established for two of the three major mammalian groups. Marsupials and eutherians ("placentals") achieve dosage compensation by inactivating one X chromosome in females. The third mammalian group, the egg-laying monotremes, diverged 210 million years ago, so knowing whether, and how, genes on the X chromosome are dosage compensated may help to unravel the evolution of dosage compensation in mammals.

We used Real-time PCR to demonstrate that three genes on the platypus X chromosome (UBE1, AR, G6PD) are transcribed at an equivalent level in cells from 8 males and 8 females. It remains to be determined whether this is achieved by X chromosome inactivation as in other mammals, or by quite a different mechanism.

If the platypus X is subjected to inactivation, it is unlikely to be under the control of *XIST* (X-Inactive Specific Transcript). We found that genes that flank *XIST* in eutherians (*CDX4, XPCT, ATRX* and *RBMX*) are not on the platypus X but map to chromosome 6. This suggests that X inactivation was brought under the control of *XIST* after the divergence of monotremes.

SNP loci association mapping in *Onchocerca volvulus*

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Treatment of *Onchocerca volvulus*, a parasitic nematode and the causative agent of River Blindness, relies solely on ivermectin. The long term intensive use of a single drug class seems certain to lead to the emergence of resistance, but the difficult biology of the organism, coupled with the fact that it occurs almost exclusively in poor West African countries and our lack of understanding of ivermectin's action against *O. volvulus* all indicate that detecting resistance will be extremely difficult. The development of genetic markers that could provide an early warning of resistance selection, as distinct from an assay for the resistance phenotype, is highly desirable since it does not require knowledge of the drug's mode of action or likely resistance mechanisms. However, because of its specificity for humans and complex, long life cycle *O. volvulus* is unsuitable for investigation by classical genetic techniques . To overcome this barrier, a combination of bioinformatic analysis of the highly redundant *O. volvulus* EST sequences with emerging techniques for linkage disequilibrium analysis is being applied to find and exploit single nucleotide polymorphisms (SNP's) as genetic markers for genetic responses to ivermectin treatment in this intractable organism.

All publicly accessible *O. volvulus* EST's were assembled into sequence contigs and the contigs searched for SNP's. Over 200 putative SNP loci were identified, 35 of which have been validated experimentally. The genotypes of individual worms were determined at 22 of these loci using genomic DNA samples of worms isolated from patients with known histories of ivermectin treatment and each pairwise combination of markers tested for linkage disequilibrium. We have defined 9 "linkage groups", each consisting of 3 loci that show linkage to each other, and have thus taken the first step toward the construction of a genetic map in *O. volvulus*. This is the first of its kind for a parasitic nematode. Furthermore, by incorporating patient treatment history, a strong statistical association has been revealed that links ivermectin exposure to one linkage group (composed of loci c777.2, c787 and 188001). This information allowed us to focus on fewer but more informative loci (including c777.2, c787 and 188001) in >600 worms for investigating selection associated with ivermectin usage . In particular, a revised analysis employing the additional genotype data has resulted in a stronger association between markers c777.2, c787 and 188001 and supports their association with patient ivermectin treatment history. The results from this study indicate the project will have significant scientific impact as the first application of emerging techniques in genetic analysis to a parasitic nematode and practical impact as the first key step in the development of a new generation of molecular-based diagnostic tools.

Swamping the Swamp gum: Habitat fragmentation and disturbance promotes hybridisation in Eucalyptus aggregata

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Altered population and habitat characteristics following the fragmentation of plant populations may accelerate rates of interspecific hybridization and lead to reduced remnant population viability. *Eucalyptus aggregata* is a woodland tree of the central and southern tablelands of New South Wales. The majority of populations are now restricted to small roadverge and remnant paddock sites where it is known to hybridize with two more common species, *E. rubida* and *E. viminalis*. This study aimed to determine if the proportion of hybrid seed can be related to population characteristics such as size, relative species frequency, isolation and flowering synchrony. Morphological and isozyme markers were used to assign seedlings from open-pollinated progeny arrays of pure and mixed *E. aggregata*, *E. rubida* and *E. viminalis* populations as either pure or hybrid. Results indicate that small and degraded populations produce higher levels of hybrid offspring. For example, in seed crops from small roadverge *E. aggregata* populations which are outnumbered by *E. rubida* and *E. viminalis* up to 45% of the offspring are hybrids. Population viability in these sites could be seriously compromised if hybrid offspring are less fit. In addition, the value of small sites as seed sources for revegetation programs is seriously compromised.

Jumping into the evolutionary "sweet spot": sequencing of the kangaroo genome.

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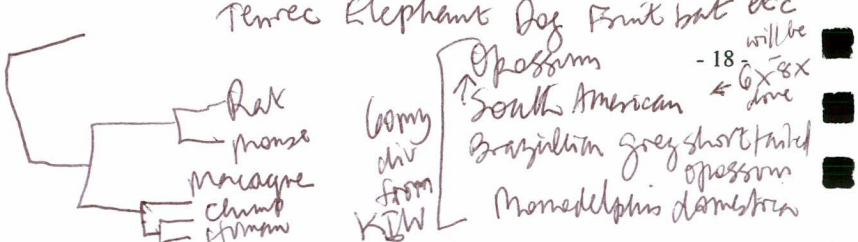
Australasian science has been given a unique opportunity to participate in a combined program with the National Human Genome Research Institute, part of NIH, in the USA. Following the success of the Human Genome Project, varying animal species are now next in the queue to have their genomes sequenced. The outcome will be the development of an evolutionary tree of genome sequences that will ultimately assist in gene prediction and gene regulation studies in the human. Professor Francis Collins has committed the NHGRI to an unprecedented agreement to match our Australian sequencing efforts read for read.

The kangaroo (tammar wallaby) genome initiative offers the opportunity to exploit a unique natural experiment. These wallabies show extraordinary adaptability to environmental challenges. They have unique features of lactation and reproduction that should be exploited for Australasia's economic benefit in the dairy industry, in livestock fertility and in the better understanding and management of human development and disease.

The project will entail generation of a 2 x total coverage of the genome, with Australia responsible for 1 x (6 million sequencing reads!). The sequences will be combined for assembly and downstream annotation. The history of the project and the potential outcomes will be described in this presentation.



Assembling an arsenal: Origin and evolution of venom in snakes

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The origin of venom in snakes has been a subject of long-standing research interest and the source of great controversy. We analyzed the origin and evolution of snake venom toxin families represented in both viperid (the most basal lineage) and elapid (the most derived) families within the diverse Colubroidea (advanced snakes), by means of phylogenetic analysis of the amino acid sequences of the toxins and related nonvenom proteins. Out of eight toxin families analyzed, five provided clear evidence of recruitment into the snake venom proteome before the diversification of the advanced snakes. In two others the nonmonophyly of venom toxins demonstrates that presence of these proteins in elapids and viperids results from independent recruitment events and one protein type was equivocal. These results provide strong additional evidence that venom evolved once, at the base of the advanced snake radiation, rather than multiple times in different lineages, with these toxins also present in the venoms of the ''colubrid'' snake families sold in pet-stores as 'non-venomous'. Moreover, they provide a first insight into the composition of the earliest ophidian venoms and point the way toward a research program that could elucidate the functional context of the evolution of the snake venom proteome.

Comparative analysis of gene clusters encoding secondary metabolites of filamentous fungi

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Genes responsible for the biosynthesis of secondary metabolites are typically clustered in filamentous fungi. We have recently cloned a cluster of genes responsible for the biosynthesis of a phytotoxic secondary metabolite, sirodesmin from *Leptosphaeria maculans*, which causes blackleg of canola (Gardiner et al. 2004). Disruption of one of the genes (encoding a nonribosomal peptide synthetase) resulted in a mutant unable to make sirodesmin. Sirodesmin belongs to the epipolythiodioxopiperazine (ETP) class of toxins produced by other fungi including the human pathogen *Aspergillus fumigatus* which makes gliotoxin, a molecule with immunosuppressive properties. We are determining the biosynthetic pathways for these molecules using a comparative genomics approach. We searched the complete genome sequences of filamentous fungi for the presence of similar gene clusters, potentially involved in ETP production. A gene cluster containing nine homologues of the *L. maculans* cluster genes was detected in *Aspergillus fumigatus*; we predict that this cluster encodes biosynthesis of gliotoxin. Gene clusters were also present in the rice blast fungus, *Magnaporthe grisea* and the wheat head scab fungus, *Fusarium graminaerum.* We are currently examining the role of particular genes in the cluster of both *Aspergillus fumigatus* and *Leptosphaeria maculans.*

Gardiner DM, Cozijnsen AJ, Wilson LM, Pedras MSC, Howlett BJ (2004) The sirodesmin biosynthetic gene cluster of the plant pathogenic fungus *Leptosphaeria maculans.* Molecular Microbiology, in press.

High local endemism of a saproxylic 'giant' springtail (Collembola) from SE Australia

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Comparative phylogeography can reveal processes and historical events that shape the biodiversity of species and communities. The phylogeography of a new endemic Australian genus and species of log-dependent (saproxylic) Collembola was investigated as part of a comparative project. Several genetic markers were used. We predicted genetic structure would correspond with five *a priori* microbiogeographic regions based on the palaeoclimatic history of the study site (Tallaganda State Forest, NSW), the species' ecological specialisation and its relatively sedentary lifestyle. We found significant genetic differences existed among regional samples ('populations'). Closely related mitochondrial DNA haplotypes were co-distributed within a single region or occur in adjacent regions, nuclear allele frequencies were most similar among more proximate populations, and inter-population migration is rare. A signature of historical vicariance was also evident: the spatial distribution of populations corresponded with four of the five putative microbiogeographic regions, and genetic contact zones were abrupt. The present-day distribution of genetic diversity in this species seems to have been influenced by three major climatic events: Pliocene cooling and drying (2.5-6 mybp), early Pleistocene wet-dry oscillations (1.2 mybp), and the more recent glacial-interglacial cycles that have characterised the latter part of the Quaternary (<0.4 mybp).

Tracking fruit flies in inland Australia

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Since European settlement of Australia, the Queensland fruit fly (*Bactrocera tryoni*) has expanded its range southward along the eastern coast of Australia. It has also moved inland, being present in almost all towns on the western slopes of NSW and northern in Victoria. It is now the largest single horticultural pest in Australia. In 1994, the Fruit Fly Exclusion Zone (FFEZ) was established in an attempt to limit range of Q-fly. This quarantine zone encompasses the Riverina and Murray Valley, which are areas of large horticultural production but only marginal habitat for Q-fly.
We have used microsatellites to investigate population structuring of fruit flies, particularly in and around the FFEZ. The questions we seek to answer are:

- What is the extent of individual subpopulations?
- What is the extent of migration between them?
- What are the origins of outbreak populations in the FFEZ?

As well as presenting data on the answers to these questions, some ecological interpretations of our observations will be discussed.

The first successful genetic manipulation of an animal parasitic nematode.

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Nematode parasites of animals have long been regarded as genetically intractable organisms. We report here the development of a genetic toolkit for *Parastrongyloides trichosuri*, a nematode parasite of brushtail possums (*Trichosurus vulpecula*). This toolkit consists of (a) inbred strains with genetic markers (SNP's and microsatellites) and techniques for single male female mating (b) heritable transformation of the parasite for the expression of transgenes and (c) RNAi of the parasite so we can knockdown the expression of target genes. We are using these tools to genetically manipulate that parasite as a potential vector for biological control of possums in New Zealand. We have also initiated a project to use these tools in the investigation of ageing in this species of nematode. We have shown that there is a 20-30 fold plasticity in lifespan in *P. trichosuri*, the biological equivalent of a 2400 year lifespan in humans. This lifespan plasticity is determined by a simple developmental switch which we can trigger, so we will use of combination of RNAi and transgenesis to determine the genetic basis of the switch and thus of this remarkable lifespan extension. The combination of this lifespan plasticity with our genetic toolkit establishes *P. trichosuri* as a potentially powerful new model for ageing research.

Wide genome comparisons reveal the origins of the human X chromosome

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The human X chromosome is the most conserved gene arrangement in mammals, although comparisons with marsupials and monotremes point to separate origins of the short and long arms 180 million years ago. Now much deeper comparisons are possible using draft sequences of the chicken, pufferfish and zebrafish genomes. These comparisons, over 310 and 450 million years respectively, reveal that the human X chromosome is composed of three evolutionary strata. Stratum 1 (most of the long arm) and stratum 3 (the distal short arm) are separate in marsupials, monotremes and chickens, but together in fish. They may have been present on a single chromosome in a vertebrate ancestor, undergone fission in a tetrapod ancestor and re-fusion in eutherians, or they may have originally been separate, and undergone fusion independently in eutherian mammals and ancestral teleost fish. A new Stratum 2 comprising the gene-rich regions of the proximal short arm and Xq28 represents an addition to the mammalian X from a single chromosome in a common ancestral vertebrate. These comparative data provide a first glimpse of the origin of the human X chromosome – and by default the Y – at the very base of vertebrate evolution. The blocks that make up the X chromosome have been strikingly conserved over all this time.

Genetic analysis of cytokinesis

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In order to identify and characterise the signal transduction pathway that drives cytokinesis, we have produced *Drosophila* strain with a sensitised background in which Rho signalling is reduced in some tissues. This blocks cytokinesis to make a small wing or eye that can are enhanced or suppressed by mutations that we know affect cytokinesis. To find new genes involved in the process we have screened a collection of enhancer-carrying transposons to identify those which can suppress the lack of Rho signalling by overexpressing an adjacent gene and restore eye morphology. This screen has identified several groups of proteins, including phosphoinositide regulators, cell cycle progression regulators and vesicle transport proteins.

We are currently concentrating on the role of one of the modifiers from this screen – the putative Rho effector kinase *citron*. Cell culture work implicates Citron in the regulation of cytokinetic furrow constriction through phosphorylation of myosin. We have shown that Citron localises to the contractile ring in response to Rho activation and is essential for cytokinesis, unlike the previously proposed effector Rock, which does not seem to be involved. We will present our analysis of *citron* mutants and the genetic interactions between *citron* and cytokinetic mutants

moesin

Functionally significant promoter and protein regions of *SPATULA*, an *Arabidopsis* gene involved in gynoecium development.

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It is the coordinated activity of many genes that produce the axial patterning, cellular differentiation, and cellular growth of the *Arabidopsis thaliana* gynoecium, to produce an apical stigma, short style, and a basal ovary. Internally, the ovary consists of placental tissue (gives rise to the ovules) and septum tissue (gives rise to the transmitting tract, required for pollen tube guidance).
The *SPATULA* (*SPT*) gene is implicated in the early developmental processes of gynoecium development. Strong *spt* mutants show a lack of medial growth early in gynoecium development that eventuates in a disruption in septum development and consequently the complete loss of transmitting tract tissue, causing a reduction in fertilisation.
SPT encodes a basic Helix-Loop-Helix (bHLH) transcription factor (162 members in *Arabidopsis*). *In situ* analysis has revealed a wider expression pattern than first considered based on the mutant phenotype, suggesting functional redundancy in other parts of the plant.
Promoter deletion experiments have revealed several functional regions responsible for temporal and spatial activation and/or repression of *SPT*. Efforts are now under way to isolate functional elements within these regions and the protein(s) targeting them. Despite the large number of bHLH genes in *Arabidopsis*, *SPT* shares homology outside the bHLH to only one other gene, *ALCATRAZ* (*ALC*). Results being presented will show that *SPT* and *ALC* have partially redundant functions. Also being presented is a comparative analysis of putative orthologs to *SPT* from a number of other closely related angiosperms. These alignments have highlighted the possibility of several other functional regions within the SPT protein besides the bHLH.

Phylogenetic relationships of African, Asian and European Suids

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Traditional taxonomy indicates that the Suidae family consists of three subfamilies; 'true' pigs (Suinae; *Sus* and *Potamochoerus*), warthogs (Phacochoerinae) and babirusa (Babyrousinae). Molecular data from some species of these subfamilies have been studied previously, and have proven consistent with the traditional classification. In the present study, we assessed the phylogenetic relationships of African, Asian and European suids, using mitochondrial 12S rRNA, cytochrome *b* and control region, and nuclear SINE PRE-1 P27 and P642 sequences, including some published sequences. Preliminary analyses show that European Wild Boar (*Sus scrofa*), Bearded pig (*Sus barbatus*), Javan Warty pig (*Sus verrucosus*) and Sulawesi Warty pig (*Sus celebensis*) clustered in a clade distinct from other suids including common warthog (*Phacochoerus africanus*) and Babirusa (*Babyrousa babyrussa*), which is consistent with traditional taxonomy. The African Red River hog (*Potamochoerus porcus*) and Bushpig (*Potamochoerus larvatus*) clustered in a clade close to the common warthog (*Phacochoerus africanus*) but distant from the Asian and European suids. This suggests that a common ancestor gave rise to these two African lineages. The present results are consistent with paleontological studies but not with the traditional taxonomy which places the 'bush pigs' (*Potamochoerus*) as a sister clade of the 'true pigs' (*Sus*) within the Suinae subfamily. This study suggests that a taxonomic review of the Suidae family is necessary. Additional analyses will be undertaken to substantiate these preliminary results.

SSR- and candidate gene- based linkage analysis of resistance and susceptibility to desiccation stress in *Drosophila melanogaster*

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A massbred population of *D melanogaster* was used to generate selected lines with increased resistance (termed D2 and D3) and susceptibility (termed D8 and D18) to desiccation stress. Following 26 generations of selection for resistance and 8 generations of selection for susceptibility, lines were inbred by single pair mating for 14 generations. After inbreeding, the lines D3.30 (mean death 21 hours) and D8.2 (mean death 5 hours) were chosen to generate reciprocal F_2 backcross mapping populations for linkage map construction and trait analysis. Each mapping population consisted of 4-day-old females that were subjected to desiccation stress and their time to death was scored. Backcross population one, (? D3.30 x ? D8.2) x ? D8.2, consisting of 204 individuals, showed a distribution pattern consistent with an effect of a single major gene or cluster of genes, with 42% of the population distributed below the mean of the D8.2 parent. In this population we have identified a region of chromosome 3R that contributes to 80% of the variation for susceptibility. Backcross population two, (? D3.30 x ? D8.2) x ? D3.30, consisting of 143 individuals, shows a similar distribution pattern with 34% of the population distributed above the mean of the parent D3.30. In this population we have identified a region of chromosome 3L that contributes to 60% of the genetic variation for resistance to desiccation stress. To refine the region of interest following the identification of genomic regions associated to desiccation stress, candidate genes identified using microarray were mapped using PCR-RFLP. Using this combined microarray and linkage map approach candidate genes for desiccation resistance and susceptibility have been identified.

ASCIZ regulates homologous recombination and apoptosis in response to DNA damage

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DNA damage response pathways are crucial for the maintenance of genome stability and the prevention of cancer, and inherited mutations in mismatch repair genes (MLH1 and MSH2) and homologous recombination genes (BRCA1 and BRCA2) are major causes of hereditary non-polyposis colon cancer and familial breast cancer predisposition syndromes. We have identified a novel human DNA damage protein termed ASCIZ that provides a molecular link between these two repair pathways. ASCIZ forms characteristic nuclear foci specifically in response to DNA damaging agents that give rise to single-stranded DNA (ssDNA) gaps via the mismatch repair pathway. ASCIZ foci contain the major recombinase RAD51 and ssDNA, indicating that they are sites of homologous recombination. ASCIZ depletion by siRNA abolishes DNA damage-induced RAD51 foci formation and dramatically increases damage-dependent apoptosis. We propose that ASCIZ functions as a lesion-specific molecular scaffold that organizes homologous recombination foci as a subnuclear structure or "recombinosome" for the repair of ssDNA gaps. ASCIZ foci formation and its role in DNA damage-induced apoptosis depend on the mismatch repair pathway. We have also isolated a novel yeast protein termed Mdt1 (Modifier of DNA damage tolerance) that is structurally and biochemically remarkably similar to ASCIZ, suggesting a conserved gene family.

Identification and characterisation of novel interactors of cytokinesis

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The activity of small G proteins is regulated by GTP exchange factors (GEFs), which stimulate GDP/GTP exchange, and by GTPase activating proteins (GAPs), which suppress activity by stimulating the intrinsic GTPase activity. The *pebble* (*pbl*) gene of *Drosophila melanogaster* is a RhoGEF that is required for Rho activation during cytokinesis.

To further understand the nature of the role of Pebble in cytokinesis, we have begun to map the genetic location of modifiers of a dominant-negative Pebble construct using polymorphic molecular markers. The modifier alleles were generated in an EMS screen for enhancement or suppression of the construct. We have a number of suppressors and enhancers, mapping to the second and third chromosomes. The technique that we are using to map the genetic location of the alleles involves using meiotic recombination with marked chromosomes. The chromosome markers are single nucleotide polymorphisms (SNPs) or indels. Once identified, theses genes can be further characterised to elucidate the mechanisms of cytokinesis.

Comparative phylogeography of the water skinks *Eulamprus heatwolei* and *Eulamprus tympanum* in southeastern Australia, with comparisons of co-distributed invertebrates at Tallaganda, NSW.

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The main strength of comparative phylogeography lies in the ability to detect shared patterns of genetic structuring. The greater the diversity of taxa compared, the greater the insights into the historical biogeography of the region. However, very few studies have compared genetic structuring in co-distributed invertebrates and vertebrates. We utilised 691 bp of the mitochondrial ND4 gene and associated tRNAs to examine population genetic structure in two species of water skink (*Eulamprus heatwolei* and *E. tympanum*) at Tallaganda on the Great Dividing Range (GDR) in southeastern NSW. Genetic structure was compared with several co-distributed invertebrate taxa, and a congruent phylogenetic break was revealed. To give this finding a greater biogeographic context, we also examined broad phylogeographic patterns in the water skinks from throughout their range. Water skinks in southern Tallaganda are more closely related to those further south in the GDR than they are to those in the geographically closer northern Tallaganda. Also, water skinks from Tallaganda were not monophyletic with nearby populations in the GDR. These findings suggest Tallaganda has been biogeographically isolated from this region of the GDR. Possible explanations for this are given in the context of historical landscape change and range contractions and expansions during the glacial cycles of the Quaternary.

Insecticide resistance and esterase patterns in two field populations of Australian *Helicoverpa armigera.*

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Helicoverpa armigera is a polyphagous species of noctuid moth occurring in Asia, Australia and Africa. Its high degree of polyphagy and ability to rapidly develop insecticide resistance have made it a significant agricultural pest. Our lab is interested in studying the genetic basis of insecticide resistance mechanisms that it has evolved. My project focuses on esterases, enzymes which are implicated in insecticide resistance in number of insect species, including *H. armigera*. Esterases are serine hydrolase enzymes catalysing hydrolysis of ester bonds, and with a few notable exceptions the *in vivo* role of the majority of esterases is unknown. Using molecular genetic and bioinformatics techniques we isolated a number of esterase genes and their sequences and expression have been examined in the two field collected strains. We have also measured current resistance levels of these two field populations of Australian *H. armigera* to pyrethoid insecticides. Since some previous studies have reported correlations with esterase expression and pyrethroid resistance we have systematically classified esterase isozymes from these strains using non denaturing polyacrylamide electrophoresis across life stages and tissue types.

Gene flow in wild populations of the native flat oyster (*O. angasi*): a species being considered for culture as an alternative to the Sydney rock oyster.

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As a result of disease and other factors that have affected productivity of Sydney rock oyster (*Saccostrea glomerata*), NSW Fisheries are investigating the culture potential of an alternative species, the flat oyster (*Ostrea angasi*) in NSW. This species is seen as an ideal candidate for culture for three reasons: i) It is an Australian endemic, ii) has relatively rapid growth, and iii) has high market value. Prior to development of any new aquaculture endeavour, it is desirable to determine the population structure of wild stocks in order to make informed management decisions regarding translocations of juveniles for culture growout, thus avoiding potential deleterious effects on natural gene pools. The current study investigated the genetic population structure of *O. angasi* in 5 estuaries in southern NSW as well as incorporating samples from across the species natural range of southern Australia in order to place levels of genetic divergence among NSW estuaries into context. Unlike *S. glomerata*, *O. angasi* brood their larvae thereby greatly reducing the time spent in the water column and hence reducing dispersal capability. It was expected therefore, that this species would display structuring over its range. Contrary to expectations, there was very little genetic differentiation across the entire natural range of the species. Furthermore, there was relatively little differentiation between *O. angasi* and its Northern Hemisphere congener *O. edulis*, raising the possibility that *O. angasi* is not an Australian endemic. This possibility is explored and the broader implications of future developments of the species in culture are considered.

Interpreting Provenance by Environment interaction in *Eucalyptus regnans* F.Muell.

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A range-wide collection of *Eucalyptus regnans* F.Muell provenances was trialled on nine sites in South-Eastern Australia. Provenance by site interaction comprised an important part of the total variance, almost as large as the provenance component.
AMMI analysis and simultaneous clustering showed the interaction to be more dimensionally complex than that observed in the related species *Eucalyptus delegatensis*, and correspondingly more difficult to interpret.
Despite this complexity, factorial regression on three independent site covariates (annual mean rainfall, radiation in the driest quarter and elevation) was able to explain 54% of the interaction, although a significant portion of the interaction remained.
Factorial regression using single environmental attributes of trial sites or provenance locations was also carried out. Individual provenance covariates effective in explaining interaction in *E. delegatensis*, such as latitude and radiation in the warmest quarter, were also among the most effective for *E. regnans*. Likewise, the site covariate elevation, found effective in *E. delegatensis*, was also able to explain a significant part of the interaction here. This suggests there may be some general associations between measurable environmental covariates and between-population adaptive variation that could be used to construct biologically meaningful seed transfer zones.

Reconstruction of predator diet from prey DNA diversity

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The prey species that any predator consumes are a key aspect of its ecology. Estimation of the identity, diversity and relative abundance of prey proves to be a surprisingly difficult problem. DNA-based species identification has only recently been applied to this area. As a species identification tool, DNA has many advantages over morphological or other biomarker methods and may provide a better means of estimating the diet of predator populations than existing methods. Our research on the diet of Antarctic krill, penguins, whale sharks, giant squid and whales has proven that prey DNA identity can be readily determined from a diverse range of predators. Current methods, problems and future potential for this new are of molecular ecology will be described.

Water balance in *Drosophila*: can early physiological decline predict aging and longevity?

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There is a general opinion among researchers that aging is the result of a cascade of damage, from macromolecular damage to organ and system failure, with loss of homeostasis progressing from the cellular to a whole-organism level. This is used to explain the many observations of measurable phenotypes declining or increasing with age. But are any of these age-related parameters reflective of the processes causing aging and death? Do they determine lifespan (i.e. biomarkers of physiological age), or are they merely consequences of chronological age? Measuring most age-related parameters is destructive. Therefore our approach essentially treats cohorts/strains of *D. melanogaster*, whose lifespans differ, as if they were individuals, allowing us to take subsamples throughout the lifespan of the cohort. It is likely that damage begins early, before population mortality accelerates, so this is our focus. Aspects of water balance, a physiologically relevant trait, were measured and correlations made of changes over time with measures of lifespan and aging.

Thermoregulation in honey bee colonies: diversity promotes stability

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Honey bee queens mate with 10 –20 males to produce colonies comprised of workers of multiple patrilines. Patrilines can vary in their sensitivity to task stimuli leading to 'task specialization' where workers of some patrilines are more likely to perform particular tasks. Direct comparisons of brood nest temperatures in genetically diverse (i.e. those sired by several males) and uniform (i.e. those sired by one male) colonies show that diverse colonies have more stable temperatures. In addition, the genotypes of workers found cooling the brood nest differed for different nest temperatures. Finally, computer simulations of colonies where workers have diverse temperature thresholds produce a more stable nest temperature than uniform ones. We show that genetically diverse honey bee colonies maintain more stable brood nest temperatures than uniform ones because diversity modulates the temperature threshold at which workers cool and heat their nest.

Mode of action of PETAL LOSS, a transcription factor involved in regulating sepal and petal development in *Arabidopsis thaliana*

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Based on its mutant phenotype, the *PETAL LOSS* (*PTL*) gene plays a role in regulating the separation of sepals and the initiation and orientation of petals (Griffith et al. 1999). PTL is a member of the trihelix transcription factor family, which is known to occur only in plants (Brewer et al. 2004). It has two DNA binding domains, defined as the trihelix domain, and a central domain predicted to be a coiled-coil.

I have investigated regions of PTL required for nuclear localization using GFP fusions. There are three candidate nuclear localization signals (NLS), but the putative NLS in the C-terminal trihelix region is sufficient to drive the whole protein into the nucleus.

The *PTL* gene is expressed at a low level during the early stages of flower development. Specifically, expression occurs in four limited zones between newly arising sepals. Mis-expression of *PTL* in a range of plant tissues results in suppression of their growth (Brewer et al. 2004). The targets of PTL may therefore be acting to arrest growth in the inter-sepal zone to establish sepal separation. I have tested whether PTL activates or represses targets using the yeast two hybrid system, and show that PTL carries an activation domain within its C-terminal region. Other members of this transcription factor family act as dimers. However, in yeast PTL acts as a monomer to activate the basal transcription machinery.

A genetic screen for novel regulators of chromosome segregation

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'Cohesin' is an evolutionarily conserved multi-protein complex thought to be the primary effector of sister-chromatid cohesion in all eukaryotes. In yeast, cohesin is loaded onto chromosomes in S-phase and maintains chromatid cohesion until the metaphase-anaphase transition. Sister-chromatid separation is then triggered by the site-specific cleavage of the Rad21 cohesin subunit. In higher species, including *Drosophila*, the bulk of 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 this we developed a dominant *rad21* allele that produces a rough eye phenotype when ectopically expressed in the developing eye of *Drosophila*. Altering the level of expression of this allele generates a dose-dependent phenotypic modification. The phenotype is also modified in individuals heterozygous for mutations in known cohesin regulators. This indicates that a genome-wide F1 dominant modifier screen for enhancers/suppressors of this phenotype is likely to identify novel regulators of chromosome cohesion. The identification and characterization of novel chromosome segregation regulators by this method is expected to provide new insights into how multicellular eukaryotes regulate the structure and stability of their chromosomes.

The origin and spread of the parthenogenetic grasshopper *Warramaba virgo*: evidence from mitochondrial DNA sequences

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parthenogenesis common in harsh environments Aust desert lizards, insects, mulga acacia

Parthenogenesis is a surprisingly rare genetic system, given the reproductive advantage it holds over sexual genetic systems. If we are to fully understand the reasons for the prevalence of sex, we must understand the constraints on the origin and spread of parthenogenesis. The Australian parthenogenetic grasshopper *Warramaba virgo* was discovered by MJD White in 1961 and was intensively studied cytogenetically until his death in 1983. There is evidence that it arose by two separate hybridization events between undescribed sexual taxa in arid Western Australia. One group of clones remained in Western Australia (the 'Boulder-Zanthus' phylad) while the other spread eastward to an ecologically similar region in western NSW (the 'Standard' phylad). We have resumed genetic study of this system and present sequence data for 600 bp of the mtDNA gene COI for both phylads of *W. virgo* and its known sexual relatives. Our data reveal low levels of mtDNA variation within both phylads of *W. virgo*, confirm their independent origins, and indicate that in both cases P169 was the maternal parent. The Boulder-Zanthus phylad closely matches the mtDNA haplotypes of south-eastern populations of P169 but the Standard phylad does not match any of the P169 populations we sampled.

W. picta P169 P125 P196 W. virgo

MJDW

W. virgo

mt seq } backing up cytological data
2 phylads of virgo
1 nested in P169 OK
other outside ???

Is virgo hybrid? 196x169 (Godfrey Hewitt). yes

same seq as one in west, very recent movement
Q5 excellent

Genetic Dissection of a Regulatory Network.

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For the geneticist, phenotypic suppressor and enhancer screens, based on well founded genetic hypotheses, performed in genetically amenable "model" organisms, have provided the cornerstone for the dissection of complex biological processes. In the era of genomics, are they still relevant?

In a screen designed to understand the molecular mechanism of carbon catabolite repression in *Aspergillus nidulans*, mutational dissection, physical analyses of the genes, and functional analyses of the proteins have uncovered many components, from the major transcription factor involved, to a regulatory ubiquitination/deubiquitination network.

Regulatory deubiquitination networks are generally not well characterized in multicellular eukaryotes, primarily due to the difficulties involved in obtaining an experimental "handle" on both the ubiquitination and deubiquitination aspects of the mechanism. The proteins identified in the regulatory network in *A. nidulans* are conserved among multicellular eukaryotes, suggesting that the mechanisms uncovered are highly conserved.

Analysis of genes involved in fatty acid β-oxidation in *Aspergillus nidulans*

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Fungi utilise a diverse range of carbon sources, including fatty acids, for growth. Fatty acids are catabolised by β-oxidation, each cycle of which reduces the fatty acid chain by two carbon atoms producing an acetyl-CoA molecule. The *Aspergillus nidulans acuJ* gene encodes carnitine acetyltransferase which shuttles acetyl-CoA between peroxisomes and mitochondria. *acuJ* is acetate and fatty acid-inducible. Acetate induction of *acuJ* is mediated by FacB, a Zn(II)2Cys6 transcriptional activator. An *acuJ-lacZ* construct containing 300bp of the *acuJ* promoter is inducible by fatty acids but not acetate. Nothing is known about the mechanism of fatty acid-regulated gene expression. Several genes have been cloned by complementation of mutants unable to utilise butyrate (4-carbon fatty acid) as a sole carbon source. One of these genes, *scfA*, encodes a transcriptional regulator required for short chain fatty acid induction of *acuJ*. Four of the genes encode orthologues of known *Saccharomyces cerevisiae* peroxisomal assembly proteins. The *pexF* (*PEX6* orthologue) mutant is sensitive to fatty acids but not acetate and lacks peroxisomes. GFP analyses confirm the presence of peroxisomal malate synthase and isocitrate lyase in the cytoplasm of the *pexF* mutant. The remaining genes encode a thiolase, an adenine nucleotide transporter, and a DnaJ-like protein.

Functional analysis of evolved differences in the Drosophila even-skipped stripe two enhancer

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The *even-skipped* stripe two enhancer (S2E) regulates _expression of a single pair-rule stripe in *Drosophila* embryogenesis, the first step in establishing segmental boundaries in the developing fly. But despite this critical role in development, S2E's differ structurally among species in the presence or absence of transcription factor binding sites, in the sequences of binding sites and in the spacing between them. Being one of the best functionally characterized eukaryotic enhancer, we investigated the functional consequences of these evolved differences by making transgenic replacements of the native *D. melanogaster* S2E with orthologous segments from three other Drosophila species. The spatio-temporal pattern of stripe two _expression is strongly conserved, but the ability to produce viable adult flies varied, as did levels of *eve* stripe two _expression. The enhancer from *D. erecta,* in particular, expresses a very weak stripe when placed in the *D. melanogaster* genetic background, does not activate the segment polarity gene *engrailed*, and is entirely lethal. In contrast, the *D. pseudoobscura* S2E, a more distant relative with a more diverged enhancer, is functional indistinguishable from the *D. melanogaster* S2E. The non-clocklike functional evolution of this essential enhancer is hypothesized to be the result of convergent co-evolution of S2E's with the bicoid and/or hunchback activation gradients. These results have important implications for understanding enhancer structure/function, mechanisms of speciation, and computational identification of regulatory modules.

The head and body lice of humans are separate species: evidence from double infestations.

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Body lice are thought to have diverged from head lice when humans began to wear clothes. This recent evolutionary divergence and incomplete lineage sorting has complicated assessments of whether head lice and body lice are separate species. We used microsatellite markers to test lice from hosts that were infested with head and body lice simultaneously, to see if the head and body lice had interbred. Our results showed that head and body lice had not interbred on a host. Assignment tests revealed that lice that had migrated among hosts always moved from head to head, and body to body, but never between heads and bodies. We conclude that head and body lice are separate species.

The use of RAFs enables determination of genetic structure within and among catchments in the Australian lungfish Neoceratodus forsteri

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The lungfish Neoceratodus forsteri, is restricted to the Brisbane, Mary and Burnett catchments in SE Queensland. It is an ancient species with a large and highly duplicated genome. To increase the resolution of previous studies, which found very low diversity, we investigated microsatellite, AFLP and Random Amplified Fragments (RAFs) markers. Our study confirmed the very low variation. however, polymorphic RAF fragments have confirmed that Lungfish populations are not homogenous within or between river systems. RAF AMOVA indicated significant partitioning of the Brisbane from Burnett and Mary catchments of 19%. This is in congruence with weak structuring previously detected using allozymes. Our results indicate that the Brisbane river population has not, as previously thought, arisen due to translocations. The use RAF markers has also resolved the low but significant structure identified by allozymes between impounded and non impounded populations, with a distinction of 21% indicating previous translocations supplementing impoundment mortalities were from the same source. We report the utility of RAFs in evaluating genetic diversity of the lungfish. In contrast to the challenges to both development and characterisation of microsatellites in lungfish, application of RAF markers was fairly direct and gave repeatability of patterns.

Characterisation of *mapmodulin*: A novel tumour suppressor homolog in *Drosophila melanogaster*

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In vertebrates, *mapmodulin* belongs to a gene class that has been implicated in numerous cellular roles *in vitro*. This study presents a molecular genetic characterisation of the sole *Drosophila melanogaster* ortholog, *mapmodulin* (*mpm*), focusing on analysing its function in development using a number of reverse genetic techniques.
Initial characterisation of *mpm* using *in situ* hybridisation showed that it is expressed in a number of disparate tissues including the CNS, mesoderm, gut and the gonads. However, analysis of the roles of *mpm* in *Drosophila* development using RNA interference (RNAi), overexpression and deletion alleles of *mpm* failed to produce a detectable phenotype suggesting that *mpm* is not an essential gene for normal *Drosophila* development. However, given the highly conserved nature of *mpm,* further analysis may yet uncover a role essential for survival.

Molecular evolution of GSTs in the *Drosophila* genus

Honours student

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Glutathione-S-transferases (GSTs) are a superfamily of ubiquitous detoxification enzymes. The evolution of these enzymes allows organisms to adapt to a wide range of environmental toxins. These may include foreign compounds found in their species-specific feeding substrates and man-made toxins such as insecticides. Here we use data from available genomic sequences to examine the evolutionary relationship of GSTs in the *Drosophila* genus. We have used 38 GST genes described in the well-annotated genome of *D. melanogaster* to identify 33 GST genes from *D. pseudoobscura*. Phylogenetic analyses reveals 27 orthologous sets with representatives from all 6 defined classes of insects GSTs (δ, ε, σ, θ, ω, and ζ). The variation in gene number between species occurs in two large gene clusters that encode δ and ε genes. Relative rate tests reveal significant rate differences among GST classes and between structural domains of the proteins.

δ & ε implicated in insecticide resistance.

looked at stat computer screen

yakuba
melanogaster
pseudo obscura
database comparison

phylogenetic trees.
rate of variation across protein

Next
1. new sequences diff genomes
2. Microarray
3. Cis elements by comp. genomics

Tolerance to the *Bacillus thuringiensis* endotoxin Cry1Ac in a laboratory *Helicoverpa armigera* strain is based on immune induction, which is transmitted by a maternal effect.

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We crossed resistant insects from a laboratory strain of *Helicoverpas armigera* with susceptible insects and examined Bt-tolerance in neonates. We noticed significantly higher tolerance in offspring from resistant females crossed with susceptible males (RxS), compared to those from susceptible females crossed with resistant males (SxR). Tolerance in RxS offspring was not as high as for the resistant line, and for SxR offspring tolerance was somewhat higher than in the susceptible line. This suggests that in addition to a genetic element, Bt-tolerance in this strain is transmitted by a maternal effect. To investigate the possible mechanism of Bt-tolerance, we examined the induction of immune responses in susceptible larvae by a sub-lethal dose of Bt-toxin. One protein of 89 kDa in size was induced within two hours of exposure to the toxin. Protein concentration peaked after 6 hours and then declined to low levels by 21 hours. The protein has similarities to *Drosophila* proteins that act as pro-coagulants. These experiments indicate an immune induction of specific larval proteins, which may be involved in coagulation reactions in the hemolymph and gut lumen.

Genetic hoofprints: is there molecular evidence of selection in domestic cattle?

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The degree to which natural selection can alter phenotypes after only a few generations has the potential to answer a number of interesting questions relating to evolution and the complexity observed at the species and genome level. We studied evolutionary-rates between human and domesticated dairy cattle (*Bos taurus*). Using roughly 550,000 bovine ESTs we searched for differences between rates in mammary and housekeeping genes. We found substantial differences between the relative rates of evolution for both groups, with milk genes showing an increase in the nonsynonymous mutation rate. This suggests a genetic response to selection and provides strong evidence for a link between genotype and phenotype, and highlights the main process responsible for changing gene frequency in domestic cattle.

Empirical evaluation of the sensitivity of selective DNA pooling to detect QTL in a half-sib design

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Genetic variation in traits of agricultural importance such as growth, fertility, production and disease susceptibility is complex in nature, where their effects are controlled by many genes called quantitative trait loci (QTL) and environmental factors. A first step towards identifying such genes is linkage mapping to broad chromosomal intervals. Since most of these genes have small effects, we need powerful experiments to identify them. However, sampling large numbers of animals to provide the necessary power and subsequent genotyping makes QTL identification a costly venture. One way to reduce the cost of a genome scan as well as of improving its efficiency is to use selective genotyping combined with DNA pooling. This technique was used to identify QTLs for milk production in Australian Holstein dairy cattle. Essentially, DNA samples from the high and low tails of the trait distribution were pooled separately within sire families to form high and low DNA pools for each family. The pools were genotyped with evenly spaced microsatellite markers spread across all of the bovine chromosomes. A simple linear model was used for the analysis of pooled genotyping data that detected markers linked to QTL.

This study represents the first instance of an empirical evaluation of the pooling methodology by comparing it to individual genotyping and interval mapping to detect QTL in a half-sib design. Errors were found to creep in at every stage of the pooling and analysis to decrease the power of the experiment compared to individual genotyping and to also cause discrepancies between the QTL detected as significant by pooling and those found significant by interval mapping. Some of the sources of these errors are identified as a first step towards providing suggestions for improvement in future experiments based on DNA pooling.

Initiation of the caspase cascade in mammalian cells.

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The mechanisms of apoptotic cell death have been strongly conserved throughout evolution. In mammals, as well as many other animals, apoptosis induced by a range of cell stress stimuli is regulated by two families of proteins: (i) the Bcl-2 protein family, which comprises both pro- and anti-apoptotic members, and (ii) the caspases, which are the enzymatic executors of the cell. Many systems have demonstrated that Bcl-2 family members act upstream of caspase activation to regulate apoptosis. In mammalian cells, it is thought by many that pro-survival Bcl-2 homologues function to prevent damage to mitochrondria and the subsequent release of cytochrome c, which can activate the initiator caspase-9 through its adaptor Apaf-1. It is widely believed that Apaf-1 and caspase-9 are essential for apoptosis of cells in response to diverse cell-stress stimuli. We have specifically examined whether these proteins are essential for apoptosis in hematopoietic cells by using mice deficient in Apaf-1 or caspase-9. Our work has demonstrated that a Bcl-2-regulated, caspase-mediated apoptotic pathway exists that does not require Apaf-1 or caspase-9, and we have defined some of the caspases involved in it. This novel alternate apoptotic pathway has relevance to understanding the etiology of some diseases.

Calving difficulty in dairy cows: the genetic background

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Calving difficulty (dystocia) is a cause of suffering or death for the cow and her calf, and inconvenience for the farmer, causing losses of about $50 million dollars a year in Australia. Dystocia may be caused by the calf or the cow, but is usually a combination of the two. Gestation length, calf size, dystocia and calf mortality all have genetic components, arising separately and largely independantly from the cow and from her calf. Dystocia can be selected against through the sire of the cow or calf. Other factors such as calf sex, breed, month of calving and cow age also influence the incidence of dystocia, and need to be allowed for when assessing bulls that may be used to minimise dystocia. Bulls that result in reduced calving problems may be identified through statistical analysis of data on individual calving records collected voluntarily by Australian farmers from all of their cows over many years. Bulls that result in reduced dystocia in their daughters do not necessarily have reduced dystocia in their own calves. Dystocia is uncorrelated with milk production, so no delaterious effects will result from selecting for easy calving bulls.

Drosophila melanogaster gene expression in response to a virulent endosymbiont

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Wolbachia pipientis is an obligate intracellular bacterium present in a wide range of insect species. While the bacterium is known for its ability to manipulate host reproductive biology, most strains cause little direct harm to host tissues. One exception is a strain called "popcorn" that infects *Drosophila melanogaster*. As the fly ages the bacterium replicates to large numbers, leading to rupture of host cells and shortened insect lifespan. This over-replication is aberrant in comparison with other *Wolbachia* infections whose densities remain stable or decline with insect age. Previous work indicates that *Wolbachia* infections do not induce or actively suppress an innate immune response in *Drosophila melanogaster*. Because the popcorn infection leads to cell rupture we predicted that this strain would likely induce host defenses. Using a microarray approach we have examined the effect of popcorn infection on host gene expression as the host ages. Overall, gene expression decreases with age in response to popcorn infection. The bacterial specific response resulting in antimicrobial peptide production is not activated. The most common class of upregulated genes, however, were serine proteases; including elastases and trypsins. Serpins, the regulators of these proteases are also highly expressed, as are other genes encoding general defense response proteins.

Conservation genetics and biogeography of the endangered mountain pygmy-possum, _Burramys parvus_.

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The mountain pygmy-possum (*Burramys parvus*) is Australia's only mammal restricted to the alpine and subalpine zone. Confined to a small geographic range (10 km^2) and listed as endangered, it is threatened due to global warming, human activities (habitat loss from ski-resorts) and feral predators (foxes and cats). Ninety percent of the remaining five isolated populations, ranging from Mt. Buller to Kosciusko National Park, were exposed to the severe bushfires that burnt large areas of alpine vegetation in 2003. Prior to the fires, the total adult female population was less than 2000 individuals. The effects of these fires on the *Burramys* population has yet to be determined. We commenced a three year research program which initiates a comprehensive genetic and ecological monitoring program on *Burramys*. The aims of this work are to establish the genetic viability of *Burramys* in response to global warming and human activities, continue monitoring sites, develop a management/conservation plan and foster close interactions with ski resort management and community groups in order to save this unique, alpine marsupial. We present results using microsatellites and detailed life history surveys on 2000 individuals sampled over 15 years, to examine factors such as effective population size [N(e)], genetic diversity, population bottlenecks, population structure, breeding system and immigration/emigration rates.

Polluting the Scientific Atmosphere? Peppered moths, spiced stories and the problem of scientific fraud.

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A recent book by science writer Judith Hooper (*Of Moths and Men* Fourth Estate, London, 2002) has effectively accused Bernard Kettlewell of making up data during his pioneering field experiments on industrial melanism in the Peppered Moth *Biston betularia*. Secondary reports of the book are more extreme, and the implication that 'Darwin's missing evidence' is still missing is now widely believed.

Later ecological geneticists are also represented as conspiring to cover up the inadequacies of Kettlewell's experiments and of the selective predation model of industrial melanism. These imputations have been challenged by people who have been involved in the work, particularly Laurence Cook (*Quart. Rev. Biol.* Dec. 2003 **78**: 399-417).

I will review Hooper's evidence and arguments. The primary accusation, against Kettlewell, is clearly speculative and also factually flawed. Other arguments are at best confused, as Cook and others have documented. Nonetheless, in two areas even these published rebuttals are inadequate. Bishop's ecological studies, especially mark-recapture estimates of selection, undermine Hooper's thesis. Also, arguments about bird predation have omitted significant published literature.

Genuine cases of scientific fraud are not trivial. Unfortunately, flimsy but well-publicised ones can also erode public trust in scientists' ethics. Ways of preventing this fall-out are needed.

The regulation of a gene family involved in glucosinolate biosynthesis in *Arabidopsis thaliana*

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Glucosinolates are a group of secondary metabolites found exclusively in the plant kingdom. These compounds and their derivatives have a range of biological activities, including protection against herbivore attack and plant recognition by specialist predators, and also influence nutritional attributes such as flavour and the anti-carcinogenic properties of many *Brassica* vegetables[1]. Glucosinolates are predominantly found within the Brassicaceae family, which includes the model plant species *Arabidopsis thaliana* and agriculturally important crops such as oilseed rape (*Brassica napus*) and various *Brassica* vegetables[1]. The structural diversity of glucosinolates is due, in part, to the secondary modification of the glucosinolate side chain. In *Arabidopsis* this modification involves the activity of members of the *AOP* gene family, which encode 2-oxoglutarate-dependent dioxygenases. The differential expression of two members of this gene family, *AOP2* and *AOP3*, between *Arabidopsis* ecotypes has been shown to result in the accumulation of either alkenyl or hydroxyalkyl glucosinolates respectively[2]. We have found that the expression of the *AOP2* gene is regulated by environmental factors such as light and temperature. The specific mechanisms behind this regulation and the regulation of the other *AOP* family members (*AOP1* and *AOP3*) are being investigated.

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AOP2" ~ cDNA [illegible] library of 2 A.thal ecotypes"
in Pitztal ont not Columbia

either AOP2 or AOP3 expressed in diff ecotypes (except Lan 4&6 neither)

The molecular identification of closely related calliphorids (Diptera: Calliphoridae) of forensic importance.

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Blowfly larvae (Diptera: Calliphoridae) are one of the most useful groups of insects utilised during the investigation of human deaths because they are among the first colonisers of a cadaver. The identification of such larvae is a critical step when estimating the the postmortem interval (PMI), as the rate of larval development can vary substantially between species. Two blowflies that have the potential to feature in forensic investigations are *Chrysomya semimetallica* and *Ch. latifrons*. The identification of these species is hampered by their almost indistinguishable morphologies, even as adults. The species were differentiated on the basis of two highly variable regions of the ribosomal DNA genes – the internal transcribed spacer (ITS) sequences. The amplification of ITS2 from both species was readily accomplished, however only ITS1 from *Ch. semimetallica* amplified successfully. The ITS2 differences correspond to 1.22 % sequence divergence between the species. A rapid, cost-effective strategy for species identification would involve PCR amplification of the ITS regions, followed by restriction fragment length polymorphism (RFLP) analysis. Despite the differences within ITS2, no restriction sites were observed that could differentiate the two species. Assessment of this possibility in ITS1 will be achievable once the remaining data is obtained from *Ch. Latifrons*.

Characterisation of the dmATP7 copper transporter in *Drosophila melanogaster*

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The human Menkes disease protein (MNK; ATP7A) is critical to normal uptake and distribution of copper to cuproenzymes throughout the body, while excess copper is incorporated into bile by the Wilsons disease protein (WND; ATP7B), then excreted. Defect in either of these proteins causes serious disease, as copper concentrations must be finely balanced to provide an essential trace element but avoid the extensive cellular damage that may result from excess copper.

To develop better treatments for patients of copper metabolic disorders, it is important to understand the structure and function of proteins involved in copper metabolism. As part of a project to establish *D. melanogaster* as a genetic model for copper homeostasis, we have investigated the orthologue of the human Menkes and Wilsons disease proteins, dmATP7. We will present data on the subcellular localisation and tissue distribution at various life stages. We will also discuss the results of functional characterisation both *in vivo* and in 'S2' embryonic cells.

Paradigm Shifts in Adaptive Enzyme Evolution

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Products of the modern chemical industry like pesticides challenge biological systems with the need to develop some qualitatively new biochemical functions. We have been analysing the mutant hydrolytic enzymes that enable resistant insects to survive exposure to pesticides, and tolerant bacteria to utilise them as mutant sources. We have also been improving these enzymes by *in vitro* evolution technologies for use in bioremediating contaminated environments. By comparing the nature of the mutations and the kinetic efficiencies of the enzymes from these three sources we can begin to understand the capacities of, and constraints on, natural systems to evolve new biochemical functions. For some chemistries we find some remarkably efficient mutant enzymes have arisen and proliferated in bacterial systems over the last few decades; some of these involve wholesale repatterning of ancestral enzyme scaffolds. The mutant eukaryote (insect) enzymes for the same chemistries are remarkably inefficient and inelegant by comparison. Other chemistries are apparently refractory to even the cleverest bacteria, if not to *in vitro* evolution technologies. We discuss the implications of these findings in the context of a concept of adaptive landscapes borrowed from theory traditionally applied to organismal evolution.

Wolbachia Genomes: A Key To Understanding a Ubiquitous Symbiosis of Insects

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The recent sequencing of the genome of the *Wolbachia* strain that infects *Drosophila melanogaster* provides an opportunity to examine characteristics that may provide insights into understanding how *Wolbachia* maintains itself within insect cells and how it is able to manipulate the reproductive biology of the hosts it infects. In addition the genome sequence will provide us with numerous tools for better understanding the evolutionary biology of Wolbachia. The Wolbachia genome shows clear evidence of steamlining but unlike other symbiont genomes contains large amounts of repetitive DNA and insertion elements. The complete genome sequence shows that Wolbachia has an intact Type IV secretion system that is likely to play a major role in communicating with host cells in which it lives. The genome also reveals an unusual abundance of genes with ankyrin repeat domains. These genes are strong candidates for playing a role in generating the phenotypes that *Wolbachia* is best known for. Finally the genome sequence has provided us with a range of sensitive markers to identify and track *Wolbachia* strains. Using an approach that examines *Wolbachia* chromosomal inversions and mini-satellite sequences it has been possible to reconstruct multiple *Wolbachia* sweeps into worldwide populations of *Drosophila melanogaster*, the most recent of which appears to correspond to the well documented P-element sweep into global *D. melanogaster* populations in the mid-1960s.

Phylogeography of an orchid mycorrhizal mutualism in the Caribbean

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Orchids depend on mycorrhizal fungi (MF) for germination. Recent studies have shown a greater degree of specialization in orchid mycorrhizae than previously thought. However, most attention has been directed at the inter-specific level. We used two approaches to investigate the degree of orchid mycorrhizal specificity and potential coevolution of MF within the Neotropical epiphyte, Ionopsis utricularioides: phylogeographic analysis at the ITS of both MF and their orchid hosts, and in vitro symbiotic seed germination. We sampled I. utricularioides and its MF broadly across the Caribbean. Fungal isolates belonged to Ceratobasidium spp, with the majority falling in a single well-supported clade. Phylogeographic structure was seen in the orchid, but fungal haplotypes were shared widely across the sampled range, indicating specificity at the species level but not the population level. These results not support a hypothesis of coevolution. Fungal isolates from across the phylogenetic range were examined for their effect on germination and seedling growth. Significant variation was seen amongst isolates. Interestingly, phylogenetically-related fungi isolated from a single site in Puerto Rico induced significantly better seed germination and seedling development than other MF.

Leaky species and the devolution of sex

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The molecular revolution has resulted in an explosion in genetic information and a re-evaluation of the evolutionary history of life. The concept of a universal tree of life comprising Bacteria, Archaea and Eukaryotes is now generally accepted, even though evolutionary relationships among this triad have proved difficult to discern. What is not generally accepted though, is a universal basis for branch demarcation at all taxonomic levels including one that endorses the rank of species. In fact there is widespread disparity on the fundamental basis of species delineation. The main impediment being acceptable criteria that allow the objective definition of species-level from higher as well as lower taxa. Most species concepts focus predominantly on sexually reproductive eukaryotes and largely exclude the greater part of cellular life. With respect to accommodating prokaryotes there are two obvious issues to deal with. The first of these is that they do not interbreed. Secondly, do they challenge the notion that species are reproductively isolated? Interbreeding encompassing true sex incorporates homologous recombination. With respect to reproductive isolation the underlying mechanism is the cessation of gene flow between populations due to the emergence of some intrinsic barrier to gene exchange. What mechanisms exist in bacteria that can be applied to delimit bacterial species? Our aim is to relate species concepts to mechanisms that distinguish and unite prokaryotes and eukaryotes and discuss the potential for a unifying concept based upon intrinsic barriers to gene exchange, in particular one that better accommodates horizontal gene transfer.

FlcA of *Azospirillum* controlling cell differentiation and attachment to plant roots

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Azospirillum spp. are free-living, nitrogen-fixing, rhizospheric bacteria, found in association with a wide range of plants. FlcA of *Azospirillum brasilense* Sp7 is a putative transcriptional regulator controlling cell differentiation from vegetative to cyst-like forms, exopolysaccharide (EPS) production, flocculation in liquid media and the pattern and extent of colonisation of plant roots.

Studies of gene expression have shown that *flcA* promoter is partially constitutive but its expression is enhanced in conditions that also permit flocculation in liquid media and increased production of EPS. Interestingly, expression of *flcA* is much higher in *flcA*$^-$, Tn5-induced mutants, than in the wild type. Since the only difference between the wild type and the mutants is the presence of the FlcA protein, we suspect that FlcA autoregulates its own gene.

FlcA forms dimers in solution and shares high homology with members of the LuxR family of response regulators, which is part of a two-component signal transduction system. So far no target gene of FlcA is known. We are currently searching for target genes and trying to elucidate other components of the *flc*- regulatory system as well as environmental conditions that effect *flcA* expression.

Insecticide Resistance in *Drosophila melanogaster*; the role of *Cyp6g1* and friends

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Insecticide resistance is one of the most widespread genetic changes brought about by human activity. In *Drosophila melanogaster*, we have recently shown that over-transcription of the cytochrome P450 gene *Cyp6g1* is involved in resistance to diverse classes of insecticides, including DDT, organophosphorus insecticides, neonicotinoids and the insect growth regulator lufenuron. The resistant allele of *Cyp6g1* is characterised by the insertion of the terminal repeat of an *Accord* retrotransposon in the upstream regulatory region of *Cyp6g1*. This allele is present in high frequency in populations of *D. melanogaster* from around the world.

Using reporter gene constructs in transgenic flies, we have determined that *Cyp6g1* is expressed in the larval and adult mid-gut and Malpighian tubules. The presence of the *Accord* insertion results in additional *Cyp6g1* expression in the fat body, potentially explaining resistance.

Additional genetic mapping in some insecticide resistance strains has indicated that other genes are also involved in both nitenpyram (a neonicotinoid) and lufenuron resistance. *Cyp6g1* is located on chromosome II, while a majority of the resistance maps to regions within chromosome III. The chromosome III contribution to resistance in these strains, however, is dependent on a resistance allele of *Cyp6g1* being present. We believe that these additional chromosome III factors may be involved in the secondary metabolism of insecticides.

Local adaptation and success of transplanted populations of *Rutidosis leptorrhynchoides* (Asteraceae)

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Local adaptation, and the question of appropriate source populations for transplanting and revegetation are important genetic issues in the restoration of threatened species and ecological communities. Despite the potential importance of these issues, there is still only limited data available to predict the scale over which local adaptation may be important, and how it relates to environmental and genetic differences between populations. This study examined patterns of local adaptation in populations of the perennial herb *Rutidosis leptorrhynchoides*. Specifically, the aims of this study were to: (i) examine the spatial scale of local adaptation, and (ii) relate differences in environment, and molecular and quantitative genetic variation between populations to the expression of local adaptation. These questions were addressed using a transplant experiment that involved 18 pairs of populations separated by a range of distances from 1 – 600 km. For each population pair, seedlings from both the target and source populations were planted into the target population soil. An environmental distance between the populations in each pair was constructed based on climatic and edaphic variables. The genetic distance between each source and target population was calculated using both AFLP markers and quantitative genetic data. Survivorship and growth of individuals were monitored to examine fitness differences between populations in each pair, and relate these differences to the environmental and genetic distance between the populations.

Linkage Analysis of a Family with Familial Partial Epilepsy with Variable Foci

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Rationale: Familial partial epilepsy with variable foci (FPEVF) is a recently described familial partial epilepsy syndrome. Linkage has been reported for FPEVF to chromosome 22q11-12 in two French-Canadian and one Dutch family. We have identified an extended Australian family with multiple members showing clinical characteristics of FPEVF. The familial inheritance of the epilepsy seen within this family is indicative of a strong underlying genetic component, with autosomal dominant inheritance. Here we report the results of genetic linkage analysis studies in this family.

Method: We conducted a genome-wide scan using 400 microsatellite markers, followed by linkage analysis to identify regions of the genome likely to contain the causative gene.

Results: Two potential candidate regions on chromosomes 22 and 19 were identified. A significant lod score of 3.45 was obtained on chromosome 22q and a multipoint lod score of 2.63 was obtained on chromosome 19, both occurring at ~30 cM from the proximal end of the chromosome. The peaks span ~8 and ~ 9 cM on chromosomes 19 and 22, respectively. Fine mapping confirmed the region of linkage on chromosome 22 as falling within the candidate regions previously identified in two French-Canadian and Dutch FPEVF families (22q11-12).

Molecular phylogeography of *Melonycteris* in the Solomon Islands

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Megachiroptera (fruit bats) genus *Melonycteris* are small, brightly-coloured nectarivorous bats, endemic to the Solomon Islands and the Bismarck Archipelago. Currently, three species of *Melonycteris* are recognised. *M. melanops* is restricted to the Bismarck Archipelago. Two species are found in the Solomon Islands: *M. woodfordi* with two subspecies and *M. fardoulisi* with four. Most subspecies are restricted to one or a few island groups within the archipelagos. The current specific and subspecific classification is based on morphology. The current (limited) understanding of Solomon Islands mammalian zoogeography is also based on morphological analyses. We report a phylogeographic analysis of mitochondrial cytochrome *b* DNA sequences from 41 samples representing most species and subspecies of *Melonycteris* from nearly all major Islands of the Solomon and Bismarck Archipelagoes, with the notable exception of Bougainville. The specific and subspecific status of taxa in *Melonycteris* were considered in light of the data, and patterns of genetic divergence between populations from different islands were evaluated in relation to archipelago geography and geological history. Classifications based on DNA phylogenies analyses agreed with morphology. Generally, sequences from each island or island group comprised a monophyletic clade. The possible routes of Island colonisation and speciation patterns suggested by the relationships of such clades were not entirely concordant with geography. The study provides a framework for further studies on phylogeography and chiropteran evolution of the region.

Circadian behaviour and speciation in tephritid fruit flies.

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Reproductive isolation based on time of mating is a feature that prevents gene flow between species in sympatry. The possible role of clock genes in setting the time of mating is therefore of interest to questions of sympatric speciation. Two sibling species of tephritid fruit fly, *Bactrocera tryoni* and *Bactrocera neohumeralis*, are differentiated by time of mating, a feature which is genetically determined and requires interactions between the endogenous circadian clock and light intensity. The *B. tyroni*, *B. neohumeralis* sibling pair is an ideal model system for identifying the genetics of species differences as hybrid flies can be readily obtained and scored, and hybrid lines selected on the basis of mating time can be used to screen for the molecular correlates of behavioural differences. We have isolated the circadian clock genes, *period* (*per*) and *cryptochrome* (*cry*), as candidates for species differentiation. Striking differences, which correlate with mating time, were revealed in regulation of *cry* expression in specific head tissues. A genetic screen for differentially expressed genes in hybrid flies lends further evidence to the hypothesis that the circadian system in the flies has a role in the mating isolation mechanism.

Two genetically distinct lines co-exist in populations of the endoparasitoid *Venturia canescens* .

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In a laboratory colony of the solitary endoparasitoid *Venturia canescens* (Hymenoptera: Ichneumonidae) two genetically distinct lines (named RP and RM) appear to coexist sympatrically. The two lines differ in a cluster of phenotypic characters, including ovarian morphology, parasitism behaviour and reproductive success. Controlled oviposition experiments showed that RM females produce fewer offspring under single egg parasitism but more under con-specific superparasitism, compared to RP wasps. Investigations of interlarval combat under *in vitro* conditions showed that the higher reproductive success of the RM line under conspecific superparasitism is due to a physiological difference between the newly hatched larvae of the two lines which results in an advantage to the RM larva independent of the order or time interval between ovipositions. Examination of field-derived wasps has found that wasps displaying the two phenotypic clusters occur in natural populations. A model, using an iterative approach with experimental data to predict the stable composition in a mixed population of wasps displaying RM and RP, suggests that the two lines will coexist sympatrically when overall rates of superparasitism are greater than 5 to 10%.

Landscapes and log-dwellers: A cockroach's eye view of the genetic effects of habitat fragmentation.

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Large-scale destruction of native vegetation generally has a profound effect on population demography. Land clearing also has the potential for more insidious effects because the genetic structure of populations may be altered by significant changes in population connectivity and the consequences of this on dispersal patterns. Log-dwelling invertebrates in particular have the potential to be adversely affected due to their dependence upon rotten logs for survival. We are examining the effects of habitat fragmentation on a local geographic scale (<20 km) using mitochondrial and nuclear markers (microsatellites, EPICs & SWAPPs) developed for log-dependent native cockroaches *Panesthia australis* collected from remnant patches of native vegetation and control plots within adjoining unfragmented forest at Tumut, NSW. Results thus far (IBD plots and spatial autocorrelation) suggest that genetic differentiation among patches has become substantial (compared to controls) after the equivalent of just five generations of isolation. The significance of this will be discussed in the context of similar studies of vertebrates and log-dwelling insects (tenebrionid beetles) that have been conducted in the same region.

deflated encodes a novel, highly conserved _Drosophila_ protein, implicated in regulation of cell proliferation.

Confident fluent

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Proper regulation of the cell cycle is essential to coordinate proliferation with differentiation and to prevent disastrous outcomes such as cancer. Some aspects of cell cycle control are reasonably well understood, however, our understanding of how regulation occurs on an organismal level remains relatively poor. Using a comparative genomics approach we have identified an evolutionarily conserved gene, which we have named *deflated* (CG18176), as a putative regulator of cell proliferation in multicellular organisms. Wildtype *deflated* expression, as revealed by RNA *in situ* hybridisation, occurs in postblastoderm proliferating embryonic cells. Three independent *deflated* alleles were generated by P-element mobilisation and preliminary characterisation reveals phenotypes very similar seen in mutants of the cell cycle regulators *dDp* and *Rbf1*. Homozygous mutant individuals become sluggish and die as 2nd instar larvae. Transheterozgous flies die as pupae, without a fully formed abdomen, presumably due to a defect in abdominal histoblast proliferation. Overexpression of wildtype *deflated* using the Gal4-UAS system results in bristle and wing defects. Progress in genetic and cell biological approaches to elucidate the role that *deflated* plays in controlling cell proliferation will be presented.

P elements jumped into deflated (wanted next gene, but worked on deflated anyway)
3 alleles, lethal at 2nd instar
no orthologs in yeast but in all metazoans
no clues about function
Is it involved in G1-S, or S ? evidence not too strong
3 expts: 1. expressed in proliferating cells in situ
2. overexpressed GAL4 UAS in wing deformed, not easy to explain (in eye, normal! no explanation)
3. where is expression required HSP heatshock 24hr intervals 9 days 3 phenotypes not easy to explain
Qs 2, good questions, answered ok

Deep divergence in Australian Onychophora: A tale of two species

Chester

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Multi component project

Organisms that have coexisted and persisted in an area over a long period of time may be expected to show similar genetic structure as a result of shared historical processes. We have been examining population structure of a range of saproxylic invertebrates throughout the Gourock range, the oldest range on the Australian mainland (96 myo). This range, also known as Tallaganda, contains a high taxonomic diversity of Onychophora (peripatus, velvet worms). Two morphospecies, *Euperipatiodes rowelli* and *Phallocephale tallagandensis*, may be found in large numbers throughout the forest. Examination of mitochondrial (mt) CO1 sequence data shows that both *E. rowelli* and *P. tallagandensis* have deep divergences and show similar phylogenetic structure that may be a result of a series of range expansion and contraction events due to glacial cycles dating back to the Miocene. *Phallocephale tallagandensis* shows strong phylogeographic structure over the forest. Allozyme data support the mtDNA sequence data and implies cryptic speciation. *Euperipatoides rowelli* shows strong population structure based on nuclear markers, morphological characters and mt CO1 haplotype frequencies. However, sequence data shows that these populations are polyphyletic with respect to mtDNA phylogenies.

Catchments will work animal barriers

E. rowelli morphology spots 4 regions
mt haplotype 5 regions biogeographic
3 microsatellites
P. tallagandensis 2 clades
9 allozymes

mt phylogeny not correlated w geographic distrib

mt phylogeny 3 correlated w geographic distrib

Qs ok

Beetles on islands of bush in a sea of pine: impacts of habitat fragmentation on two species of Adeliini at Tumut, SE Australia.

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Anthropogenic activities continue to cause massive fragmentation and reduction of forest area world-wide. With fragmentation and reduction of habitat recognised as the greatest threats to biodiversity, the implementation of improved, informed and conservation-based forestry practices is essential, and requires a greater understanding of the responses of different organisms to forest fragmentation. While genetic techniques can add invaluable insights to fragmentation studies they have rarely been employed, particularly for multiple species. Combining ecological data with genetic information obtained from five allozyme loci, we investigated the impacts of forest fragmentation on two log-dwelling beetles with different life histories in an "islands of bush in a sea of pine" model at Tumut in NSW. Both the relatively mobile *Adelium calosomoides* and the less mobile *Apasis puncticeps* showed reduced mobility and gene flow in fragmented compared to continuous forest: steeper isolation-by-distance and stronger local structure revealed by spatial autocorrelation were both in agreement. There was also evidence for *Ad. calosomoides* being more impacted at larger spatial scales and *Ap. puncticeps* at finer ones. Analysis of patch and species characteristics revealed that genetic and demographic structure may be influenced by: log degradation class for both species, fragment age and distance from continuous forest for *Ad. calosomoides*, and desiccation intolerance and fragment age + distance from continuous forest + number of potential dispersal barriers for *Ap. puncticeps*. Thus fragmentation of forest impacts mobility and gene flow in both species.
This ongoing project is part of a program investigating a suite of saproxylic (rotting log dwelling) invertebrate pairs in "islands of bush in a sea of pine" at Tumut NSW - a site which has hereunto been a model for a range of animal taxa excluding invertebrates.

Double sex-linked translocation chains: segregation in a new race of *Delena cancerides.*

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Delena cancerides incorporates five well characterised chromosomal races. The ancestral form has a completely telocentric karyotype, while the other races are saturated for different combinations of Robertsonian fusions. Three of these races form chains of chromosomes at male meiosis due to fixed fusion heterozygosity. One race has a chain of three chromosomes, another a chain of five, and the most spectacular, a chain of nine chromosomes. These chains include a sex chromosome, and segregate as a complex XY system. This ensures that karyotypic integrity is maintained over successive generations, and the races remain stable.

We have recently found a new, distinct race of *D. cancerides*. At male meiosis this race forms two chains of five chromosomes. The presence of X chromosomes on both chains ensures a stable inheritance pattern, although coordination of the two chains is essential. This is the first stable, double chain system found in any organism, and the coordination of segregation patterns between two distinct translocation chains is unprecedented. Analysis of this chromosomal race will permit the investigation of the origins of fusion heterozygosity in *D. cancerides*, and may shed light on basic principles of meiosis such as the mechanisms behind chromosomal segregation, and sex chromosome evolution.

(Polymorphism) races with lots of different numbers in chains up to chain of 12 or more
A bit melodramatic

Q? Intersex spiders? No

The role of the *lozenge* gene in apoptosis and cell recruitment in the developing *Drosophila* eye

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Mutations in the *lozenge* (*lz*) gene of *Drosophila melanogaster* elicit a pleiotropic set of adult phenotypes. These include severe compound eye perturbations resulting from defective recruitment of photoreceptors R1/6 and R7, cone and pigment cells. In this study, we show that Lz possibly functions downstream of Ras1/MAPK signalling in both cell survival and cell recruitment in third instar eye development. In *lozenge* null mutants, apoptosis occurs prior to *lozenge*-dependent cell fate specification. Although expression of the caspase inhibitor p35 eliminates death in *lozenge* mutants, the *lozenge* mutant eye phenotypes persist because other normal Lozenge functions are still lacking. These results lead to the proposal that Lozenge is required to contribute to the repression of cell death mechanisms, creating a permissive environment for the survival of undifferentiated cells in early eye development. Lz may function downstream of, or in parallel to, Ras1/MAPK signalling in this process. In this study, we also present evidence that suggests that Lz functions downstream of Ras1/MAPK signalling in R7 cell recruitment. We show that the Ras1/MAPK antagonist Tramtrack69 (Ttk69) regulates *lz* gene expression in a cell specific manner. Removal of Ttk69 results in the development of ectopic, *lz*-expressing R7 cells in third instar eye discs. The ectopic R7 cells were shown to be dependent upon Lz function for development. Over-expression of Ttk69 results in down-regulation of *lz* expression in all *lz*-dependent cell types. If Lz is re-introduced into this background via an alternate promoter, *lz*-dependent R7 cells are rescued. This suggests that the loss of R7 cells in Ttk69 over-expression lines was largely due to the removal of Lz.

Latitudinal clines without chromosomal inversions?

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Clinal patterns arise when there are continuous changes in traits or genes over space. They are widely considered to be a way of testing whether natural selection is acting on traits. Latitudinal clines have been demonstrated for many quantitative traits (e.g. body size, development time, heat and cold resistance) in *Drosophila melanogaster*. It is suggested that the clinal variation found in these quantitative traits is the result of adaptation to different environmental conditions, with temperature or temperature-related factors the main selective agents. Recent work, however, has shown an association between body size and a clinally varying cosmopolitan chromosomal inversion in *D. melanogaster*. This suggests that the clinal patterns found for body size and thermal resistance in *D. melanogaster* may be the result of clinal changes in inversion frequencies, rather than the result of selection acting directly on these quantitative traits. *Drosophila simulans*, a sibling species of *D. melanogaster*, does not harbour cosmopolitan chromosomal inversions, and thus provides a natural system to examine the effects of clinal selection acting on quantitative traits independent of chromosomal inversions. Here we present our initial characterisation of clinal variation in *D. simulans* collected from the eastern coast of Australia and discuss the implications for studies of natural selection and QTL mapping.

The Rho GTP exchange factor Pebble is required for the Drosophila mesoderm epithelial to mesenchymal transition

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In *Drosophila melanogaster,* the Rho family of proteins, regulators of the actin cytoskeleton, function as molecular switches to control a wide range of cellular processes including cell shape change, cell adhesion and cell cycle progression. *pebble (pbl)*, a putative Rho guanine nucleotide exchange factor (Rho GEF), is known to be required for cytokinesis. Cells in *pbl* mutant embryos fail to divide at cycle 14 of mitosis resulting in embryonic lethality. In addition to a failure in cytokinesis, *pbl* mutants display a mesoderm epithelial to mesenchymal transition defect. We show that mesodermal cells in *pbl* mutant embryos appear more tightly adhered to neighbouring cells and extend very few processes compared to wild type. We demonstrate that these phenotypes can be rescued by expression of a wild type *pbl* cDNA and by a site-directed mutant form of *pbl* that is incapable of rescuing the cytokinetic phenotype. Interestingly, we further show that the GEF function of Pbl is required for mesoderm cell migration, demonstrating that Pbl activation of the Rho family of small GTPases is required for at least two independent actin based processes during *Drosophila* development.

clear
fluent white paper
easy to follow

in situs to pebble, twist
ventral mesoderm → mesenchyme
Concl. defect meso → mesenchyme
GTPase exchange activity of Pbl required
Next? too fast.... targets?
Qs? ✓✓

Reverse Genetic analysis of the *RGK* gene family of *Drosophila melanogaster*

mostly methods → K/Os
K/Os have no phenotype yet

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The Ras superfamily of small GTPases have diverse roles in many aspects of cell biology and development. The family is composed of six subfamilies, the Ras, Rho, Ran, Rab, Arf families and the least characterised RGK family. GTPases of the RGK family are closely related to the Ras subfamily but have unique structural characteristics that imply that they are not regulated in the same manner as a typical GTPase. Analysis of the four mammalian RGK family members has shown that they have two major roles: regulation of the cytoskeleton through interactions with the Rho pathway, and control of calcium signalling by direct modulation of calcium channel levels at the cell surface. Like mammals, *Drosophila* has four RGK family members, which do not share individual orthologous relationships with the mammalian genes, as both families have undergone independent expansions from a single common ancestor gene. Here we present basic characterisation of the RGK gene family in *Drosophila* and the production of gene targeted mutant alleles of these genes by both the "ends in" and "ends out" methods.

RGKs what do they do?
1. Phylogenies
2. Expression: proliferating tissues
3. Gene targeting by homologous recomb
"ends in" using new system
RGK1 19/27,380 genes 1 in 1500 gametes
RGK2 8/60,733 " 7500 "

3. "ends out"
≈ mouse K/O system
RGK3 2 N 20,000 genes

4. [diagram] 2R
all K/Os viable, fertile
all K/Os triple also viable fertile
RGK4 on X, not yet K/O in 170,000

5. Try screen for
eglA in triple 1/2/3
- RNAi?
Qs?

Sex chromosomes and *DMRT1* in the tiger snake

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In reptiles, sex determination is accomplished either by genetic factor(s), often on sex chromosomes (GSD) or by the effect of the temperature at which eggs are incubated (temperature-dependent sex determination, TSD).

Like birds, snakes have ZZ male/ZW female sex chromosomes. As with birds, some snake families (like the Boidae) possess sex chromosomes that are virtually homomorphic, whereas others (such as the recently diverged tiger snake *Notechis scutatus*) have strongly heteromorphic sex chromosomes. Snakes and bird sex chromosomes are similar in size and morphology, so it is possible that they are genetically homologous and share the same sex-determining gene. A putative avian sex-determining gene *DMRT1* maps to the chicken Z in a region homologous to a sex-reversal region on human 9p. Unlike other Z chromosome genes, DMRT1 shows no dosage compensation in males, and is expressed in the testes during gonadogenesis, suggesting an important dosage sensitive role in male determination and differentiation.

We karyotyped male and female tiger snakes and identified heteromorphic sex chromosomes. We also isolated and characterised *DMRT1* in this species and mapped it to the short arm of the tiger snake Z. Surprisingly, however, *DMRT1* also produces a strong signal on the terminal region of the W. This is the first time a gene has been shown to be conserved between avian and reptilian sex chromosomes. Our results support homology between bird and snake sex chromosomes, and raise questions about a putative sex determining function of *DMRT1* in tiger snakes.

(In progress) Larger spots than expected from single copy gene. Correlative. Could be repetitive DNA in phage clone? NOT BKM repeat. Still testing it out......

Qs? David Rowell evolution "phylogeny misinterpreted". Other snakes? No, lizards chrom SD, temp SD. Yes, pythons

Transcriptional regulatory mechanisms in yeast and human cells

Kevin Struhl

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School

ChIP

The following issues will be discussed:

Markus Proft: HOG1 kinase moves to 3' end of 28 target genes carried by pol II

1) The response to osmotic stress: novel roles for transcriptional corepressors and MAP kinases
2) Global organization of the yeast genome: intrinsic histone-DNA interactions are primarily responsible for preferential accessibility of promoters (promoter regions histone free or low nucleosome (80% of yeast genes))
3) Genomic loci with incomplete Pol III transcription complexes in yeast
4) Unbiased location of p53, Myc, and Sp1 binding sites on two human chromosomes, and their relationship to non-coding RNAs
5) Pol II occupancy and paused transcription complexes in human cells

(not seen in yeast) Affymetrix collab also → haematopoiesis in vitro, retinoic acid, vitamins

ETC 1-8 binds only TFIIIC; ZOD1 mostly + TFIIIB + pol III — don't know if transcribed yet, function yet

(Tom Gingeras, Affymetrix) Hidden transcriptome of human chr 21, chr 22 (2% of genome), human cell lines

65% unknown RNAs

Sites for: Myc 745 sites, Sp1 375 sites, p53 49 sites → 29% also, 61% also — many assoc. with non-coding RNAs. What do they do???

Antisense RNAs? Regulatory RNAs? Modify chromatin structure? Are they markers of enhancers?

Biological function? — disorder + specific phenomenon — occurs in living cells

Phylogeographic congruence and long-term environmental refuges: did a Regional Forest Agreement cream off a biodiversity-generating hotspot?

Paul Sunnucks[1], Ryan Garrick[1], Chester Sands[1], Sherryn Ciavaglia[1], Mark Blacket[1], Noel Tait[2], David Rowell[3]
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Comparative phylogeography can provide information about (1) genetic-geographic patterns, and (2) inferences about the events and mechanisms leading to those patterns. We are conducting fine scale (~100km) comparative phylogeography of rotting-log-dependent (saproxylic) invertebrates. The study area, Tallaganda in the Great Dividing Range of SE NSW, has been geologically stable for tens of millions of years, during which the area has experienced radical shifts in the spatial distribution of forest. In cold, dry periods, forest retreats from uplands into sheltered, wetter gullies. Under an *a priori* model, the east-facing, lower-lying Eastern Slopes Region (ESR) offers the most significant high-quality refuge during harsh times. Two velvet worms, two terrestrial flatworms and a giant springtail show strong congruence in phylogeographic patterning, including that the ESR appears to have been repeatedly an important biodiversity refuge. The ESR currently contains relatively high endemism; it also contains the highest quality production forest, and in the recent Regional Forest Agreement was allocated entirely to State Forest (production) rather than National Park (no harvesting). Thus the region with the highest current endemism is also the most important for producing / maintaining future biodiversity, but is the most likely to suffer detrimental human impacts.

Recent Human Evolution: What Genes Really Tell Us.

Alan Templeton
Department of Genetics/Department of Biology, Washington University, St. Louis

Phylogeographic inferences are frequently drawn from the analysis of an evolutionary haplotype tree of a single DNA region or type of DNA, such as mitochondrial DNA. However, a phylogeographic event is only detectable in principle if an appropriate mutation or mutations occurred in the right time and place in the evolutionary history of the genetic variation being screened. In addition, like all forms of statistical inference, phylogeographic inference is subject to both false positives and false negatives. One way of both increasing the resolution of phylogeographic analyses and reducing the error rates is to survey multiple DNA regions and cross validate phylogeographic inferences across these DNA regions. A formal hypothesis testing framework based upon likelihood ratio tests is used to test hypotheses about recent human evolution based upon ten different DNA regions. The analyses shows that there were three major expansion events out-of-Africa in the last 2 million years. Moreover, gene flow between African and non-African human populations was established at least by 600,000 years ago, and the last out-of-Africa expansion event involved interbreeding with nonAfrican populations, not their replacement. These results have implications for understanding the meaning of race in current humanity.

Cytoplasmic organellar DNA has contributed massively to the genetic complexity of the nucleus during endosymbiotic evolution

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Genome sequencing has revealed that many large contiguous segments of mitochondrial and chloroplast DNA sequences, sometimes even complete organelle genomes, are integrated into eukaryotic chromosomes. This indicates that a deluge of DNA from organelles is constantly bombarding the nucleus. This process – that underlies endosymbiotic gene transfer - is a ubiquitous, ongoing, and natural mechanism that pervades nuclear DNA dynamics. As a result, cytoplasmic organelle autonomy has been abolished and nuclear complexity has been increased by a continuous influx of mitochondrial and chloroplast DNA. Recent experiments have shown that chloroplast DNA transfer to the nucleus occurs at frequencies that were not previously conceived, suggesting that the process generates a high level of nuclear and cytoplasmic genetic novelty that is unique to the eukaryotic lineage.

Nuclear Export of the Transcriptional Activator AreA occurs via the CrmA Exportin in *Aspergillus nidulans*.

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Department of Genetics, University of Melbourne, 3010, AUSTRALIA

The *Aspergillus nidulans areA* gene encodes a GATA DNA-binding zinc finger transcriptional activator of many genes required for nitrogen catabolism. AreA levels and activity are controlled by autogenous regulation, differential mRNA turnover and interaction with the NmrA and TamA proteins. We have shown that an epitope-tagged AreA protein hyperaccumulates in the nucleus under nitrogen starvation conditions independent of these controls. Transfer from nitrogen starvation to media containing a nitrogen source triggers rapid exit of AreA from the nucleus. AreA nuclear hyperaccumulation and nuclear exit correlates with changes in nitrogen catabolic gene expression.

We have shown that residues 60-423 or 844-876 of AreA are dispensable for its nucleocytoplasmic redistribution. AreA contains a putative leucine-rich binding motif for CrmA, the homologue of the *Schizosaccharomyces pombe* CRM1 exportin. CRM1 function is inhibited by the drug Leptomycin B (LMB), via interaction with a specific cysteine residue. We isolated the *crmA* gene and mutated the corresponding threonine residue to cysteine. The $crmA^{T525C}$ mutant grows normally and retains normal AreA nuclear accumulation and exit in the absence of LMB. Unlike the wild-type, this mutant is LMB sensitive and in the presence of LMB, AreA nuclear export is inhibited. These results indicate that CrmA is the major AreA exportin.

The effects of cyromazine treatment on *Drosophila melanogaster* – a genetic approach

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The insecticide cyromazine is classified as an insect growth regulator. It has been used to control pest species such as the housefly (*Musca domestica*), the Australian sheep blowfly (*Lucilia cuprina*) and the pea leaf miner (*Liriomyza huidobrensis*). The physiological effects caused by cyromazine include necrotic lesions and irregular hardening of the cuticle, elongation of the larval insect body and death. The molecular mode of action of cyromazine has not been determined. Although cyromazine resistance exists in the field, mechanisms of resistance are unknown. Physiological studies have been carried out to assess the effects of cyromazine. Here we describe the genetic responses observed due to cyromazine application. Using *Drosophila melanogaster* as a model organism, we have used an integrated approach of deficiency kit analysis, microarrays and mutagenesis combined with mapping to assess the effects of cyromazine.
We have devised a deficiency mapping method for genome wide screening of genes with the potential to mediate insecticide resistance. Genomic regions conferring increased tolerance to cyromazine have been identified. Many genes showing cyromazine-induced changes in expression, detected on microarrays, map to these regions.
Mutants have been identified in *D. melanogaster* that show resistance to cyromazine. Using polymorphic molecular markers (single nucleotide polymorphisms (SNPs), restriction fragment length polymorphisms (RFLPs) and microsatellites) and recombination mapping, we have refined the location of *Rst(2b)Cyr* to a region of 1 map unit which contains 70 putative open reading frames. Further analysis using molecular markers will refine this region to allow identification of a single candidate gene.

Evidence for
Sympatric speciation by host shift in the sea

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Current Biology (in press)

The genetic divergence and evolution of new species within the geographic range of a single population (sympatric speciation) contrasts with the well-established doctrine that speciation occurs when populations become geographically isolated (allopatric speciation). Although there is considerable theoretical support for sympatric speciation, this mode of diversification remains controversial, at least in part because there are few well-supported examples. We use a combination of molecular, ecological, and biogeographical data to describe sympatric speciation by host shift in a new species of coral-dwelling fish (genus *Gobiodon*). We propose that intense competition for preferred coral habitats drives host shifts in *Gobiodon* and that the high diversity of corals provides the source of novel, unoccupied habitats. Habitat selection and host fidelity promote reproductive isolation and ultimately lead to species divergence. Our results are analogous to sympatric speciation by host-shift in phytophagous insects. The fundamental similarity between these fishes and insects is a specialised and intimate relationship with their hosts that make them ideal candidates for speciation by host shifts. Ironically, the extreme habitat specialisation that has generated divergence of this new species of fish now places it at extreme risk of extinction due to the global decline in coral reef health.

Coral gobies

need reproductive isolation for sympatric speciation
host coral | change in species of

2 types of speciation
— usual one same host coral
— new one moves to new host coral

Patching up common human cancer

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Common human tumours can also exist in rare inherited forms of the disease showing high penetrance. When this occurs genetic and physical mapping strategies can be brought to bear to isolate the gene concerned. In some cases the gene responsible for the rare inherited form of the disease leads to the identification of a gene or genetic pathway which is perturbed in the common, sporadic form of the disease. Through mapping and isolating the gene for a rare inherited form of basal cell carcinom utilising patients presenting with naevoid basal cell carcinoma syndrome we identified the mammalian homologue of the drosophila segment polarity gene *patched*. Also, it appears that the *patched* pathway is mutated not only in the common form of basal cell carcinoma but also in a variety of other solid tumours. This talk will overview this field and also present data on the use of a number of model organisms to dissect *patched* pathway function in tumorigenesis and mammalian development.

Altered Gene Expression Levels Linked to Fenvalerate Resistance in Cotton Bollworm, *Helicoverpa armigera*

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The ANO2 strain of *Helicoverpa armigera* is 50-fold resistant to the pyrethroid insecticide fenvalerate; largely due to a single gene, *RFen1*, on Linkage Group 13. The cDNA-AFLP technique was used to search for changes in gene expression levels controlled by or linked to *RFen1*. Larvae from a segregating backcross family were genotyped at a linked marker locus, enabling prediction of their fenvalerate resistance status without exposure to insecticide. mRNA was isolated from individual larvae and used to make cDNA, which was processed for AFLPs (double restriction digestion, ligation to adapters, amplification by two rounds of PCR, and resolution of labelled fragments on a sequencing gel). 256 different primer combinations were tried and a total of 426 bands showing differences between resistant and susceptible groups were identified. Three were cytochrome p450s, with similarity to *Depressaria pastinacella* CYP6AE1 and *Helicoverpa armigera* CYP4S1 and CYP4AP1. The latter was upregulated whereas the other two were down-regulated in the resistant group. In addition, two carboxylesterases were down-regulated and one was up-regulated in the resistant group. Currently, real time RT-PCR is being used to verify the results and full length sequences of these potential detoxification genes are being sought using rapid amplification of cDNA ends (RACE).

Frequency-dependent selection maintains clonal diversity in asexual organisms: evidence from natural populations of the earth mite species *Penthaleus major*

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It is generally not known how asexual organisms maintain diversity. One explanation not previously tested involves negative frequency-dependent selection. This type of selection is assumed to play a role in maintaining genetic variation within sexual species, yet even for these species few studies have demonstrated it in natural populations. Here we use field mesocosm experiments with mites from different origins to measure selection in the asexual blue oat mite species, *Penthaleus major*. Allozyme-defined clones in the mesocosms exhibit negative frequency-dependent selection. Fitness differences among the allozyme-defined clones were similar regardless of the geographic origin of the mites. The same allozyme-defined clones from different areas grouped together based on their similarity for amplified fragment length polymorphisms, indicating that *P. major* clones are closely related genetically regardless of their origin. Regression equations predict the equilibrium frequencies for these allozyme-defined clones which fit previous experiments/surveys. The results indicate that negative frequency-dependent selection maintains clonal diversity in natural populations of *P. major*, and we propose that it is a general explanation for persistent diversity in asexual organisms.

Are the dingoes on Fraser Island the last remaining pure dingoes in the wild?

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When dingoes arrived in Australia 5,000 years ago with Austronesian explorers, they were the domestic dog of the day. Only a limited number of related animals can have been introduced as all of their mitochondrial D-loop sequences have a common recent ancestor. The dingo is an example of the early stages of the domestic dog and is more closely related to the Asian breeds of today (Shiba Inu, Akita) than European domestic dogs. We have exploited this to identify genetic markers that can differentiate dingoes from European dogs. The dingo is an Australian icon that is under threat and disappearing from the wild where it is being replaced by hybrids with domestic dogs. The close relationship of dingoes and dogs means that there are no barriers to interbreeding. The process is difficult to stop once started and has taken a strong hold in east coast populations such that most contain ~80% hybrids. The dingoes on Fraser Island have been presented to tourists as pure but "dingo experts" say otherwise? DNA testing of 20 microsatellite loci chosen for their differences in allele distributions between dingoes and dogs do not support claims of large amounts of hybridisation in 39 dingoes culled in 2001.

Gestational Diabetes as a model for Type 2 Diabetes

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Gestational diabetes mellitus (GDM) is an elevation of plasma glucose that occurs in 5% of pregnancies in Australia. It is likely that the same genetic predisposition to the late onset Type 2 diabetes underlies GDM. Many women who experience GDM go on to develop Type 2D later in life. In GDM, subjects are young and parents are usually available. This makes study of GDM a good model for Type 2 diabetes. We are testing regions of the genome for evidence of genes involved in GDM that have shown evidence of association with Type 2 Diabetes. We have tested a group of over 200 affected sib pairs for markers on a region of chromosome 10. Suggestive evidence of genes involved in GDM in the region is found using the whole data set. Partitioning the data set using factors known to be associated with the onset of diabetes, such as age at symptoms, waist-to hip ration, body mass index, can lead to large improvements in the significance of the association. This support the hypothesis that the same genes contributing to type 2 diabetes are also contributing to GDM and we can study GDM to help identify some of them.

Fine-scale genetic structure of inshore bottlenose dolphins (*Tursiops aduncus*) in the Hunter Region, NSW

Joanna Wiszniewski[1,2], Luciana Möller[1,2], Simon Allen[1] and Luciano Beheregaray[2]
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Inshore bottlenose dolphins (genus *Tursiops*) live in fission-fusion societies, also called communities, where individuals join and leave schools on a fluid basis yet maintain long-term associations with specific individuals. For these animals, it has been hypothesized that factors such as disjunct distributions and philopatry influence genetic structure by promoting isolation between groups. Alternatively, extensive ranging patterns, dispersal and occasional movements by individuals may enhance genetic exchange between adjacent communities. This study investigates fine-scale genetic structure and dispersal patterns between five dolphin communities inhabiting the Hunter Region (NSW) using eight polymorphic cetacean microsatellite loci. Preliminary results based on fixation indices (Fst and Rst) suggest that the extent of gene flow between these communities is largely dependent on the type of habitat they reside in (embayment vs open coast). Communities on the open coast showed high levels of gene flow. By contrast, despite smaller geographic distances, there was a lower level of gene flow between communities living within an embayment and those inhabiting the adjacent open coast.

Poster Abstracts

(in alphabetical order)

Comparative mapping of HSA1q with respect to porcine chromosomes further refines evolutionary breaks

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QTL for economically important traits have been discovered on porcine chromosomes (SSC) 9 and 10. Previous comparative mapping suggested that regions of SSC9 and 10 along with SSC4, 6 and 14 share homology with human chromosome 1 (HSA1). The refinement of the evolutionary breakpoints between pig chromosomes and HSA1 allows us to utilise human genome information to identify positional candidate genes for the QTL. To further refine the boundaries of the evolutionarily conserved segments between humans and pigs, genes from the public human genome sequence for HSA1 were used in a BLAST of porcine expressed sequence tag (EST) database. Matching ESTs were used to design porcine specific primers. This allowed the assignment of genes to pig chromosomes using the INRA somatic cell hybrid panel (INRA-SCHP) and/or the high resolution radiation hybrid panel (IMpRH) to determine whether gene order was conserved. Sixteen genes from HSA1 were localised onto porcine chromosomes. Three genes were mapped to SSC4 (ALDH9A1, TNRC4 and UMPK), nine to SSC9 (ATF3, CACNA1E, C1orf16, FNBP2, GLUL, MAPKAPK2, PEPP3, PTGS2, and RAB7L1) and four to SSC10 (CYB5R1, EPHX1, RGS2, and RGS18). The physical mapping of these genes has contributed to refinement of evolutionary breakpoints between SSC4, 9 and 10 and HSA1.

This work was supported by Australian Pork Limited (APL) project 1756.

Transformation of *Thielaviopsis basicola*: A tool to study the host-pathogen interaction at a molecular level.

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The soil-borne fungus, *Thielaviopsis basicola* is a plant pathogen of the deuteromycetes. Different strains are capable of attacking a wide range of host plants causing black root rot, a seedling disease. In Australia, *T. basicola* was first detected on sweet pea in the state of Queensland in 1930. Since then, it has been reported in all states except the Northern territory. It is now estimated that the incidence of black root rot of cotton in New South Wales may reach 95% by the year 2004. Control strategies based on cultural practices, biocontrol agents, chemical fungicides, systemic induced host resistance and genetically modified host resistance have not yet solved the loss of important economic agricultural yield. Although, some progress has been made in elucidating the steps involved in the infection process of host plants by *T. basicola*, little is known about the key genetic factors that could characterize the pathogenicity genes of this fungus. Insofar, no molecular tools, such as mutagenesis via integrative transformation have been developed for this pathogen. We are in the progress of constructing *T. basicola* random mutants via integrative transformation using PEG-mediated and *agrobacterium tumefaciens*-mediated transformation (ATMT). The mutants will be screened and those with altered pathogenicity will be analysed using Southern blot and marker rescue technique. Currently, we have managed to produce protoplasts only, a requirement for PEG-mediated transformation. ATMT is still under progress.

Interaction of two *Arabidopsis* PCNA homologues with *Arabidopsis* AtRad30, a putative homologue of human and yeast polymerase eta

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Polymerase eta (Pol η) contributes to UV resistance through bypass of UV-induced DNA damage via translesion synthesis. Interaction of yeast (*Saccharomyces cerevisiae*) and human Pol η, encoded by *hRAD30A/XPV* and *RAD30*, respectively, with the sliding clamp PCNA is essential for Pol η activity. We have cloned *Arabidopsis* cDNA sequences predicted to encode homologues of Pol η (AtRad30) and PCNA (AtPCNA-1, AtPCNA-2). Expression of *AtRAD30* in a yeast *rad30* deletion mutant did not restore UV resistance. This lack of complementation could have been due to a failure of AtRad30 to interact with yeast PCNA, a possibility we confirmed in yeast two-hybrid studies. However, we found that AtRad30 was able to interact with AtPCNA-1 and AtPCNA-2. Nonetheless, co-expression of *AtRAD30* and *AtPCNA-1* or *AtPCNA-2* in the *rad30* mutant also failed to restore UV resistance. The apparent failure of AtRad30 to operate in yeast might reflect divergence in its C-terminal region which lacks a C_2H_2 zinc binding motif present in human and yeast Pol η. Alternatively, the fault may lie with the plant PCNA homologues. Currently, we are testing their functionality in yeast by determining whether yeast strains expressing *AtPCNA-1* or *AtPCNA-2* produce viable clones after deletion of the yeast PCNA locus.

Elimination of CYP21 as a candidate gene for androstenone boar taint.

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1. Centre for Advanced Technologies in Animal Genetics and Reproduction, University of Sydney. 2. Animal Genetics and Breeding Unit, University of New England.

The Cytochrome P450 steroid, 21 Hydroxylase (CYP21), is a positional candidate gene for boar taint caused by an excessive level of androstenone. This gene contains 9 Introns and 10 Exons and is located in the SLA (Swine Lymphocyte Antigen) region on chromosome 7. Sequencing of four sires from an Australian mapping pedigree has revealed 36 SNPs. 22 of these are in non-coding regions, and those in coding regions do not cause changes in the amino acid sequence of the protein. A SNP at position 2329 (Intron 7) could influence the use of a theoretical alternative splice site. However quantitative analyses have shown that segregation of this SNP in the sire families has no impact on androstenone levels. RT-PCR and sequencing have provided no evidence of the use of this alternative splice site in testis RNA. CYP21 can be excluded as the locus responsible for the SSC7 androstenone QTL.

The role of RacGAP50C during cytokinesis and in the organisation of the interphase cytoskeleton

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The RacGAP50C-PAV kinesin-like protein complex is known to play multiple roles during cytokinesis. The microtubule bundling activity of the RacGAP50C-PAV complex is required for the formation of the central spindle, but the role of the contractile ring-localised RacGAP50C-PAV, and the importance of the synergistic interaction with the RhoGEF pebble at the contractile ring is less clear. RacGAP50C, previously shown to be a GAP for Rac/Cdc42, is functionally converted to a RhoGAP through phosphorylation by Aurora B during cytokinesis. We have shown that overexpression of an Aurora B kinase phosphorylation-deficient RacGAP50C (S419A) results in cytokinesis failure in Drosophila S2 cells. We speculate that constant activation by pebble, and inactivation by RacGAP50C is required for the Rho directed constriction of the contractile ring.
RacGAP50C overexpression in S2 cells resulted in a dramatic alteration of the interphase cytoskeleton. RacGAP50C overexpressing cells grow long, non-filopodial processes, similar to axons. We are currently investigating which Rho family G-protein needs to be inactivated for the process formation. Preliminary data show, that the best candidate is Rac2.

Interactions of the replication initiator protein RepA with the origin of replication in IncB plasmid.

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Replication of pMU720 requires the synthesis of the initiator protein, RepA, which is rate limiting for replication. Purified RepA was used in different chemical modification assays *in vitro* to characterize its interaction with DNA target sites in the origin of replication of this plasmid (ori^B). It was found that RepA protected a 68-bp region of ori^B, which contains four copies of sequence motif 5'-AANCYGCAA-3'. Scanning mutagenesis identified this sequence as specifically recognized by RepA. Binding of RepA to the three functional sites RB1, RB2 and RB4 was in an ordered and sequential manner and the spacing between sites are critical for origin activity *in vivo* indicating that RepA molecules bound to ori^B may interact with one another to promote and optimise origin function. Using $KMnO_4$ footprinting assay, an open complex (spanned one turn of DNA helix) was detected at a downstream region of RB4. Mutational analysis verified several requirements for efficient unwinding of ori^B which are: (i) the presence of both RepA and DnaA (ii) the native positioning of the RepA boxes (iii) presence of the 6-mer 5'-TCTTAA- 3'. This 6-mer was found to be highly conserved in three distantly related plasmids pMU720, pMU604 and pSW800.

Comparative population genetic structure of Indian Ocean bottlenose dolphins (*Tursiops aduncus*) and short-beaked common dolphins (*Delphinus delphis*) in South Australian waters

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Indian Ocean bottlenose dolphins (*Tursiops aduncus*) and short-beaked common dolphins (*Delphinus delphis*) are distributed parapatrically in coastal waters of South Australia (SA). Several individuals from both species die each year entangled in nets for aquaculture of blue fin tuna near Port Lincoln, Spencer Gulf. There is a concern that this mortality may negatively impact on the viability of local dolphin populations. This study is conducting a comparative analysis of the genetic diversity and structure of bottlenose dolphins and common dolphins in SA waters using sequences of the mitochondrial DNA (mtDNA) control region and microsatellite markers. Tissue samples (*T. aduncus*=112, *D. delphis*=125) available for this study were collected from carcasses by the South Australian Museum over the last 14 years, and also by biopsy sampling of living animals. Preliminary single stranded conformation polymorphism analysis of approximately 460-bp of the mtDNA control region suggests higher haplotypic diversity for bottlenose dolphins than for common dolphins.

Mutation of spo-11 and its effect on meiotic recombination in Neurospora crassa.

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The *SPO11* gene is carried by numerous organisms, including humans, and is thought to code for the enzyme that catalyses the initiation of all meiotic recombination. We have generated three *Neurospora crassa spo-11* mutant alleles.

When homozygous, all three alleles reduce fertility and spore viability, and an analysis of the remaining viable spores suggests extensive chromosome non-disjunction during meiosis. While we expected that, as in other organisms, mutation of *spo-11* would reduce or abolish recombination in Neurospora, our data indicate otherwise. The least altered allele, $spo\text{-}11^{RIP3}$, appears to increase allelic recombination near the recombination hotspot cog^{L}, but has no detectable effect on flanking marker exchange nearby. The remaining two alleles, $spo\text{-}11^{RIP1}$ and $spo\text{-}11^{RIP2}$, appear to elevate both allelic recombination initiated from cog^{L} and the frequency of flanking marker exchange nearby. Conversely, recombination in another region of the Neurospora genome seems to be reduced in $spo\text{-}11^{RIP1}$ homozygotes, suggesting that cog^{L}- initiated recombination may be spo-11p independent.

Characterisation of T-DNA insertion mutants of γ-glutamylcysteine synthetase in *Arabidopsis*

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Glutathione (GSH) is involved in a number of different processes in plants including defence against heavy metals and oxidative stress. GSH is synthesised in a two step pathway by the enzymes γ-glutamylcysteine synthetase (GCS) and glutathione synthetase. Two mutant alleles of GCS are already known in Arabidopsis, *rml1* and *cad2*. *cad2* plants contain 30% of wild-type GSH levels and have a wild-type phenotype but *rml1* plants have less than 2% of wt GSH levels and do not grow roots.
Three new T-DNA insertion mutant alleles of GCS have been identified from the SALK database and characterised. These mutants cause a recessive embryo lethal phenotype that can be rescued by exogenous GSH. However, rescued embryos do not survive past the seedling stage. It is likely that the GCS T-DNA mutants are null alleles of GCS and this suggests *rml1* is not a null allele because *rml1* mutants have a less severe phenotype.
Using these mutants we intend to study the effect of expressing GCS only in specific tissues of Arabidopsis by using an enhancer trap expression system to express GCS in isolated tissues in the *rml1* and GCS T-DNA mutant background.

Overexpression of cytochrome P450 genes and insecticide resistance in the sheep blowfly, *Lucilia cuprina*

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Some cytochrome P450 enzymes are able to detoxify xenobiotic compounds. In insects the over-expression of specific cytochrome P450 genes has been shown to be responsible for resistance to a broad spectrum of insecticides. *Lucilia cuprina* is a major pest of sheep in Australia and New Zealand. We have cloned 31 P450 genes in *L. cuprina* including members of the *Cyp4*, *Cyp6*, *Cyp9* and *Cyp12* genes, families that have been implicated in resistance in other insect species. To identify the resistance-associated P450s, the expression levels of the *Cyp6*, *Cyp9* and *Cyp12* genes were investigated in susceptible strain and a recently isolated field strain that is resistant to chemically diverse insecticides – diflubenzuon and lufenuron. Three genes (*Cyp6A2*, *Cyp6A8* and *Cyp12A2*) are highly up regulated in the resistant field strain. One or more of these genes may be responsible for the metabolic detoxification of insecticides. The mapping of these genes and the overexpression and resistance phenotypes is in progress.

A fishy tale of molecular evolution in the genus *Chironomus*

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Larvae of the genus *Chironomus* are often able to survive high levels of pollution. In order to understand the molecular basis of this pollution tolerance, we have used a degenerate primer approach in isolating known heavy metal responsive genes. One of these genes is Cu/Zn superoxide dismutase (SOD1). Amplification and sequencing of this gene from an Australian species, *Chironomus duplex,* revealed a sequence motif with greater similarity to SOD1 from fish and oyster species than to *Drosophila* and mosquito homologues. A novel intron that is not present in any known SOD sequence was also found. RT-PCR revealed that this gene is being transcribed in larvae which are an aquatic life-stage. *In-situ* hybridization onto polytene chromosomes of *Chironomus duplex* showed a single band on cytological position 4b of arm D. The same fish-like SOD1 sequence was also found in another Australian species, *Chironomus tepperi.* Further work includes sequencing SOD1 from other *Chironomus* species and getting their full length SOD1 sequences. Based on the phylogenetic distribution of the fish-like sequence motif we hypothesize that it may reflect an adaptation to aquatic environments.

Weapons of Mass Transformation: Biolistic DNA Delivery in *Helicoverpa armigera*

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Helicoverpa armigera is Australia's most significant pest of cotton crops causing extensive yield losses each year. The use of broad spectrum insecticides to control *H. armigera* outbreaks is rapidly losing its effectiveness due to resistance build up. My project will assess the feasibility of using RNA interference (RNAi) to specifically knock down essential growth and development genes in *H. armigera* as a potential species-specific control measure. Traditionally the introduction of gene constructs into insects has been performed via microinjection. To circumvent this technically challenging and relatively low-throughput technique I will develop biolistics as a novel way of introducing transgenes into *H. armigera* embryos in a high throughput manner. Biolistic gene delivery uses a helium-driven gene gun to "shoot" microscopic gold particles coated in DNA into insect eggs. Using this method, a transposon-based reporter gene can be introduced into insect eggs allowing stable integration of the reporter gene. Biolistics will also be used to introduce RNAi constructs into *H. armigera* as a way of studying the parameters of RNAi in a new insect and testing the function of targeted genes.

Hsr-omega repeat-length polymorphism of _D. melanogaster_: geographical variation, and trait and marker associations

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Clinal latitudinal variation occurs down the eastern coast of Australia in an important heat stress gene (*hsr-omega*) that produces two RNA transcripts but no protein product. This 8bp-indel variation (*hsr-omega*$^{L/S}$) at the 5', non-repeat, end of the gene has been strongly associated with heat tolerance variation in this species. Here we report on a multi-allelic, hyper-variable, polymorphic site at the 3` end of the gene, the *hsr-omega* repeat-length polymorphism. Natural populations of *D. melanogaster* were examined for latitudinal and altitudinal variation in repeat-length. Average repeat-length demonstrated a strong negative association with latitude, which was robust over three years of screening (2000 (r_s= -0.684, $p<0.001$), 2001 (r_s= -0.740, $p<0.001$) and 2002 (r_s= -0.556, $p<0.01$)), but no altitudinal association was detected. Linkage disequilibrium was assessed in one population located centrally in the latitude cline, to help tease apart associations between *hsr-omega* repeat-length and ten other clinally varying genetic markers on chromosome three, including *hsr-omega*$^{L/S}$ and *In(3R)P*. The data indicated that repeat-length was not associated with *In(3R)P*, was mildly associated with *hsr-omega*$^{L/S}$ (D`= 0.57), and that there was strong linkage disequilibrium between *In(3R)P* and *hsr-omega*$^{L/S}$ (D`= 0.72, $p < 0.001$ after Bonferroni correction). A family study using this same population, investigated associations between *hsr-omega* repeat-length variation and variation in heat tolerance, cold recovery and body size. No association with heat tolerance or body size occurred but a borderline association with cold tolerance was detected.

Comparative mechanisms of hypertrophy between ovine and murine muscle models

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Muscle is functionally important for locomotion and as a source of meat. Muscle is highly responsive to changes in functional demand and overload can lead to hypertrophy, an increase in cell size. Hypertrophy can arise through the expression of transcription factors involved in myogenesis. The first of the transcription factor genes coding for muscle-specific proteins are the myogenic regulatory factors (MRFs), part of the basic Helix-Loop-Helix (bHLH) transcription factors. The subfamily consists of MyoD (also known as Myf-3), Myf-5, myogenin (Myf-1) and MRF4 (Myf-6 or Herculin), primary and secondary MRFs involved in expression and hypertrophy. The null hypothesis of this study is that *in vitro* treatments do not induce myotube hypertrophy by acting on transcription factor expression and binding to DNA. After differentiation is induced, ovine and murine cell cultures are treated with Tumour Necrosis Factor (TNF), Insulin, Insulin-like Growth Factor-1 (IGF-1) and Adrenaline. Whole cell protein is collected and nuclear protein extracted to carry out western blotting and electrophoretic mobility shift assays (EMSA). Results examine the effects of treatments on gene and protein expression and the induction of hypertrophy using computer modulated programs.

Cloning and Mapping of the Genomic Region containing the Tammar Wallaby (Macropus *Eugenii*) Orthologues of MHC genes.

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Major Histocompatability Complex (MHC) molecules are central to development and regulation of the immune system in all jawed vertebrates. MHC Class III cytokine genes from the Tumor Necrosis Factor core family, including Lymphotoxin-β (LT-β), Tumor Necrosis Factor-α (TNF-α) and Lymphotoxin-α (LT-α) are well studied in human and mouse. Orthologues have been identified in several other eutherian species and the cDNA sequences have been reported for a model marsupial, the tammar wallaby.
Comparative genomics can help to determine gene function, to understand the evolution of a gene or gene family, and to identify potential regulatory regions. We therefore cloned the genomic region containing the tammar LT-β, TNF-α, and LT-α orthologues by "genome walking", using primers designed from known tammar sequences and regions conserved in other species. We isolated a tammar BAC clone containing all three genes, and used FISH to map it to tammar chromosome proximal 2q (the first chromosomal assignment of any marsupial MHCIII gene). These tammar genes show similar intergenic distances and the same transcriptional orientation as in human and mouse. Gene structures and sequences are also similar, except that the Tammar LT-α gene lacks an intron between exons 1 and 2. By comparing the tammar, human and mouse genomic sequences we were able to identify candidate regulatory regions for the tammar genes.

Towards positional cloning of *Rst(1b)Cyr*, a mutant conferring cyromazine and lufernuron resistance in *Drosophila melanogaster*

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The insect growth regulator insecticide cyromazine, has been widely used for over thirty years on Dipteran pests *Musca domestica*, ((the house fly) and *Lucilia cuprina*, (the sheep blowfly). Cyromazine is involved in disruption of chitin synthesis and poisoning induces cuticular lesions, which result in death. However, like many insecticides, the molecular target and mode of action of cyromazine, is unknown.
Due to the availability of powerful genetic tools, including the genome sequence, we have been using *Drosophila melanogaster* as a model organism for studying cyromazine resistance. Six cyromazine mutants have been generated by chemical mutagenesis followed by selection at the (LC95) (Daborn *et.al*, 2000). In each case, the genetic basis for low to moderate resistance levels is monogenic. One of these mutants, *Rst(1b)Cyr*, also has cross resistance to lufenuron, a chitin synthesis inhibitor. In order to elucidate the molecular mechanism of resistance, we are using the positional approach to clone *Rst(1b)Cyr*.
Rst(1b)Cyr was localised to a region of the X Chromosome flanked by phenotypic markers. Recombination mapping using P-transposon insertion sites, phenotypic markers, microsatellites and Single Nucleotide Polymorphisms (SNPs) has refined the region to approximately 27kb containing five open reading frames. None of these have an obvious connection to chitin synthesis. Sequencing the coding regions of these five open reading frames has not shown a difference between *Rst(1b)Cyr* and a susceptible strain, white. Expression levels of the five remaining open reading frames at different life stages will be investigated to help elucidate this gene.

The *Aspergillus nidulans sarA* gene encodes an L-amino acid oxidase

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In *Aspergillus nidulans* the major nitrogen regulatory gene is *areA*, encoding a positively acting DNA binding protein. During growth in the absence of preferred nitrogen sources AreA activates the expression of genes involved in alternative pathways of nitrogen assimilation. Wild-type strains of *A.nidulans* grow poorly on a number of amino acids as the sole nitrogen source, whereas strains that contain the mutant allele *areA102* grow strongly. Mutation of *sarA* abolishes the strong growth associated with *areA102*, and the *sarA* gene was cloned by complementation of this phenotype on L-histidine. Alignment of the predicted product of *sarA* with the *Neurospora crassa* homologue suggested that *sarA* encoded an L-amino acid oxidase. This was confirmed by the creation of a *sarA* gene inactivation strain, and we have found that over expression of *sarA* is able to mimic the *areA102* phenotype on L-histidine. LAOs catalyse the conversion of L-amino acids to their corresponding keto acids, hydrogen peroxide, and ammonium. Growth tests have shown that a range of amino acids serve as substrates for LAO in *A.nidulans*, and have demonstrated the existence of additional pathways for the catabolism of some amino acids.

Directed evolution of barley β-D-glucan endohydrolases

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Similarities in the primary sequences and three dimensional structures of the barley (1-3)-β-D-glucan endohydrolase isozyme EII and (1-3,1-4)-β-D-glucan endohydrolase isozyme GII suggest they are closely related in evolutionary terms, yet they perform completely different functions. While the (1-3)-β-D-glucanase capable of hydrolyzing the linear, substituted and branched (1-3)-β-D-glucans often found in fungal cell walls appears to be involved in plant protection, the (1-3,1-4)- β-D-glucanase is responsible for digestion of the starchy endosperm cell wall during germination of barley grains (Hrmova and Fincher, 2001). Possibly in response to the hostile environment it encounters, the (1-3)-β-D-glucanase has evolved to be significantly more thermostable than the (1-3,1-4)-*β*-D-glucanase (Stewart *et al.* 2001). We are using an *in vivo* gene diversification technique developed in *Neurospora crassa* (Catcheside *et al.* 2003) to investigate the molecular basis for their functional divergence and to generate a more thermostable (1-3,1-4)-*β*-D-glucanase intended for industrial use.

Catcheside, D. E. A., Rasmussen, J. P., Yeadon, P. J., Bowring, F. J., Cambereri, E. B., Kato, E., Gabe, J. & Stuart, W. D. 2003, 'Diversification of exogenous genes *in vivo* in *Neurospora*', *Appl. Microbiol. Biotechnol.*, 62: 544-549.

Hrmova, H. and Fincher, G.B. 2001, Structure-function relationships of β-D-glucan endo- and exohydrolases from higher plants. Plant Mol. Biol., 47: 73-91.

Stewart, R., Varghese, J., Garret, P., Hoj, B. & Fincher, G. 2001 'Mutant barley (1-3,1-4)-β-glucan endohydrolases with enhanced thermostability', Protein Eng., vol. 14: 245-253.

Sex determination in Nile tilapia (*Oreochromis niloticus* L.): isolation and physical mapping of sex-linked DNA markers

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The Nile tilapia is one of the most important species in worldwide finfish aquaculture and is an important model species for the analysis of the early events in sex chromosome evolution. We obtained genetic evidence that *O. niloticus* has genetic sex determination (GSD) with an XX female – XY male sex determination system. To search for sex-linked or sex-specific markers, we screened gynogenetically produced XX and YY Nile tilapia and diploid control groups for amplified fragment length polymorphisms (AFLPs). Family level bulked segregant analysis (BSA) screening of XX and YY gynogenetic family pools and individuals (XX and YY gynogenetics and XX and XY control individuals) identified three Y-linked (*Oni*Y425, *Oni*Y382, *Oni*Y227) and one X-linked (*Oni*X420) AFLP markers. Single locus PCR assays were developed for these markers. Tight linkage was demonstrated between the AFLP markers and the sex locus within the source families. However, these markers failed to consistently identify sex in unrelated individuals, indicating recombination between the markers and the sex-determining loci. *O. niloticus* bacterial artificial chromosome (BAC) clones, containing the AFLP markers, hybridised to the long arm of chromosome 1. This confirmed previous evidence, based on meiotic chromosome pairing and FISH probes obtained through chromosome microdissection, that chromosome pair 1 is the sex chromosomes.

An *in vitro* model for intramuscular fat in beef cattle is effected by sire genetic estimated breeding value, steer age and cell treatment with thiazolidinedione

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International markets for beef are sensitive to eating quality attributes of which intramuscular fat (IMF) is an important component. However, in the live animal IMF is difficult to measure and genetic selection is one of the limited methods that cattle producers have to influence this trait. This study has used serum from steers of different ages and selected for genetic divergence in IMF for treatment of the 3T3-L1 murine adipogenic cell line. This was done to investigate the potential of this *in vitro* cell culture model to be used as a tool to select cattle that have the phenotypic and genetic propensity to enter the expensive feedlot finishing system that produces beef with high levels of IMF that attracts a premium price in specific international beef markets.
The 3T3-L1 adipogenic cell line was treated with serum prepared from blood collected from steers at 12 and 24 months of age that were bred from sires of high and low genetic merit for IMF. In order to determine the maximum limit for adipogenesis, cells were also treated with and without the thiazolidinedione, ciglitazone. The effect of the treatments on glyceraldehyde 3 phosphate dehydrogenase activity and peroxisome proliferation activator receptor (PPAR) expression were assessed using spectrometry and western blotting, respectively.
The results show that the treatments effect adipogenesis and that this may be related to the expression of the adipogenic transcription factor PPAR. This preliminary study provides some insight into the future methods that may be developed for cattle producers to economically assess IMF development.

Markers of copper deficiency in *Drosophila melanogaster*.

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Extreme copper deficiency results in growth retardation, neural degeneration and other pleiotropic defects that cause death in the first few years of life. The effects of marginal deficiency are less clear, but may include an increased susceptibility to infection, impaired neurological function and an elevated risk to a range of diseases. A lack of informative *in vivo* markers for copper deficiency and a limited understanding of the genetic response to copper deficiency make establishing a minimum "safe" dose very difficult. Our work has established *Drosophila* as a useful system in which to investigate copper deficiency. Two *in vivo* markers for copper deficiency are proposed, the subcellular localisation of *dmATP7* and adult pigmentation. Copper dependent hypopigmentation is being used to uncover the genetic response to copper deficiency.

Expression Peroxisome Proliferator Activated Receptor (PPAR) and Lipogenic enzyme activity on bovine whey and soy protein isolates fed minipig

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Obesity is a disease that is associated with excess white adipose tissue. Currently obesity is a major health epidemic in developed countries worldwide. Obesity is associated with many health problems such as hypelipidemia, hypertension and diabetes. The research being conducted involves looking at the effects of high protein and modified carbohydrate diets, in controlling body composition. The minipig is an ideal human model for this work as they are genetically obese with body weights of approximately 120-140 kg which mimics the weight and composition of an obese human. The diets being used in this study contain either bovine whey protein which contains the satiety-inducing compound glycomacropeptide (GMP) or soybean protein isolate which contains the cholesterol lowering isoflavones, genestein and diadzin. Each of these diets is fed to 16 individually housed minipigs *ad libitium* at 80% and 160% of their normal requirement. Experimental analysis will involve the use of colorimetric techniques to determine the expression of lipogenic enzymes present in adipose tissue biopsies as well as the expression of the transcription factor PPAR. Fat composition within the minipig will also be determined using dual x-ray absorptiometry (DXA).

HIV and the Human Genome

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The aim of this study is to investigate the extent of homology between HIV and Human genomes and proteomes. The study uses bioinformatics tools and current data bases to perform a systematic comparative analysis. The literature contains numerous reports of HIV and human sequence similarities and epitope cross reactivity which is consistent with the hypothesis of mimicry, self- recognition and subsequent immune regulation.

Infection by human immunodeficiency virus type 1 (HIV-1) retrovirus initially attracts a host immune response. However, the interaction between the virus and the human immune system leads to an ineffective immune response and the chronic course of the disease. This process is not clearly understood.

Endogenous homologs of retroviruses are integrated and dispersed throughout the human genome. These endogenous retroviral genes are widely expressed both during development and constitutively in human tissues and may be recognised as self by the host's immune system. Similarity between an exogenous retrovirus such as HIV and endogenous homologs or other sequence elements could lead to the exogenous virus being recognised in the same way. Results of the initial comparative analysis are presented.

Detailed genomic comparison of the human Xp and wallaby 5p regions

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Comparative genomics has been extremely useful in studying the function and evolution of the mammalian genome. The Kangaroo is a new comparative candidate that offers the benefit that its genome is sufficiently diverged to mask random noises, while still providing a large array of orthologous sequences for analysis.

In this project I will study, in detail, a region of the short arm of chromosome 5 from the tammar wallaby. This region is of particular interest because it shares synteny with human chromosome Xp, so it will be possible to compare gene content and regulation in autosomal and X-borne regions that are identical by descent. Our ultimate aim is to build a complete physical map of the tammar 5p region. The comparative data obtained will allow us to identify important functional elements of the mammalian X chromosome such as X inactivation, sex determination and mental retardation.

I have therefore screened a tammar BAC library and recovered a 200kb, 5p specific clone containing homologues of the human Xp genes -- ZFX and Eif2S3.

Determining the biosynthetic pathway for sirodesmin production

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Sirodesmin PL is a phytotoxin produced by the fungus *Leptospharia maculans,* which causes blackleg disease of canola (*Brassica napus*). A cluster of genes with predicted roles in the biosynthesis of sirodesmin PL has been cloned. The roles of the individual genes in this cluster are being examined in three different ways. Firstly, the intermediates in the sirodesmin PL biosynthetic pathway are being characterized by HPLC in isolates that are deficient in sirodesmin production. Secondly, the effect of knocking down the expression of genes in the putative sirodesmin cluster via RNAi interference (RNAi)i is being examined. Thirdly, mutants created by insertional mutagenesis are being screened for loss of sirodesmin production via an antibacterial assay.

Gene clusters involved in biosynthesis of fungal epipolythiodioxopiperazine toxins

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Secondary metabolites from filamentous fungi are produced by pathways where genes encoding the enzymes are typically clustered in the genome. We have recently cloned a cluster of genes with roles in the biosynthesis of a phytotoxic secondary metabolite, sirodesmin PL from the plant pathogenic fungus, *Leptosphaeria maculans*. Sirodesmin belongs to the epipolythiodioxopiperazine (ETP) class of toxins produced by other fungi including the human pathogen *Aspergillus fumigatus* which makes gliotoxin, a mycotoxin with immunosuppressive properties. We have searched the genome sequences of filamentous fungi and found homologous gene clusters in *Aspergillus fumigatus* (but not in *A.nidulans*, which does not make gliotoxin); we predict that this cluster encodes biosynthesis of gliotoxin. Also homologues are present in the genome of the rice blast fungus, *Magnaporthe grisea* and of the wheat head scab fungus, *Fusarium graminaerum.* We are using comparative genome analyses to determine the minimum set of genes responsible for the biosynthesis of the ETP moiety.

Transport proteins for the fungal secondary metabolites, sirodesmin and gliotoxin

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Both Leptosphaeria maculans, the causal agent of blackleg disease of canola, and *Aspergillus fumigatus*, an opportunistic human pathogen, produce toxins (sirodesmin and gliotoxin, respectively) of the epipolythiodipiperazine (ETP) class. These molecules contain a disulphide bond which is thought to confer toxicity via redox reactions and cross linking dithiols of target proteins. The genes encoding their biosynthesis are clustered and include putative toxin efflux pumps of different classes, namely an ABC transporter (for sirodesmin) and a major facilitator superfamily type transporter (for gliotoxin). We have mutated the *Leptosphaeria maculans* ABC transporter (*sirA*) from the sirodesmin biosynthetic cluster and shown that the resultant mutant still makes sirodesmin, but has increased sensitivity to both sirodesmin and gliotoxin. We have carried out complementation experiments in the *sirA* mutant with both itself and the putative gliotoxin efflux pump (*gliA*) from *Aspergillus fumigatus*. Both *sirA* and *gliA* mediate resistance in *L. maculans* to gliotoxin, but only *sirA* appears to alter sensitivity to sirodesmin. Complementation of a knockout *Saccharomyces cereviseae* strain of the ABC transporter, PDR5, known to confer resistance to ETPs is being carried out to confirm that these *L. maculans* and *A. fumigatus* proteins act as transporters for their predicted substrates.

Correlated responses following selection for desiccation and starvation resistance: towards genetic dissection and gene identification

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A number of selection experiments have been carried out for stress resistance with Drosophila to explore the genetic basis of adaptive responses and patterns of correlated responses that might influence evolutionary trajectories for these traits. Here we describe new sets of selected lines that are being used to go beyond the phenotypic level and understand the genes underlying trait interactions. As a starting point, we here describe the replicated responses to selection of the lines, and the physiological basis of the selection response. We then compare our results to those obtained in previous studies and outline the strategy we are using to go beyond the phenotypic level, including the use of microarrays and QTL mapping to identify candidate genes.

Genetic and molecular characterisation of a novel Major Facilitator Superfamily protein implicated in zinc homeostasis in *Arabidopsis*

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Zinc is an essential micronutrient required for metabolism in all cells. In higher eukaryotes it is the second most abundant micronutrient after iron and Zn deficiency is possibly the most wide spread limitation on crop yields world-wide. Although candidates for uptake, storage and vascular translocation have been identified in *Arabidopsis*, our understanding of the mechanisms underlying Zn homeostasis remains limited. A number of protein families have been implicated in Zn transport, including Zrt- Irt-like Proteins (ZIP), Cation Diffusion Facilitators (CDF) and CPx P-type ATPases. In the current study, we have identified a novel mutant of *Arabidopsis thaliana* which shows sensitivity to elevated Zn. The mutation is in a gene encoding a member of the Major Facilitator Superfamily (MFS). Members of this protein family transport a wide range of substrates, but no characterised member has been implicated in Zn metabolism. The protein shows less than 50% similarity to any non-plant protein in the databases, however, two homologues exist in *A. thaliana*. This study involves the characterisation of mutants in these three genes, and the molecular characterisation of the gene conferring Zn sensitivity.

Directed evolution of human growth factors in *Neurospora crassa*

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This project aims to generate novel human growth factors (hGF) by utilising the *in vivo* gene diversification system developed for the filamentous fungus, *Neurospora crassa* (Catcheside *et al.*, 2003). Directed evolution of hGFs in Neurospora will utilise the high frequency of meiotic recombination initiated at the cog^L recombination hotspot to diversify hGF DNA sequences juxtaposed to cog^L during a sexual cross. Specifically, targeting vectors will be used to transplace a functional hGF gene between *his-3* and cog^L. Repeat-Induced Point mutation (RIP) is a natural phenomena occurring in the pre-meiotic phase of Neurospora's sexual stage that results in G:C to A:T transition mutations in duplicated sequences (Selker, 1990). Thus, an additional non-functional hGF gene will be ectopically transformed into Neurospora to induce low frequencies of RIP, generating hGF alleles *in vivo*. Additional hGF variation is created by subsequent meiotic recombination shuffling the hGF alleles created by RIP. This has the additional advantage of potentially separating deleterious mutations. Progeny from the cross will be screened to identify novel hGF variants.

Catcheside, D. E. A., Rasmussen, J. P., Yeadon, P. J., Bowring, F. J., Cambareri, E. B., Kato, E., Gabe, J. & Stuart, W. D. 2003, 'Diversification of exogenous genes *in vivo* in *Neurospora*', *Appl. Microbiol. Biotechnol.*, vol. 62, pp 544-549.

Selker, E.U. 1990, 'Premeiotic instability of repeated sequences in *Neurospora crassa*', *Ann. Rev. of Genetics*, vol. 24, pp 579-613.

Sex, size and success: factors affecting multiple paternity and reproductive success in the brown antechinus

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Factors that impact upon reproductive success directly affect the inclusive fitness of individuals and the viability of naturally breeding populations. This study investigated the factors that influence male and female reproductive success in free-living *Antechinus stuartii*, using microsatellite markers to determine paternity. The small marsupial, *A. stuartii,* has a short, synchronised mating period after which all males die. Females subsequently store sperm for up to two weeks. This study confirmed that both male and female *A. stuartii* are promiscuous and that multiple paternity within litters is common. Increased mating frequency will increase male reproductive success, however, no relationship was observed between effective mating frequency (number of inseminated females) and the male characters of body mass and scrotal size. Interestingly, both of these male characters were significantly related to paternity success (number of offspring). This suggests that these physical characters affect fertilisation success rather than mating access. The mechanism by which large males achieve increased paternity success is likely to be via extended copulation times that function as mate-guarding. Increased paternity success of males with large scrotal size is likely to be via increased allocation of sperm per ejaculate. Sperm storage and high levels of multiple paternity suggest the existence of sperm competition in *A. stuartii*.

Identification of susceptibility loci in complex disorders

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Identification of susceptibility loci in complex disorders involves a genome-wide screen of thousands of individuals. The recent development of more efficient screening technologies has made gene identification more achievable by the ability to screen large numbers of genotypes in a short amount of time. The Neurodegenerative Disorders Centre (NDC), through the support of GlaxoSmithKline, has established a semi-automated high-throughput genotyping facility with both microsatellite and single nucleotide polymorphism (SNP) capabilities. The microsatellite section of the facility is a flexible and robust system involving over 450 highly informative markers for a comprehensive genome screen. Regions of interest can be further refined using the SNP genotyping component of the facility which utilises several robotic systems allowing automation and rapid generation of results. Previous screens for complex disorders including a metabolic syndrome, asthma and osteo-arthritis have been carried out at the NDC. Opportunities also exist for academic researchers to access the resources of this facility.

Phylogenetics and population biology of the south-eastern Australian holly-leafed *Grevillea* species.

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Grevillea R.Br ex Knight (Proteaceae) is represented in Australia by ca. 360 species, making it the third largest plant genus in the country. Within the 20 recognised taxa representing the '*G. aquifolium* Group' found in south eastern Australia, 13 are currently recognised as rare, vulnerable or endangered on the ROTAP list. A number of these geographically disjunct taxa are narrowly endemic and may be at risk from altered land management strategies. Therefore, the major aim of the proposed project will be to obtain information of direct benefit to their conservation management. Sequence data from three cpDNA regions (trnT/trnL spacer, rpl16 intron, psbA-trnH spacer) and nuclear rDNA ITS regions will be used to assess the phylogenetic relatedness of members of the '*G. aquifolium* Group' and help clarify the current taxonomy. Initial cpDNA sequence data obtained to date suggest a close genetic affinity of many of the Victorian species. However, a more complete phylogenetic analysis including additional data needs to be conducted. Attention will then focus on one of the ROTAP listed species within Victoria (*e.g.*, *G. montis-cole* subsp. *brevistyla*) to investigate the population structure using molecular markers. Aspects of the breeding system of the species will also be investigated and related to population genetic data. The current management practices will be assessed and suggestions made as to future management to ensure long-term viability of threatened populations.

Molecular ecology of Bogong Moths

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Bogong moths *Agrotis infusa* are distributed throughout south-eastern Australia, and are known for their spring flights to mass aestivation sites in alpine regions. In autumn the moths return to their breeding grounds to mate. The larvae are significant pests of crops. Some summer aggregations have been found to contain high levels of arsenic. This toxin is present in high concentrations in the droppings of small alpine mammals, such as the Mountain Pygmy-Possum *Burramys parvus*, for which Bogong moths are an important prey. Adult moths do not feed in the alps, so it is likely the arsenic is derived from their breeding regions where the caterpillars feed, and is related to past use of arsenic as an insecticide. The fact that different long-term accumulations of moth bodies contain consistently different arsenic levels suggests that particular aggregations are derived from different geographic regions with varying larval exposures. We are using mitochondrial and nuclear DNA markers to attempt to characterise summer aggregations and distinguish potential source populations. We hope to identify the geographic sources of arsenic contamination. Here we present results of DNA analyses of adult moths collected from aestivation sites in the Victorian Alps and the Snowy Mountains in NSW and ACT.

Effects of genetic polymorphisms on gene expression and muscle hypertrophy

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Skeletal muscle makes up the greatest percentage of muscle in mammals, its primary role is in locomotion. MyoD, Myf5, myogenin and MRF4 transcription factors are involved in myogenesis. Occasionally spontaneous DNA mutations arise due to deletions, substitutions and insertions. Known single nucleotide polymorphism (SNP) mutations that effect muscle include the Callipyge (CLPG) mutation. Qualitative and quantitative methods showed that the CLPG mutation is an A-G polymorphism and its locus is at the terminal region of chromosome 18. Evidence supports the paternal polar overdominance model of gene action. The CLPG mutation causes dramatic effects on the development of normal muscle in the hind region, carcass composition, shape and meat quality. Aim of this experiment is to determine if selected myogenic transcription factors are affected by genetic polymorphisms. DNA and RNA are isolated from cell cultures (POM and C2C12) at 3 and 10 days post differentiation and polymerase chain reaction is carried out to detect expression of the CLPG gene and differences in transcription factors. Tissues are collected *ex vivo* and PCR is carried out for CLPG mutation and transcription factors. The results from molecular studies determine the effects of genetic polymorphisms on gene expression as a result of the CLPG mutation.

P-type ATPase heavy metal transporter genes involved in essential zinc homeostasis in Arabidopsis thaliana

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In Arabidopsis there are eight P_{1B} heavy metal transporting ATPases. This study is focussed on three closely-related members of this group, HMA2, HMA3 and HMA4. Genetic analysis has shown HMA2 and HMA4 are essential for zinc homeostasis: an *hma2,hma4* double mutant is severely zinc deficient (1). Symptoms include chlorosis, stunting and failure to develop pollen, and the phenotypes are reversible by the application of exogenous zinc. In contrast, the role of HMA3 *in vivo* is less clear. Promoter-GUS reporter constructs show *HMA2* and *HMA4* have parallel expression patterns in vascular tissues and in developing anthers. The expression pattern for *HMA3* is less clear and appears to vary depending upon the genetic background. Expression of the HMA proteins in yeast has effects on cadmium resistance and zinc dependence. We are using multiple mutants and further analysis of gene expression to better understand the role of HMA3 and we are manipulating *HMA2* gene expression *in vivo* to investigate the apparent role of HMA2 (along with HMA4) in the delivery of zinc to pollen in developing anthers.

(1) Hussain et al. Plant Cell 16: 1327-1339.

Testing Parentage using Microsatellites in Saltwater Crocodiles

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Fifteen microsatellite loci were evaluated in farmed saltwater crocodiles for use in parentage testing. One marker (C391) could not be amplified. For the remaining fourteen, the number of alleles per locus ranged from 2 to 16, and the observed heterozygosities ranged from 0.219 to 0.875. The cumulative exclusion probability for all fourteen loci was 0.9988. The eleven loci that showed the greatest level of polymorphism were used for parentage testing with an exclusion probability of 0.9980. Using these eleven markers on 107 juveniles from 16 known-breeding pairs, a 5.6% pedigree error rate was detected. This level of pedigree error, if consistent, could have an impact on the accuracy of genetic parameter and breeding value estimation. The usefulness of these markers was also evaluated for assigning parentage in situations where maternity and/or paternity may not be known. In these situations, a 2% error in parentage assignment was predicted. It is therefore recommended that more microsatellite markers be used in these situations. The use of these microsatellite markers will broaden the scope of a breeding program allowing progeny to be tested from adults maintained in large breeding lagoons for selection as future breeding animals.

Genetic Improvement of Saltwater Crocodiles

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By implementing an improvement program based on reproductive performance, juvenile growth and juvenile survival, superior juveniles will be selected as future breeding animals to increase the profitability of crocodile farms. It was, therefore, the aim of this study to create a plan for a practical genetic improvement program at a commercial crocodile farm (Janamba Croc Farm), thereby establishing an industry-wide genetic improvement program to be called CrocPLAN. Data were collected from Janamba Croc Farm and analysed to obtain relevant genetic and phenotypic parameters (heritability, repeatability and correlation) for breeding objectives and selection criteria. Relative economic weights were estimated and combined with estimated crocodile breeding values (CBVs) into a crocodile economic selection index ($CESI). Each $CESI value was expressed as a dollar ($) deviation from the herd average. Using the breeding pairs from Janamba, the highest ranked pair had a $CESI value of +$4,748, whereas the lowest ranked pair had a $CESI of -$5,257. The response to selection was predicted to be $324 increase in profit per breeding pair per annum. This result reinforces the potential for implementing CrocPLAN on saltwater crocodile farms in Australia. It also illustrates the potential for similar selection programs to be implemented using other crocodilians.

Gene silencing using RNAi in *Leptosphaeria maculans*, the blackleg fungus of canola

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Leptosphaeria maculans, the causal agent of blackleg disease, can cause severe yield losses to the Australian canola (*Brassica napus*) industry. We have begun a reverse genetics approach to understand gene function in this fungus. Few genes have been targetted so far because at least 7 kb of flanking DNA is required for homologous recombination and up to several hundred transformants need to be screened to achieve gene disruption. To tackle this problem we are developing gene silencing by RNAi. We have developed a hairpin vector using Gateway technology which allows rapid creation of gene silencing constructs. To show proof of principle, we have silenced an exogenous gene, thymidine kinase, from *Herpes simplex*, which had been transformed into *L. maculans*. When this gene is active, the fungus cannot grow on certain pro-drug thymidine analogues. We have also applied this technology to silence a gene, acetyl transferase, in the biosynthetic pathway for sirodesmin PL, a major secondary metabolite which is thought to play a role in blackleg disease. The HPLC profile of the transformants show a higher ratio of deacetyl sirodesmin (the substrate of this enzyme) to sirodesmin (the product of the reaction) than in the wild-type, suggesting a role for this gene in the final step of the sirodesmin biosynthesis pathway. Transcript levels in transformants are currently being determined by real time PCR. We are now developing constructs to silence other genes in the sirodesmin biosynthetic pathway.

Identification of genetic interactors of the *Drosophila* Rho-GEF, *pebble*.

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Rho belongs to a family of small GTPases that are important regulators of the actin cytoskeleton. The *Drosophila pebble* gene encodes a putative Rho GTP exchange protein (Rho-GEF), which activates Rho and subsequent downstream pathways. Pebble is essential for the formation of the actin-myosin contractile ring during cytokinesis. Pebble is also required for the epithelial-mesenchymal transition of the embryonic mesoderm, another process dependent on the actin cytoskeleton. To identify novel genes that interact with *pebble*, a dominant negative form of *pebble* was generated by deletion of 53 codons within the GEF-encoding domain. Expression of dominant negative *pebble* in the developing *Drosophila* eye results in a rough eye phenotype. This phenotype has been used in an ongoing dominant modifier mutant screen to identify new genes that interact with *pebble*. One dominant suppressor (*inops*) has been mapped to a small region on the third chromosome, and candidate genes are being sequenced. Some *inops* mutations are recessive lethal, but a transheterozygous *inops* allelic combination gives a dramatic adult phenotype of small eyes, and deformed legs and wings, indicating that *inops* is important for *Drosophila* development. Results of the dominant modifier screen and the mapping of the *pebble* interacting loci will be presented.

Effects of bovine whey and soy protein supplemented diets on biochemical signalling pathways involved in muscle development in pigs

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Muscle is a dynamic reservoir of bound and unbound proteins (amino acids) that are constantly broken down and regenerated to meet metabolic demands. Insulin-like growth factor 1 (IGF-1) is a small peptide growth factor that controls muscle growth through the insulin signaling pathway. Insulin axis involves the phosphorylation of Insulin Receptor Substrate-1 (IRS-1) in response to insulin *in vivo* which in turn activates the phosphatidylinositol-3-kinase (PI-3K) pathway. PI-3K pathway stimulates muscle protein synthesis and the uptake of glucose with the most prevalent glucose transporter isoform expressed being GLUT4. Creatine kinase (CK) system efficiently regulates energy transfer and is believed to be involved in the metabolic regulation of energy fluxes and oxidative phosphorylation. Forty eight pigs were fed with 100%, 140% and 180% of the RDI for protein (whey and soy) supplemented diets for 20 weeks and blood samples obtained at weeks 1, 5, 10, 15 and 20. Muscle biopsies were taken at the end. Plasma insulin levels are measured by RIA and Quantitative PCR and Western blotting used for IRS-1, IGF-1, CK and GLUT4 genes. These analyses will indicate the effects of both whey and soy protein sources and levels on muscle development through the insulin and IGF axes.

Neutral evolution of the SRY gene in the Pinnipeds

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The pinnipeds include three carnivore families, (Phocidae, Otariidae and Odobenidae), with diverse breeding systems and levels of sexual dimorphism, with the potential for competition between the sexes to maximise fitness. Here 19 pinniped, and 5 terrestrial carnivore (two mustelid, one Ursid, one canid and one felid) *SRY* gene sequences were investigated to determine whether high rates of divergence occur in the terminal regions of the SRY gene and whether such divergence may be the result of selection acting on the protein sequence. Maximum Likelihood methods employing codon substitution models were used to estimate rates of non-synonymous and synonymous substitutions along branches of the phylogeny, and between codon sites along the sequence. Rates of non-synonymous substitution were suggestive of possible positive selection or lack of selective constraint. The ratios between rates of synonymous and non-synonymous substitutions were not significantly variable between branches in the phylogeny, and no variation in selective pressure could be detected between codon sites in the terminal regions of *SRY*. This suggests a lack of functional constraint as the probable cause of the divergence between species.

X inactivation of the Marsupial SLC16A2 Gene

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X chromosome inactivation (XCI) is the mechanism that achieves dosage compensation of all X linked genes between males and females. Marsupial XCI differs from the eutherian process as it is less stable, paternal rather than random and tissue specific. Suggesting that there have been fundamental changes in the mechanism during the evolution of the two lineages.

In addition some eutherian X linked genes have been shown to escape XCI. In the case of marsupial X linked genes it is unknown which are silenced and which escape XCI. Hence determining the X inactivation status of genes on the marsupial X chromosome is of great interest for later comparative studies.

In this study the 3' UTR of the tammar wallaby (macropus eugenii) SLC16A2 (solute carrier, family 16, class A, member 2) gene was used to screen a BAC library. The isolated BAC containing the marsupial SLC16A2 mapped to the terminal end of the long arm of the marsupial X chromosome by DNA FISH. This clone was used in RNA FISH experiments on female and male cells to determine the X inactivation status of the gene. The results indicate that the marsupial SLC16A2 gene is silenced on the inactivated X.

Comparative genome mapping of pig and sheep for economic traits

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Current traditional breeding methods that use quantitative genetic evaluation are based on the pedigree and phenotypic information of sheep and the pig population. For traits that are difficult to assess such as lean meat yield, eating quality and subcutaneous fat microsatellite markers have become a popular tool to assess genetic heritability and for livestock selection in genetic studies. One strategy used to detect the homology of genes between species is comparative mapping. This allows for the identification of sections of pig and sheep chromosomes that carry QTL that correlate with phenotypes that are desirable. The aim of this project is to create a fine scale map of known QTL by using both microsatellites and single nucleotide polymorphisms (SNP). The resulting data will be analysed to precisely determine the position of the QTL. In addition, Targeted Identification of Polymorphic sequences (TIPs) will be used to find SNPs in this region. These SNPs will also be typed across sheep and pigs in an attempt to find the gene of interest. Results indicate that known pig microsatellite primers for economic traits have been successfully amplified in sheep.

Western grey kangaroo population structure and the possibility of hybridisation with eastern grey kangaroos in the wild

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Management and conservation plans require a comprehensive understanding of many aspects of a species in order to be effective, particularly in the long term. Modern genetic techniques are capable of clarifying questions which ecological studies have left unanswered. The taxonomic classification of the western grey kangaroo, *Macropus fuliginosus* has, in the past, been the subject of considerable confusion, due to the presence of morphological variation and a poor understanding about the species population structure. Furthermore the occurrence of hybridisation between the two species of grey kangaroo in captivity coupled with sightings of putative hybrids in the wild has added to the confusion. Therefore, the aim of this project is to examine both the contemporary and historical population structure of the western grey kangaroo, its phylogeography and dispersal patterns as well as its relationship to the eastern grey kangaroo (*Macropus giganteus*). While the project is still in its early stages various microsatellite loci are currently being examined, several of which have already been identified as having fixed differences between eastern and western grey kangaroos. Extensive sampling has also begun in an attempt to answer these questions. Here we present an outline of the plan which will be used in this project.

DNA Quality and Your Results

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The Australian Genome Research Facility is a high throughput organization, which provides state of the art DNA analysis technologies, to support gene discovery and genomic exploration by research institutions and industry.

As it is a high throughput facility, established protocols are utilised. Previously, no optimisation or quality control of samples occurred at the AGRF. Therefore the turnaround and pass rate of the results supplied by the AGRF could be directly correlated to the quality and accurate quantification of DNA samples provided by the client.

We have identified that DNA extraction and purification protocols, contaminants present in samples and inaccurate concentration readings provided, have all been contributing factors toward the quality of the output data. To improve data quality, the AGRF is introducing DNA quality control procedures through Real-Time PCR.

The AGRF will henceforth be able to predetermine whether the quality of samples provided is adequate for our high throughput system. Through DNA quality control and client education we expect to improve turnaround times and increase pass rate ensuring the data provided will be most complete and of the highest quality.

Gene duplication, selective sweeps and fitness costs of P450s implicated in insecticide resistance.

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Cyp6g1 is a cytochrome P450 gene that is over-expressed in insecticide resistance strains of *D.melanogaster*. Frequencies of the resistant allele, which has an Accord transposable element 290 nt upstream of the transcription start site, are high in populations across the world. Here we report on the results of fitness cages that show that the resistance allele is less fit in the laboratory when reared in the absence of insecticide. Surveys of molecular variation at the *Cyp6g1* locus in Australian field populations reveals at least five variants of the Accord insertion. This contrasts with the paucity of diversity in closely linked genes. We describe the patterns of linkage disequilibrium around this gene extending out to *Cyp12d1* - another P450 gene 1Mb away. There is polymorphism for a *Cyp12d1* gene duplication. Our data indicate that this duplication is in the process of become fixed. The importance of P450 gene duplication to insecticide resistance will be discussed.

What genetic markers can elucidate about wobbegong sharks?

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Genetic markers allow for the identification of individual dispersal, fine-scale population structure and cryptic speciation. They are particularly useful for studying large marine predators such as sharks, where movement patterns and population structures are difficult to document because animals are long-lived and may move widely between breeding events. Here we describe the development and functionality of two types of genetic markers (microsatellites and mitochondrial DNA, mtDNA) for a declining group of wobbegong sharks (*Orectolobus* spp). Like several coastal shark species, the spotted wobbegong (*O. maculates*) and the ornate wobbegong (*O. ornatus*) have life history and ecological characteristics that have rendered them particularly vulnerable to anthropogenic impacts, particularly targeted fishing. Recently, a distinct "dwarf" morphotype that matures at a significantly smaller size than the known morphotype has been identified, implying taxonomic uncertainty for the group. We are using a set of *Orectolobus* microsatellite markers and mtDNA sequence data to test for reproductive isolation among sympatric wobbegong morphotypes and elucidate large and fine-scale patterns of population structure (such as differences in the dispersal rates between sexes). Determining these demographic parameters along with resolving the taxonomic uncertainties will have direct implications for monitoring commercial catches and establishing conservation strategies for wobbegong sharks.

The cytokinetic role of *Drosophila* Citron kinase in Rho GTPase signalling.

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The Rho effector kinase Citron has been implicated in cytokinesis in vertebrates. Citron is required for cytokinesis in some, but not all, cell lineages during mammalian development and in cultured *Drosophila* cells. Here we report a detailed genetic analysis of the *citron* ortholog of *Drosophila melanogaster*. We find that Citron can bind RhoA and that Citron localises to the contractile ring in a RhoA-dependant manner. We demonstrate that *Drosophila citron* is expressed in proliferating tissues but is downregulated in differentiating tissues. Phenotypic analysis of mutants showed that *citron* is required for cytokinesis in every tissue examined, mutant cells exhibiting multinucleate and hyperploid phenotype. In imaginal disc cells, but not embryonic PNS or larval brain cells, the cell division defects were accompanied by elevated levels of cell death. Significantly, genetic interaction analyses showed that Citron promotes the Rho cytokinesis pathway in proliferating tissues. Our analyses of another Rho effector kinase Rok failed to show its role in Rho signalling during cytokinesis. We provide therefore the first *in vivo* evidence that Citron is a downstream effector of Rho cytokinesis signalling.

Microsatellite markers for the invasive fruit fly pest species Bactrocera papayae

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The dorsalis complex (defined by the Oriental fruit fly, *Bactrocera dorsalis* (Hendel)) comprises at least 52 species and includes some of the most economically important and destructive pest species of the Asia-Pacific region such as the papaya fruit fly, *Bactrocera papayae* (Drew and Hancock). *B. papayae* is native to southeast Asia (Thailand, Malaysia, and Singapore), Indonesia and Kalimatan although in 1992 it was detected in Papua New Guinea where it was thought to have arrived from Asia through Irian Jaya. *B. papayae* has also been detected in traps on the Torres Strait Islands since trapping started in 1993. In October 1995 it was detected in Cairns, although it is thought to have established up to 18 months earlier. An eradication programme was implemented in 1996 and it was declared eliminated in 1998. A set of at least 20 microsatellite markers have been identified for *B. papayae*, which includes a set of markers, isolated from *B. papayae* together with a larger set of markers previously isolated from *Bactrocera tryoni*. Microsatellite analysis was performed on samples of *B. papayae* from Cairns, Torres Strait, Papua New Guinea and Malaysia in order to determine if the origin of the invasive populations can be established.

The *Drosophila dead ringer* gene is required for adult fly vision

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The *Drosophila* protein Dead ringer (Dri) is one of the founding members of the ARID family of transcription factors. *dri* is required for the normal expression of a range of genes during embryogenesis and is essential for viability. Expression of *dri* continues beyond embryogenesis. In the larval eye, *dri* expression is restricted to the R1-R6 and R8 photoreceptor cells. Utilising *dri* mutant somatic clones spanning the entire eye, the function of *dri* in the adult *Drosophila* eye was examined. Using electroretinograms, *dri* was found to be required for normal vision in the adult fly. In the adult eye, *dri* expression continues in R1-R6 cells, an expression pattern identical to the light-sensing Rhodopsin 1 (Rh1) gene, *ninaE*. Analysis of the cellular structure of *dri* mutant eyes revealed the rhabdomeres degenerated in an age-dependent manner. Similarly, mutations in *ninaE* also exhibit retinal degeneration. Further analysis into the hypothesis that Dri regulates *ninaE* expression will be presented.

Field assessment of black leg disease among genotypes of *Brassica napus*

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Canola is a major oilseed crop in Australia and contributes approximately $500 million annually to the national economy. Blackleg, caused by the fungus *Leptosphaeria maculans,* is the most severe disease of canola world-wide and results in significantly lower yields in affected crops. We are currently developing F_4-F_6 reference populations of recombinant inbred lines (RILs) from the progeny of resistant and susceptible crosses. Three crosses of canola (F_4 RILs), BLN2367 TT x Ag Emblem (designated 012 WT), BLN 2385TT x Surpass 400 (designated 030WT) and 44C71 x Surpass 400 (designated 074NC) were investigated in a field study at Horsham and Lake Bolac, Victoria in 2003. Results show that leaf lesion and stem canker size and stem lodging increased with time following infection but that there were differences between the crosses. Disease developed more quickly and to a greater extent on the susceptible canola lines (012 WT than that on the resistant lines (074 NC and 030WT). Nine hundred leaf samples are currently being characterised for co-segregation of blackleg resistance using SSR, ISSR and microsatellites. This research will contribute significantly to an understanding of disease resistance in canola and assist the development of new varieties with increased resistance to blackleg disease.

Avian Evolution Using Complete Mitochondrial Genome Sequences

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Despite decades of study using a variety of data (morphological, palaeontological, immunological, DNA hybridization, short DNA sequences etc), a number of questions regarding avian evolution remain unresolved. For example, the relationships between bird orders and the times of origin of modern bird lineages. It is held that most of the interordinal diversification of modern birds occurred over the 15 million years following the mass species extinction at the Cretaceous/Tertiary (K/T) boundary (65 million years ago).

There is increasing confidence from molecular studies that these questions are now answerable, given a range of sufficiently long enough DNA sequences – for example, complete mitochondrial (mt) genomes. However, only a few avian orders are currently represented by complete mtDNAs. Moreover, analysis of the few bird mt genomes available has lead to conflicting hypotheses about the root of the avian tree. In order to address these issues, our group has sequenced many complete avian mitochondrial genomes. Adding these taxa to the mt genome dataset provides improved taxon sampling of long avian DNA sequences. This should aid in resolving bird phylogeny and in identifying the deepest branch in the avian tree. Our molecular dating of survival across the K/T boundary indicates that some of the avian interordinal diversification occurred before the K/T boundary.

Lipase Expression in *Helicoverpa armigera*

Ariadne Tan-Kristanto[1], David Heckel[1-2], Phil Batterham[1]
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Twelve genes putatively encoding lipases expressed in larval midgut were discovered through EST studies in the cotton bollworm (*Helicoverpa armigera*). To examine their role in this species, feeding studies and real-time RT-PCR were performed. Larvae were fed one of three diets: 1) normal medium; 2) the same medium without the usual added oil; 3) the second medium with 80% of the oil-containing wheat germ removed. The dietary treatments do not affect the time or survival rates to pupation. Pupal weights in treatments 2 and 3 and 10-day larval weights in treatment 3 were lower than in treatment 1. Real-time RT-PCR was performed on cDNA from midguts taken from larvae in the three treatments, as well as from all life stages and various organs within fifth instar larvae on control diet. Results indicate the potential importance of regulation of this gene family in adaptations to varying lipid levels present in the natural diet of the fruit- and flower-feeding larval stage.

An historical view of clinal variation along the east coast of Australia

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Clinal variation in natural populations may be directly related to natural selection or the by-product of non-adaptive processes related to population structure and history. The alcohol dehydrogenase and glycerol-3-phosphate dehydrogenase loci of *Drosophila melanogaster* have been extensively used to examine evolutionary processes and provide evidence that clines are maintained by selection gradients[1-4].

We revisit work from the early 80's and compare the degree and pattern of the genetic polymorphisms in these genes between then and 'current-day' samples. Flies were collected from seventeen populations spanning the eastern coast of Australia in 2002 and again in 2004. Using a bi-pasa based PCR method, we studied the distribution and relative frequencies of $Adh^{-S/F}$ and $Gpdh^{-S/F}$ frequencies throughout the cline in both years. We compare the slope of these clines to early estimates by Oakeshott *et al.* (1982), allowing inferences regarding the processes that affect latitudinal patterns and clinal variation at these two loci, and stability of these over time. Additionally, in the same samples we examined linked polymorphisms for *In(2L)t* and for two common insertion/deletion variants that occur in the first intron of the *Adh* gene.

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[4] Van 'T Land, J. *et al.* 2000. Evolution **54**:201-209.

Evolutionary relationships inferred from the TEF1 gene among the rust fungi.

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The rusts (Basidiomycetes: Uredinales) are a large and diverse group of obligate biotrophic fungi that include many of the most important and devastating agricultural pathogens - especially of cereals (stem, leaf and stripe rusts). Despite their economic importance, the understanding of evolutionary relationships within the group is poor and based largely on the morphology of fungal structures, and to a lesser extent, on host identity and life cycle stages. Better knowledge of the phylogenetic relationships among rusts is likely to increase our ability to predict the potential for host shifts to and from native plants and crops and may also provide a conceptual framework for understanding the evolution of the intricate life cycle histories found within the group.

A fragment of the Translation Elongation Factor 1-alpha gene (TEF1), including 4 exons and as many as three introns was amplified and used to investigate relationships among different taxonomic groups of rusts. Amplification of the TEF1 gene showed variation in the intron sequence, position of the introns and the number of introns amplified. While exons provide information among more distantly related rusts, the introns appear to be most useful with regard to delineating species level relationships.

Arabidopsis AtXPD and Atp44 participate in transcription and/or nucleotide excision repair in yeast

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XPD/Rad3 and 44 are two of the nine sub-units in the TFIIH protein complex that functions in transcription and nucleotide excision repair (NER) in humans and yeast. We have cloned cDNAs potentially encoding the *Arabidopsis* XPD (>81% conservation with human XPD) and p44 (86% conservation with human p44, including a conserved C4 zinc finger motif) proteins. Functional complementation studies revealed that expression of AtXPD or Atp44, respectively, in yeast *rad3*or *p44* mutants temperature-sensitive for transcription permitted growth at the restrictive temperature. This indicated that the *Arabidopsis* proteins can operate in transcription as part of yeast TFIIH, implying a similar function in the plant. Although expression of *Atp44* also corrected the UV sensitivity of the yeast *p44* mutant, AtXPD was unable to restore UV-resistance in a UV-sensitive yeast *rad3* mutant. This suggests that Atp44 also functions as part of TFIIH in NER whereas the Rad3 helicase activity necessary for NER in yeast is not provided by AtXPD. The XPD/Rad3 and p44 proteins have been shown to interact in human and yeast TFIIH. Yeast two-hybrid studies confirmed an interaction between Atp44 and AtXPD, and we are currently examining interactions between AtXPD and yeast p44, and Atp44 and yeast RAD3.

AtRev7, an _Arabidopsis_ homologue of the human and yeast polymerase zeta Rev7 subunit, restores UV resistance in a yeast _rev7_ mutant

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The human and yeast (*Saccharomyces cerevisiae*) *REV3* and *REV7* genes encode polymerase zeta (Pol ?). This polymerase contributes to UV resistance, and is necessary for UV-induced mutagenesis, because it is involved in error-prone replication of UV-induced DNA damage (translesion synthesis). The catalytic subunit of Pol ? is encoded by *REV3*, and *REV7* encodes a subunit that stimulates translesion synthesis. We have isolated and characterised an *Arabidopsis* cDNA (*AtREV7*) predicted to encode a Rev7 homologue (51% and 68% conservation to the yeast and human proteins, respectively). We determined that *AtREV7* is constitutively expressed in flower, leaf and root tissue, and that its expression partially complements the UV sensitivity, but not the defect in UV mutagenesis, conferred by deletion of yeast *REV7*. The latter result suggested that rather than being a genuine Rev7 homologue, AtRev7 might suppress UV sensitivity in the *rev7* mutant through a Pol ?-independent pathway. However, expression of *AtREV7* failed to restore UV resistance in a *rev3 rev7* double mutant linking the function of AtRev7 to Pol ?. Collectively, these results suggest that AtRev7 can interact with Rev3, the catalytic subunit of yeast Pol ?. Currently, we are performing yeast two-hybrid studies to test this hypothesis.

Translation of a second open reading frame in *drosophila* dicistronic mRNAs is dependent on the absence of internal *AUG* codons in the first open reading frame.

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Translation of eukaryotic dicistronic transcripts requires deviations from the general rules of translation. Ribosomes are presumed to dissociate from the transcript after termination at the end of the first open reading frame (ORF). Translation of a second ORF would require re-initiation of translation after an inter-cistronic region. Recently dicistronic transcripts have been identified in *D.melanogaster* and have been shown to efficiently translate a second ORF after the previous translation of a large (up to 2.5kb) upstream ORF. A common factor of these dicistronic transcripts is the absence of internal methionine residues encoded by the 5'ORF. To identify the significance of the absence of in-frame AUGs, a series of dicistronic constructs were created with differing numbers of in-frame AUG codons in the 5'ORF with the green fluorescent protein (GFP) encoded by the 3'ORF. The levels of re-initiation for each construct were identified by transfecting *Drosophila* S2 cells and assaying for fluorescence. Extracts were also subjected to Western blot analysis to determine relative protein levels. Results revealed that a single AUG insertion decreased GFP expression levels by up to 58% and up to 77% of normal expression levels when three AUGs were added to the 5'ORF.

Genetic variation and the occurrence of population bottlenecks in wild Javan rusa deer and Fallow deer within Australia

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Javan rusa deer (*Cervus timorensis russa*) and fallow deer (*Dama dama dama*) are two of six introduced deer species that persist as wild populations in Australia. Rusa deer were first introduced into the Royal National Park NSW nearly a century ago and has Australia's largest population. During the 1830's fallow deer were first introduced to Tasmania and is Australia's largest population. Using Artiodactyla microsatellite markers genetic investigations of both of these species were carried out.
Results from rusa deer indicated that 24 markers (64.9%) were polymorphic and had a mean of 2.29 alleles/locus. This is substantially lower than that seen in source populations from New Caledonia. Total exclusion power for the first and second parent was 0.944 and 0.996 respectively and historical documentation of a population bottleneck was supported by this molecular data ($P<0.001$).
Results from fallow deer revealed 10 polymorphic markers (27%) with an average of 2.488 alleles/locus. Samples were collected from three sub-populations (~100km apart) and an analysis of molecular variance showed 91% of the genetic variation is within the populations and 9% among the populations. Genic sub-structuring was significant for two population combinations (F_{ST}=0.1217 and 0.1104). A population bottleneck was evident from molecular data ($P<0.001$) coinciding with historical documentation. Despite low allelic diversity evident in both species, the suite of polymorphic loci identified show promise for applications in population genetics, including parentage analysis and population substructuring within Australia.

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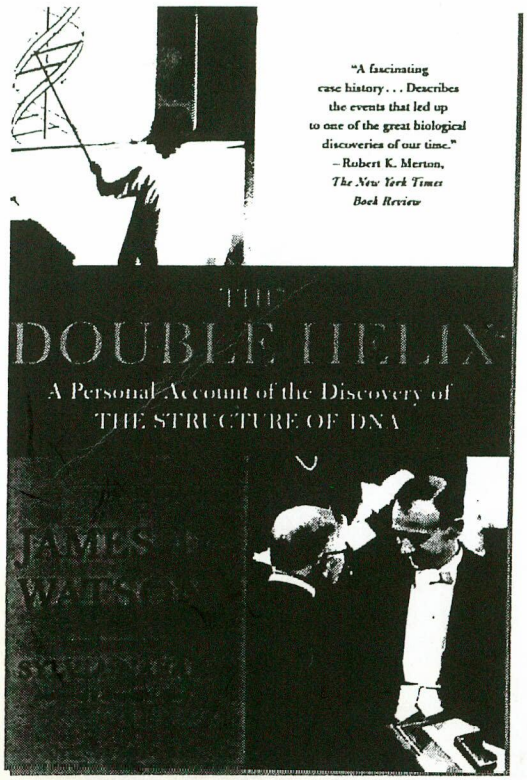

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